APVS 2023

Asian Pig Veterinary Society Congress 2023
Taipei, Taiwan

July 30-August 2, 2023

ABSTRACT BOOK

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Acknowledgments

Acknowledgments

Dear members, colleagues, and friends:

On behalf of the 10th Asian Pig Veterinary Society (APVS) Organizing Committee, I am pleased to announce that the 10th APVS Congress will take place from 30th July to 2nd Aug 2023 in Taipei, Taiwan ROC. I look forward to welcoming you in person and also online.

The Congress was initially scheduled in 2021 but had been postponed because of the pandemic. Now the COVID restrictions are easing, the APVS 2023 will take place successfully with my confidence. I look forward to welcoming you in person or online.

Pig health and production management are facing new threats and challenges in the post-COVID era, particularly in Asia. International dialogues on related veterinary approaches are essential to forming new and innovative attempts for producers and veterinarians.

APVS 2023 will include four topics:
1. Transboundary Diseases
2. Viral Diseases
3. Bacterial and Other Infectious Diseases
4. Nutrition, Animal Welfare

Oral and poster presentations are all welcome and will be given in person or virtually.

Taipei is a modern cosmopolitan city with a well-developed infrastructure. Subways, railways, and high-speed trains are convenient to access. The town is famous for museums as well known for friendly people and exquisite foods. The Congress will be held at Taipei International Convention Center, and your participation and contribution will be highly appreciated, and I trust you will have a wonderful stay.

Looking forward and Best Wishes.

Ming-Tang Chiou DVM, PhD.
President, APVS 2023 Organizing Committee
Professor, Department of Veterinary Medicine, National Pingtung University of Science and Technology

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History of APVS Congress

2003 Seoul, Korea

2005 Manila, Phillippines

2007 Wuhan, China

2009 Ibaraki, Japan

2011 Pattaya, Thailand

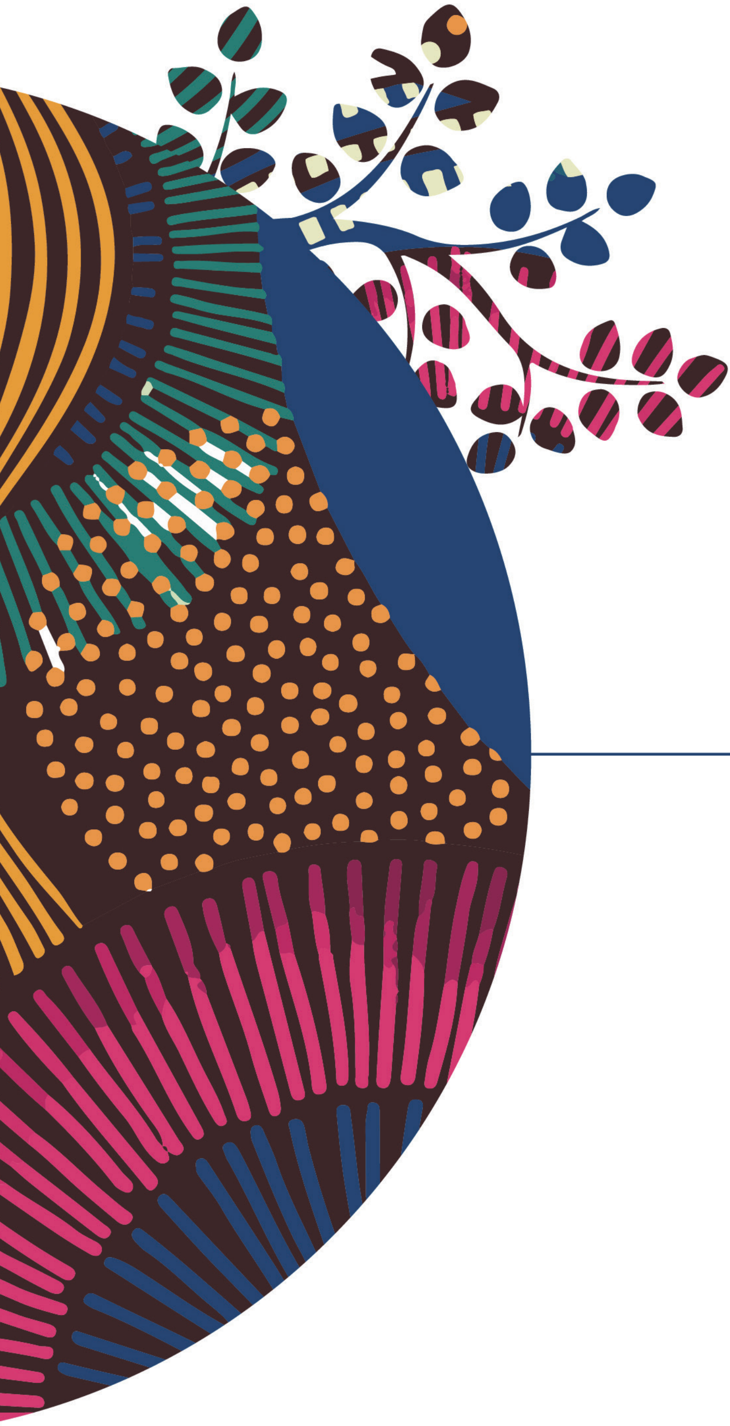
2013 Ho Chi Minh, Vietnam

2015 Manila, Phillippines

2017 Wuhan, China

2019 Seoul, Korea

2023 Taipei, Taiwan



Country Reports

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China Country Report 2023

Qigai He[1]
[1]*State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University*

C TRENDS IN PIG PRODUCTION AND DISEASE CHALLENGES IN JAPAN

Dr. Mali Miyashita[1]
[1] *Eckstein Swine Service*

Pig production in Japan
Statistics of the Ministry of Agriculture, Forestry and Fisheries (MAFF)[1] indicate that the number of pig farms and sows are declining. Whereas ten years ago 5,270 farms were registered, there were only 3,570 farms in 2022, of which 65% are farrow to finish operations. Sow numbers declined from 885,300 in 2012 to 789,100 in 2022, a reduction of 11%, yet the total number of pigs raised declined to a lesser extent (-8.2%) over the past 10 years, possibly reflecting improved genetics, better management, and disease control. At the same time, farms tend to get larger (2,493 heads/farm in 2022 vs. 1,810 heads/farm in 2012). On the consumer side there is a clear trend towards increased consumption of pork[2]. The per capita pork consumption was 13.2kg per year in 2020, yet the self-sufficiency rate of pork in the same year was only 50%[1]. The gap is filled by imports mainly from the USA, Canada, Mexico, and Spain.

Disease status
Japan is free of swine vesicular disease (SVD), African swine Fever (ASF) and foot and mouth disease (FMD)[1]. In April 2010, there was an outbreak of FMD in the southern part of the country, which resulted in the culling of 297,808 animals[3]. FMD-free status was resumed in 2012[1]. Through rigorous Aujeszky's disease (AD) eradication programs, 40 prefectures have maintained free status or have eradicated AD, whereas 7 prefectures are still in the final stages of eradication . Classical swine fever (CSF) was eradicated in 2007, but 11 years later, in September 2018, a farm in central Japan was found positive for CSF. After this initial outbreak CSF spread rapidly to surrounding areas probably by means of infected wild boar. Oral vaccination of wild boar has not been successful in containing the spread of disease , partly due to lack of adequate vaccine doses, partly due to the mountainous landscape of Japan, making it difficult to effectively place vaccine baits. Vaccination of domestic pigs started in November 2019, and as of date, only the two islands of Kyushu and Hokkaido are free of CSF and exempt of vaccination. Despite vaccination, sporadic outbreaks in domestic farms are still reported. As of date (July 2023), 86 farms have been affected and the number of culls has surpassed that of the FMD outbreak in 2010. A new strain of porcine endemic diarrhea (PED) entered Japan in 2013, causing a nation-wide outbreak involving more than 800 farms[1]. PED still occurs sporadically, especially in the winter season, and remains to be endemic in some pig-dense regions. Porcine respiratory and reproductive syndrome (PRRS) is endemic and together with newly emerging highly pathogenic strains, it continues to pose a major challenge in disease control.

Productivity
To benchmark productivity of individual farms, a unique benchmarking system was launched in 2011 (PigINFO by the National Agriculture and Food Research Organization) and since 2022 conducted by the Japanese Association of Swine Veterinarians (JASV). Noticeable improvements in the numbers of weaned and marketed pigs have been seen in the past 10 years. Whereas the mean number of pigs marketed/sow/year was 21.58 (24.53 for top 10 farms) in 2012, this number increased to 24.53 (29.58 for top 10 farms) in 2022[4].

Current challenges

Apart from the disease challenge of CSF, Japanese farmers are struggling with increasing feed prices and energy costs, as in many parts of the world. Feed prices started to increase at the end of 2020, and have continued to rise to levels unseen before. Low crop yields in 2020, COVID-19, the war in Ukraine, and weak yen to USD exchange rate are factors pushing prices up. Whereas the average feed cost was 535.5USD in 2015 (calculated at exchange rate 130 JPY/USD), it has increased to 743.6 USD in 2022[2]. Increased variable costs will severely impact profitability for most farms and further push the trend to reduction in farm numbers.

References

1. Ministry of Agriculture, Forestry and Fisheries (www.maff.go.jp)
2. Animal Media Inc., Tokyo, Japan
3. Prefecture of Miyazaki HP (www.pref.miyazaki.lg.jp)
4. Japan Association of Swine Veterinarians (JASV) Benchmarking Report

SWINE PRODUCTION AND DISEASES IN KOREA

Prof. Jason Lee (Joo Young Lee)[1]
[1]*Choong Ang Vaccine Laboratories Co., Ltd.*

Introduction
Recent years, swine population in South Korea has steadily maintained and slightly increased. As of 2022, standing swine population is approximately 11.1 million heads (i.e. Sows: 1 million heads). While the number of pig farms has dramatically decreased every year, the average herd size has increased from 1,831 head per farm in 2018 to 1,953 head per farm in 2022 (Table 1). With respect to the production systems, a multi-site operation system has been introduced in large swine farms though single site operation is still common in Korea.

Major diseases
Some major diseases such as ASF, FMD and PED are still the challenge for swine industry. In addition, Influenza A Virus in Swine (IAV-S) is getting the attention recently.
The 1st ASF outbreak in Korea was September 2019. ASF outbreak was reported in farming pigs and wild boars almost at the same moment. However, majority of ASF cases are found in wild boars. (i.e. 3,141 cases in wild boars and 36 cases in farming pigs, as of June 2023 (Table 2).
FMD is another difficulty. Most recent FMD outbreak in swine was April 2018 and FMD is more or less settled down, thanks to strict policy such as the vaccination (i.e. Vaccination schedule: Sow 3-4 weeks before farrowing, Boar every 6 month, Fattening pigs 1st shot 8 weeks old, 2nd shot 3-4 weeks after 1st shot), and checking antibody at slaughter house (i.e. If SP antibody titer is below 60% in sows and less than 30% in fattening pig, approx. 5,000 USD will be fined). More FMD cases are in the cattle.
Regarding PED, there were 230 cases in 2022 and 50 cases until April 2023, according to Korea Animal Health Integrated System (KAHIS) (Table 3). Non-S-INDEL PED virus which has significant pathogenicity is the dominant in Korea while S-INDEL PED virus which has less pathogenicity is common in Europe. It is the fact that every 2-3 years, Korean swine farms suffer from PED outbreak. It is the observation that PED situation is critical in Jeju island in 2022 and 2023, based on KAHIS data.
IAV-S is one of major pathogen for PRDC in Korea and it happens mainly from August to February in Korea. Abortion, death & culling in sows, decrease in the number of weaned piglets, and the growth retardation after weaning are typical symptoms with acute swine influenza. With endemic swine influenza, delay in marketed age, increase in medication cost (more than two times) and the number of sick pigs, and decrease in the growth rate after weaning (3-7%) are mainly observed. Based on the serological test against IAV-S on 40 one-site operation farms in nationwide from summer to spring in 2021-2022, 97.5% of the farm were antigen- or antibody-positive while 95% of the farms were antigen- and antibody-positive at the same time (Table 4).
In addition, NADC34-like PRRSV, which causes high mortality and abortion, was isolated in July 2022 in Korea for the first time.
Other bacterial diseases caused by *Heamophilus parasuis*, *Streptococcus suis*, and new serotypes (7 and 12) of *Actinobacillus pleuropnemoniae* have been continuously increasing.

Conclusions and discussion
The Korean swine industry have made a substantial improvement on pork quality and safety, biosecurity and production system. Farmers and veterinary professionals have also made great efforts on swine disease control and eradication to increase productivity. In order to achieve sustainable development of swine business, Korean pig industry will have to

overcome various obstacles including disease control, the increasing labor cost, lack of reliable labors, alternatives of antibiotics, and more importantly, environmental factors (manure treatment, odor complaints and soil contamination etc.). Lastly, animal welfare is also important considerations.

Acknowledgement and references
Changhee Lee, Gyeongsang National University
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Seongho Shin, Optipharm
Won-Hyung Lee, XP Bio.
Won-Il Kim, Jeonbuk National University
Young-Ho Joo, Chunhajeil Feed

Table 1. Changes in swine production in recent years

Items	2018	2019	2020	2021	2022
Number of farms	6,188	6,133	6,078	5,942	5,965
Pig inventory (k head)	11,332	11,279	11,078	11,216	11,123
Number of sows (k head)	1,063	1,026	1,001	1,023	995
Number of pigs/farms	1,831	1,839	1,823	1,888	1,953
Number of slaughter pigs	17,369	17,825	18,330	18,383	18,556
Pork imports (k ton)	463	421	310	333	442
Consumption/capita/y(kg)	27.0	26.8	27.1		
Feed production (k ton)	6,554	6,850	6,921	6,932	7,032

Data source: Korean Statistical Information Service (KOSIS)

Table 2.
-ASF outbreak in farming pig

Year	2019	2020	2021	2022	2023. 6	Total
Total cases	**14**	**2**	**5**	**7**	**8**	**36**

-ASF outbreak in wild boar

Year		2019	2020	2021	2022	2023. 6	Total
Total		**55**	**856**	**964**	**878**	**388**	**3,141**
Carcass	Sample (Positive)	706 **(50)**	3,287 **(814)**	2,454 **(848)**	2,109 **(761)**	1,102 **(372)**	9,666 **(2,845)**
Capture	Sample (Positive)	48,651 **(5)**	93,963 **(42)**	69,489 **(116)**	74,627 **(117)**	25,064 **(16)**	312,678 **(296)**

Data source: Ministry of Agriculture, Food and Rural Affairs (MAFRA)

Table 3. The reported cases of PED by province in 2022-2023

Year	Month	Province									Sum
		GG	KW	CB	CN	JB	JN	KB	KN	JJ	
2022	Jan	2			4	8	1	3	15		33

	Feb	3			4	13	6	2	1	1	30
	Mar	3			11	9	7	4	2	20	56
	Apr	7	2	1	8	9	8	3		18	56
	May			1		1		1		12	15
	Jun				3				1	6	10
	Jul				3	1	1		1	2	8
	Aug					1	1			2	4
	Sep									3	3
	Oct					1				6	7
	Nov				2	2		1		1	6
	Dec	1		1	1					2	5
	Total	**16**	**2**	**3**	**36**	**45**	**24**	**14**	**20**	**73**	**233**
2023	Jan							2		5	7
	Feb	2			1	4	1	4	1	13	26
	Mar				2	1	1	2	3	7	16
	Apr								2	3	5
	Total	**2**	**0**	**0**	**3**	**5**	**2**	**8**	**6**	**28**	**54**

Data source: Korea Animal Health Integrated System (KAHIS)

Table 4. The seasonal IAV-S antigen- and antibody- positive rate in 2021-2022

	Summer	Autumn	Winter	Spring
Antigen positive	33/40 (82.5%)	36/40 (90.0%)	34/40 (85.0%)	33/40 (82.5%)
Antibody positive	18/40 (45.0%)	21/40 (52.5%)	23/40 (57.5%)	19/40 (47.5%)

Data source: Prevalence of swine influenza virus in pig farms, Korea

PHILIPPINES Swine Industry Updates

Dr. Angel Antonio C. Manabat, DVM, FPCSP[1]
[1]*Philippine College of Swine Practitioners*

Introduction:
The Philippines has an ever growing population which was last pegged at around 117.3M people, with a per capita consumption of pork at 15.5 kilos. Pork is one of the most preferred protein by Filipinos. Although, recently, chicken has overtaken pork in terms of consumed meat volume at 16.6 per capita consumption, it is expected that consumption may favor pork again when pork supply concerns and prices stabilizes. The price of pork has fluctuated due to a number of diseases that has hit the Philippines, like Porcine Epidemic Diarrhea (PED), PRRS, and the most devastating one, African Swine Fever (ASF) which continue to ravage the island since it was first detected in July 2019.

Based on data from the Philippine Statistics Authority (PSA), the country still has about 10.18 million heads of pigs which they say was about 4.2% higher compared to previous year (2022) of 9.77M heads. Compared to pre ASF time, PSA, in their January 2019 report, showed the pig population of the country to be at 12.71 million heads which meant we have lost around 20% of our pig population. Some industry analysts however feel the country may have lost more, based on gathered outbreak reports both official and rumored, estimating that the loss could be around 50 to 60% of the population.

African Swine Fever. The country is now at the end of its 4th year of dealing with ASF, which continue to be active, recently entering previously negative areas. Unlike other countries that has had ASF and where the virus spread quickly, the Philippine ASF situation is more like a long slow burn, as can be seen in the ASF maps below. Being a country composed of 7000+ islands, it was thought that the virus would not overwhelm the country, but as experienced by other countries affected by ASF, it wasn't the virus making changes in how it is transmitted, it is the people who helped the virus cross the many island it has infected. The pattern of ASF breaks are either started in area where food garbage, both local and foreign, are dumped or later came out near the highways in which pork or pigs were transported. Biosecurity implementation which government and private groups pushed as the needed ASF prevention strategy was not internalized enough by many farmers which led to the breaks. Uncontrolled selling and movement of sick pigs further worsened the situation.




Repopulation Strategies – The Philippines Department of Agriculture (DA) thru the National Livestock Program (NLP) has launched the INSPIRE and the Bantay ASF Program (Babay ASF), a twin program that is aimed to further highlight biosecurity implementation and support strategic repopulation of previously affected regions, in the target to recover former pork production volumes. More than 8,000 farmer beneficiaries has received support in terms of piglet and feeds for sentinel growing, which is a requirement before areas are given more budget for breeder loading.

Swine Veterinarians – After the ASF outbreaks, unsurprisingly, the number of veterinarians engaged in swine production has gone down as seen in the attendance to conferences and conventions. Those that are still committed to support the swine industry like the members of the Philippine College of Swine Practitioners (PCSP) and other independent swine veterinarian, have been involved in as many technical activities such as training to support the government INSPIRE and BabAy ASF programs. They have conducted dozens of seminars to different stakeholders to spread knowledge regarding ASF and other swine industry issues and concerns. PCSP has been part of Technical Working Groups (TWG) for ASF, Swine Animal Welfare Code, GAHP, AMR, Organic Swine Code of Practice and review of Government GM Animals and Animal Product Regulations and FMD Risk Analysis workshops to site a few. PCSP has also engaged with partnerships with swine associations like the National Federation of Hog Farmers Inc. and the Pork Producers Federation of the Philippines to take on pressing concerns of their different member farmers. Also together with the key industry movers of the Province of Batangas in island of Luzon, probably the best province in terms of ASF control and management, we worked in the developing and implementing a Compartmentalization system amidst ASF, in the hope being able to continue business for committed member farmers to produce and sell ASF free animals and products that could be acceptable for movement even into ASF free zones.

Materials and methods:
The presentation was completed with the use of references from government and private resources.

Conclusions and discussion:
The Philippine Swine Industry is probably in its worst state in its known history. Only thru the combine efforts of all stakeholders, government and private sectors, swine veterinarians and experts, could the country ever even to start imagining that we could rebound and return to pre ASF populations and production levels.

Acknowledgement and references:
Philippines Statistical Authority
PIC Philippines Inc.
Babay ASF sa Barangay 'BabAy ASF" Facebook Page
Department of Agriculture – National Livestock Program, and Bureau of Animal Industry

Thailand Country Report 2023

Dr. Metta Makhanon, *DVM, MBA, PhD.*[1]
,[1]*Thai Swine Veterinary Association*

Introduction:
After ASF emerging in ASIA since 2018, Thailand and other Asian countries were under stress and searching for the effective process to protect our swine industries from this terrible virus. However, in January 2022, Thailand DLD announced the official African Swine Fever outbreak to the world. During 2022, farms were infected, depopulated, and repopulated, then, re-infected, and depopulated again and again. We learned form the previous infected countries in Europe and Asia for the biosecurity, vaccination, disinfection, and had chosen several methods from research and practice to be our owned solution. Our attempt resulted in the improvement of pig population and disease control from the starting of outbreak until now. Furthermore, the increased biosecurity of farms can also reduce other diseases problems. We found that several farms can find the best solution of their farms by "The uplevel biosecurity" and they are success for repopulation and protection their farms from ASF even their farms location are in the infected areas.
TSVA issued a Clinical Practice Guideline (CPG) at the end of 2022 in the topic "For Uplevel Biosecurity on African Swine Fever". This CPG is different from other CPGs TSVA did before because this CPG is based on the practical knowledge from the farmers and farm veterinarians who being success in ASF handle. It is not the booklet from the technical reviews but this CPG is the summary of all practical biosecurity in farms and being as the MUST HAVE for successful repopulation and protection from ASF.
Moreover, in 2023, Thai DLD and Ministry of Agriculture and Cooperatives start to control farms with Good Agricultural Practice (GAP) since May 2023, all farms having pigs from 1,500 heads and over must have the GAP certificate and from August 2023, all smaller size farm having pigs from 500-1,499 heads must have the GAP certificate in order to produce hygienic pork for consumption. GAP is the rules not the voluntary practice in Thai pig industry. Farm veterinarians have to prescript for vaccines and medicines for GAP farm being one of the major points of the CPG as well as disease management and welfare. TSVA corporate with local DLD team and the swine producer association in each region to educate the small to medium sizes farms for biosecurity and GAP practice by the TSVA mobile seminars**.**

Materials and methods:
<u>CPG for Uplevel Biosecurity on African Swine Fever</u>
Selection of the target farms from all part of Thailand as the following criteria:
- Successful farms have been success to repopulation at least 50% of the total pig s before infection and the negative status has been continue not less than six m onths after the repopulation
- ASF naive farm where there has never got infection while their neighbors are all infected
- Farm sizing: Small and medium (less than 500 sows or 5,000 pigs) and large (mo re than 500 sows or 5,000 pigs) farms

Method: Interview with the opened end questions and summary their practice to be the guideline for uplevel biosecurity according to farm sizes
Results:
Farm information:
Large farms (5 farms)
- Location: Western, Eastern, and Northeast parts

- Farrow to finish 3 farms, Breeder 1 farm, and fattening 1 farm
- Both Opened house system and Evaporative cooling system (EVAP)
- Continuous flow, AIAO, WTF and batch farrowing
- Water sources: underground, and surface water and chlorine treatment
- Feed: farm own feed mixing and commercial feed from feedmills
- Infected farms have improved structure and farm management until their rep opulation are success

Small and medium farms (6 farms)

- Location: Western and North part of Thailand
- Farrow to finish farms (6)
- Both opened house system and Evaporative cooling system (EVAP)
- Continuous flow, AIAO, batch farrowing, and WTF
- Underground and surface water, mountain tab water disinfected by chlorine
- Commercial feed, no swill feeding
- Practice from infected and depopulated-repopulated farm and naïve farm in t he infected area

<u>Critical points of ASF entry: Large farms</u>

1. **Workers**

- Workers in selling unit (selling piglets or finishers) entered the farm area wit hout sufficient washing and changing their clothes, the starting of outbreak is from the unit these workers entry to work
- Selling unit workers' family members work in different units of farm, the star ting of outbreak is from the unit these workers work for
- Going out and have interaction with workers from infected farms

2. Vectors

- Birds, rodents, and insects are the key vectors carried virus from infected far m surround, or from the pig carcass in the infected farms. Those vectors sho wed positive ASF from screening swabs
- Cockroach inside the farm drainpipe (sewer) caused the internal transferring a mong units especially when they were attacked by insecticide in the opened area (no control of the pipe area before applying insecticide)
- Dogs and cats ate the infected carcass and showed positive ASF from screeni ng swabs

3. Management

- Using the same syringe gun for application of oral hormone in gilts
- Don't change needles and syringes during mass medication
- Opened houses or EVAP houses have the same chance to get infection

Critical points of ASF entry: Small and medium farms

- Workers enter the raising area without washing and disinfection (even the wa shing room is available)
- High viral load surround farm area
- Pig buyers enter the raising area to purchase pigs
- Swill feeding
- Bring pork from outside to cook or eat in farm

Conclusions and discussion:

Conclusions of uplevel biosecurity for ASF control

Large farms (≥5,000 heads or ≥500 sows)

- Investment in the important structure, working process, and staff managemen t as well as the farm audit routinely are necessary.
- Critical point is to facilitate farm workers to be convenient to washing, disinf ection, and changing cloths to farm uniform. And to regulate workers to stay in their owned working unit, stay in farm until finish the batch, and no foo d from outside.
- Control and eradicate of vectors, routinely
- Setting farm laboratory (Real-tine PCR) for ASFV detection for the faster dete ction since in large size farm, the clinical observation is not as fast as smalle r farms.
- Due to the number of workers and stakeholders in large size farm, the clear and enough details of regulation to uplevel farm biosecurity is necessary. M oreover, the motivation and punishment have to be clear and accepted by all stakeholders.

Small and medium farms (<5,000 heads or <500 sows)

- ASF free farm, the naïve farm, and the successful repopulation in the infecte d region have the following biosecurity:
- Never let visitors go inside the farm
- Feed pigs with commercial feed, no swill feeding
- Strictly control the invade of external virus as follows:
  - Focus on the quality of washing and disinfection
  - Changing to farm cloths although there is no uniform, the cloths to ware during working have to be separated from their personal cloths
- Strictly control the spreading of virus between houses as follows:
  - Boots have to be changed at every house.

- Disinfection of car/truck used in farm work.
- Reducing direct contact to animals.

- **Focusing on strict management process than spending money for constructio n**.
- Opened house and EVAP have the same risk level of infection.

Acknowledgement and references:
Thai farm owners and farm veterinarians who share their valuable experience and practice.

Reference:

2022 Clinical Practice Guideline for Uplevel Biosecurity on African Swine Fever, TSVA, ISBN 978-616-91341-5-2.

Pig production and health in Vietnam over the challenging period

Assoc.Prof. Nguyen Tat Toan[1,2,3]
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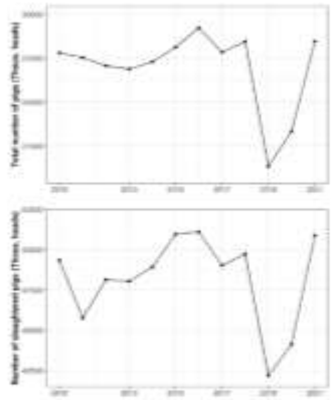
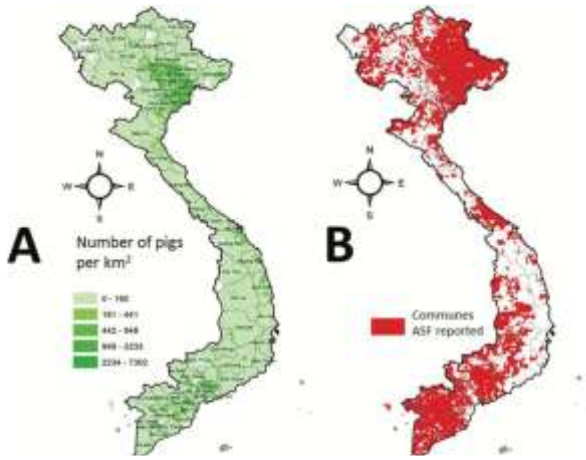
Introduction
Vietnam is one of the most important pork markets in the world due to the traditional pig production with high demand and centralization process for husbandry in the country. Specifically, Vietnam is the second-largest pork producer in Asia, only behind China, and the seventh worldwide. Also, it is the largest per capita consumer of pork in Southeast Asia and the sixth in the world. An estimation from IPSOS (2016) found that the amount of pork consumption in Vietnam was 22.2 kg per capita, relatively low – just one-third of that in the US (107kg) and half of the amount within the EU (76kg) - and will be increasing and lead to the optimistic view for pig production. However, in reality, until now the demand for pork is still stable with an estimation of 23.5 kg per capita (VIR, 2022) due to many factors such as COVID-19, ASF outbreaks, political incidents, and economic distress. These factors have made a lot of influence on pig production in Vietnam with dramatic changes in which some difficult issues and promising points for future production can be seen and will be discussed in this paper.

Current change in pig production of Vietnam
Over the years, our country's pig production has had great fluctuations in the total herd and meat production, in which the total pig herd reached the highest in 2016 (29.1 million heads), decreased to 27.4 million heads in 2017 (due to the overestimation of the market), increased again in 2018 (28.1 million heads), then suffered a record drop in 2019 to only 19.6 million due to African swine fever (Figure 1 & 2) with a third of deaths from pig population and the very low demand during COVID-19. Figure 1 clearly shows the change in the pig population of Vietnam. Then the pig population recovered very slowly because ASF outbreaks were still going on in 2020 and many farms after the outbreaks tried to repopulate without success. The total cost of ASF for society in Vietnam was estimated to be 0.9-1.8% of GDP (Nguyen-Thi et al., 2021). This huge consequence was explained by the high proportion of pig production revenue in the national economy. In particular, the pig production revenue should be 20-25% (and 40% from poultry, 30-35% from cattle) while it was 65-70% before ASF (Nam, 2019). This urges the government to have appropriate strategies for the economic components.





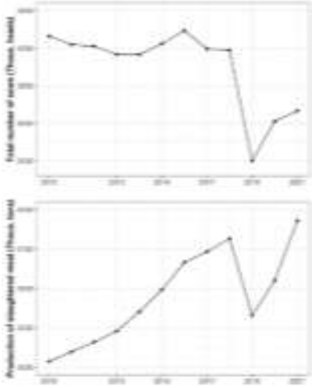


Figure 1: Number of pigs during 10 years with the sharp drop due to ASF (Lai et al., 2022)

Figure 2: Vietnamese pig holding and ASF communes during 2019 (Woonwong et al., 2020)

During 2021 and 2022, the government established strict procedures to guide farm-owners to restock and some initial successes of vaccine development have been gained. Then the total pig herd gradually recovered in 2020 (22.0 million heads) and continued to grow to reach 28.1 million heads in 2021. However, the recovery was made with a change in pig population structures. Most small and medium scale farms could afford the difficulties before and after the ASF while large scale and intensive pig farms have survived and come back to raise pigs. According to the Vietnam Livestock Association, there were about 45-50% of large farms suspending production while 70-75% of mid and small farms and livestock households temporarily stopped re-herding because the input costs have surged while the selling price is low. The change will have many effects such as better management for the government in terms of disease surveillance and technology application, but also consequences such as dealing with lack of skilled workers and the issue relating to control of the whole market or monopoly.

Difficulties for pig production

With the change in number and structure, pig production has been experiencing many challenges. The most important thing is the fluctuation of slaughter pig price which can be used as a measurement of cost-benefit for pig investment (Figure 3). When comparing the price with the average production cost of the smallholder farmers (about 55.000-60.000 VND per kilo of pigs (VietnamPlus, 2023)), we can understand small scale households could not maintain their business. And the intensive pig farms have also struggled during this fluctuation so-called “price storm” and cannot survive unless they manage production well. Reports from these farms revealed that the cost for a kg of slaughter pigs is 52.000 VND, ranged from 48.000 to 55.000 VND which seem to be good but are all higher than that from other parts of the world. The average cost of production in the EU in 2021 for the countries above was £1.50 per kg of slaughter pig (~45.328 VND) (AHDB, 2021). That means our pig producers must do more in the market nowadays.

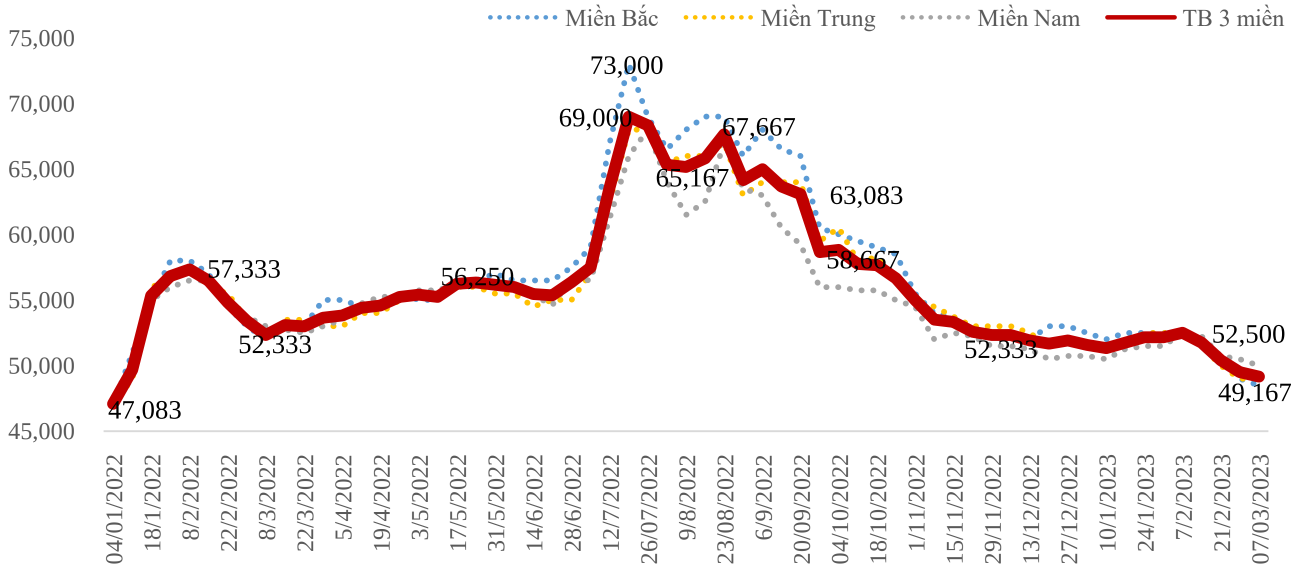


Figure 3: Slaughter pig price (VND/kg) from Jan 2022 to Mar 2023 of the North (blue dots), Middle (yellow dots), South (grey dots) areas of the country (the thick red line is the average)

As mentioned earlier, the change from small scale to large scale of pig production might lead to many issues. Many big enterprises with strong investment budgets seem to be incompatible when they can survive and control the breeding herds and productions, in turn they also can control the market price. At the same time, the investment to build many new farms in rural or wild areas will lead to many environmental problems for the countries. Some examples are the waste causing air and water pollution, loss of land for agriculture, deforestation, spillover, emerging diseases.

Health Problems

The low benefit of production as mentioned above is considered to be the pig health issues when up to 30% cost for pig production is for medication (vaccination and antibiotics). Many diseases were prevalent although during this period, ASF seems to be over discussed, thus other diseases become neglected. However, according to one study (Pham et al., 2017), PRRS, FMD, and PED were always costly for farmers prior to ASF. The losses per pig holding due to PRRS were the highest: 41% of gross margin for large farms, 38% for fattening farms and 63% for smallholders. Cost incurred by FMD was lower with 19%, 25% and 32% of gross margin of pig holding in large farms, fattening farms and smallholder, respectively. The cost of epidemic diarrhea was the lowest compared to losses due to PRRS and FMD and accounted for around 10% of gross margin of pig holding. These estimates provided critical elements on swine disease priorities to better inform surveillance and control at both national and local level (Pham et al., 2017).

Excluding ASF, PRRSV had been well controlled by commercial vaccines belonging to two groups: HP-PRRSV and US-PRRSV. However, recently, especially in 2021, pigs in many farms that have used the attenuated PRRS vaccine for a long time still showed clinical signs associated with PRRS. A study reported that in these types of farms, the stillbirth rate was 14.3%, and the incidence of stillbirth at the litter level was 68.9% (Lanh & Nam, 2022) considering PRRS with potential emerging strains. From these farms, the genetic analysis showed that besides the conventional highly pathogenic PRRSV subtype (HP—PRRSV), there were isolate belonging to NADC-like group, lineage 1, sublineage 1.4, which were reported in Vietnam for the first time; and considered complex pathogenicity and various level of protection from vaccination (Nguyen et al., 2022). FMD is the major problem for cattle and

pigs in Vietnam, thus their vaccination is compulsory. However, recently there have been many outbreaks despite vaccination. Department of Animal Health had to investigate and found that FMD viruses in Vietnam belong to 3 topotye (strains) of type O: O/ME-SA/PanAsia; O/SEA/Mya-98; and O/ME-SA/Ind2001e In which, strain O/Ind2001e accounted for the majority and have not appeared since 2018 (Palinski et al., 2019). PED is still sporadic in some farms depending on biosecurity level.
Beside these prioritized diseases, many other health problems should be considered especially when antibiotic reduction policies become strict. These are ileitis, *Mycoplasma suis* (Eperythrozoonosis)… which were reported in many farms. As a result, food safety issues are also rising (esp. streptococcus suis, residue). Particularly, since the beginning of 2023, a number of streptococcus infections have been recorded across the nation and the majority of cases have involved people eating pork products such as blood pudding, or those who slaughter pigs (The Straits Times, 2023). To combat all issues, biosecurity should be improved. The average biosecurity scores of surveyed pig farms were always lower than those from the global average scores (Cuc et al., 2020). So raising awareness on biosecurity for farm owners and workers is very important.

Bright side
Although there were many obstacles for pig production in Vietnam, some good news might help us believe in a brighter future. The first thing is the benefit from restructured farm scales such as investment, high technology application. New development of the ASF vaccine was an important optimistic view for the future of pig production. The government has also issued many policies to encourage sustainable development such as connected food supply, innovation, and technology support. Among those, the farm's commitment to ensure safety seems to be the good part to deal with diseases in the future. This program is called "đăng ký an toàn dịch bệnh" in which farmers have to follow many guidelines on biosecurity and report, surveillance, and report on health condition in farms to have the certificate which will help products easy to enter strong supply chain and have compensation when culling animal for outbreak. From that program and many communications, most farmers after ASF have recognized the value of farm biosecurity and aim in that direction. A good pig veterinary system seems to be achievable.

Conclusions:
Currently it is a hard time for Vietnam's pig production due to the consequences from ASF, COVID 19, and other problems. With any other obstacles, pig production structure will change drastically. But we believe in the bright side to make the difficulties to become opportunities.

Author contributions: Assoc. Prof. Dr. Nguyen Tat Toan, Assoc. Prof. Dr. Le Thanh Hien, Assoc. Prof. Dr. Do Tien
Duy, Dr. Nguyen Thi Phuoc Ninh, MSc. Nguyen Thi Thu Nam; Nong Lam University, Ho Chi Minh City, Vietnam.

References:
AHDB (2021). *Pig cost of production in selected countries*. From https://projectblue.blob.core.windows.net/media/Default/Pork/Pork%20MI%20files/CostPigProduction2021_221117_WEB.pdf (Retrieved July 2, 2023)
Cuc, N. T. K., Dinh, N. C., Quyen, N. T. L., & Tuan, H. M. (2020). Biosecurity Level Practices in Pig and Poultry Production in Vietnam. *Advances in Animal and Veterinary Sciences*, 8(10). https://doi.org/10.17582/journal.aavs/2020/8.10.1068.1074
Lanh, D., & Nam, N. (2022). High stillbirth rate in a swine farm in Vietnam and associated risk

factors. *Journal of Advanced Veterinary and Animal Research*, 9(1), 13.
https://doi.org/10.5455/javar.2022.i564
IPSOS (2016). Vietnam Meat Market:
https://www.ipsos.com/sites/default/files/2016-08/meat-market-in-vietnam.pdf (Retrieved July 5, 2023)
Nam B. N. N. V (2019). *Phải thay đổi cơ cấu chăn nuôi*.
https://nongnghiep.vn/phai-thay-doi-co-cau-chan-nuoi-d242518.html (Retrieved 2019, May 31).
Nguyen, N. H., Tran, H. A. T., Nguyen, T. Q., Nguyen, P. B. T., Le, T. H. T., Lai, D. C., & Nguyen, M. N. (2022). Phylogenetic analysis of porcine reproductive and respiratory syndrome virus in Vietnam, 2021. *Virus Genes*, 58(4), 361–366.
https://doi.org/10.1007/s11262-022-01912-w
Nguyen-Thi, T., Pham-Thi-Ngoc, L., Nguyen-Ngoc, Q., Dang-Xuan, S., Lee, H. S., Nguyen-Viet, H., Padungtod, P., Nguyen-Thu, T., Nguyen-Thi, T., Tran-Cong, T., & Rich, K. M. (2021). An Assessment of the Economic Impacts of the 2019 African Swine Fever Outbreaks in Vietnam. *Frontiers in Veterinary Science*, 8, 686038. https://doi.org/10.3389/fvets.2021.686038
Palinski, R. M., Bertram, M. R., Vu, L. T., Pauszek, S. J., Hartwig, E. J., Smoliga, G. R., Stenfeldt, C., Fish, I. H., Hoang, B. H., Phuong, N. T., Hung, V. V., Vu, P. P., Dung, N. K., Dong, P. V., Tien, N. N., Tho, N. D., Dung, D. H., & Arzt, J. (2019). First Genome Sequence of Foot-and-Mouth Disease Virus Serotype O Sublineage Ind2001e from Southern Vietnam. *Microbiology Resource Announcements*, 8(10), e01424-18. https://doi.org/10.1128/MRA.01424-18
Pham, H. T. T., Antoine-Moussiaux, N., Grosbois, V., Moula, N., Truong, B. D., Phan, T. D., Vu, T. D., Trinh, T. Q., Vu, C. C., Rukkwamsuk, T., & Peyre, M. (2017). Financial Impacts of Priority Swine Diseases to Pig Farmers in Red River and Mekong River Delta, Vietnam. *Transboundary and Emerging Diseases*, 64(4), 1168–1177. https://doi.org/10.1111/tbed.12482
The Straits Times (2023). *Vietnam on alert after human cases of pig-related disease discovered*.
https://www.straitstimes.com/asia/se-asia/vietnam-on-alert-after-human-cases-of-pig-related-disease-discovered (Retrieved July 2, 2023)
VietnamPlus. (2023). *Meat producers forecast to see more positive results*.
https://en.vietnamplus.vn/meat-producers-forecast-to-see-more-positive-results/252808.vnp (Retrieved July 2, 2023)
VIR (2022). *Vietnam’s pork industry faces shrinking consumption and domestic supply*.
https://vir.com.vn/vietnams-pork-industry-faces-shrinking-consumption-and-domestic-supply-94218.html (Retrieved July 2, 2023)

Pig production and diseases in Taiwan 2023

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Introduction
The pig industry in Taiwan comprises 5,991 pig farms with 520,000 sows. These figures are from the latest pig inventory survey conducted in November 2022 (Fig. 1). Results from these surveys show that the number of pig farms and the pig inventory has continuously declined year by year, especially for small-scale pig farms, and the issues of high feed cost and African Swine Fever (ASF) in the neighbor countries. The rate of pork self-sufficiency is about 85% in Taiwan, and pork imports come mainly from the USA and Canada. Recently, ASF has been a major pre-border disease widespread in Asian regions. The risk of ASF incursion has increased, and we have strict control measures in place for our border control to minimize the risk of ASF outbreak in Taiwan. We have imposed a heavy fine to stop travelers from carrying meat products if they violate the regulation.

Challenges in the Pig Industry
Major challenges in the pig industry in Taiwan include:
Generational change ;
A need for more farm laborers ;
Demands for more young veterinarians to provide services to pig farms.
Some solutions have been provided for the pig industry, and the government has funded several projects to train swine veterinary specialists in the veterinary schools in which Master's Degree courses and specialist pig veterinary training courses have been established. Hands-on practical farm management training has been introduced to train the younger generation of farm staff about data registration, batch production system, and adjusting pig flow to maximize farm productivity. There are some demonstrative farms for introducing this concept to farmers and showing farmers how to implement these programs. An innovation for training young swine veterinarians is training farms where they can participate in daily farm activities and learn to plan operations such as pig flows in the farms they consult. A website is provided for swine producers and veterinarians to access information to improve their knowledge of pig production and health management. They can access the website at: http://pmtw.atri.org.tw/. The website provides videos of our training courses and photos of various common swine diseases.

Pig diseases
Foot and Mouth Disease (FMD) and Classical swine fever (CSF) are the targeted diseases for eradication, with the first control by compulsory vaccination. After years of surveillance of non-structural protein and serum-neutralizing antibody tests in the auction markets and pig farms, the results demonstrated no FMD case in the field after 2014. In 2017, Taiwan was recognized as an FMD-free country with vaccination by the OIE and stopped all FMD vaccination in July 2018. After 23 years, we regained an FMD-free country without vaccination in June 2020. With the experiences of FMD control and eradication, our next target disease for eradication is CSF. We have not had disease outbreaks for the past 17 years, and no virus circulation in our national sentinel pig surveillance for the past two years. Then the cessation of CSF vaccination in our fattening pigs began on January first this year. The fattening pigs are near without SN titers of CSF, and no field case has been reported. And stop all the CSF vaccination on July first, including sows 2023. If we do not have other CSF cases till 2024, we are qualified for the application for official recognition by OIE of free CSF status. Then, Taiwan will be the only country without FMD, CSF, and ASF in Asia. In 2014,

Porcine Epidemic Diarrhea (PED) was introduced in the farrowing units and still causes severe losses in newborn piglets. Currently, other important diseases on pig farms in Taiwan are those caused by Porcine Reproduction and Respiratory Syndrome virus, Porcine circovirus type 2, Swine influenza virus, and co-infection with other pathogens such as Mycoplasma, E. Coli, Salmonella and Streptococcus causing a post-weaning respiratory syndrome which results in severe losses in the nursery piglets.

Control and management

The strategies for controlling the problems are mainly through good husbandry and sound production management to maintain pig flow using batch production, all-in/all-out systems, and vaccination programs in sows. Antibacterial treatment of major diseases, such as those caused by E coli, Salmonella, and Streptococcus, is carried out where indicated. The whiteboard recording system and PigChamp have been introduced to monitor the important numbers of sows per batch. With the “sow board" (Fig. 2), we can monitor the pregnant sows per batch and then provide timely advice on what intervention is needed if severe losses occur.

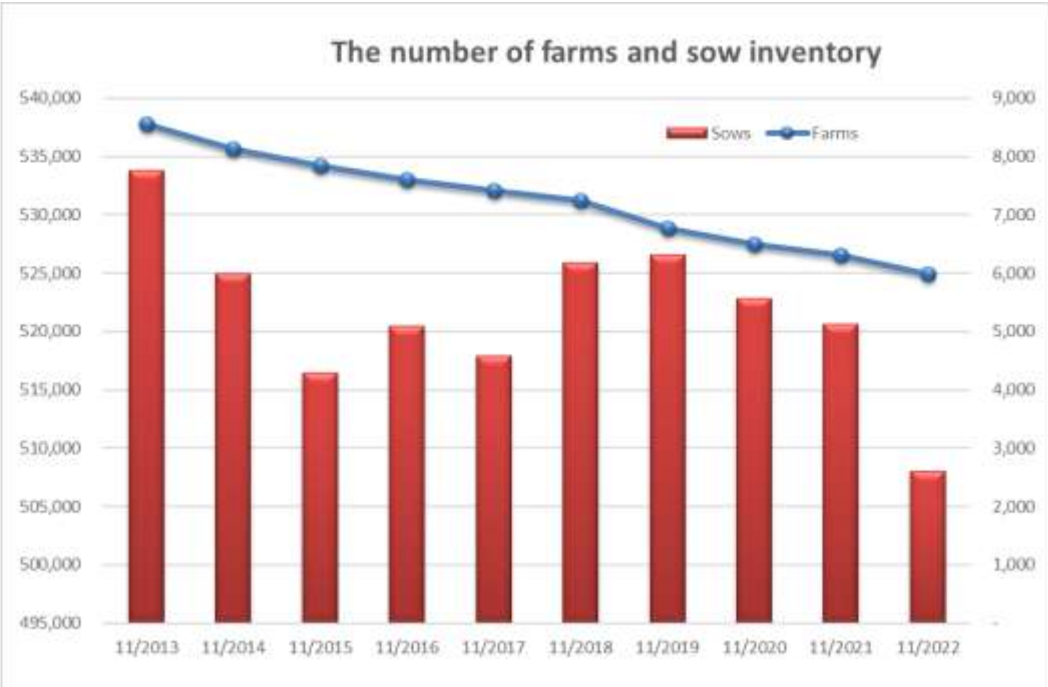

Fig 1. The number of farms and sow inventory from 2013 through 2022.

Fig 2. White board recording system has been introduced for monitoring the number of sows per batch.

Conclusions

After working with FMD for more than 23 years, Taiwan finally became FDM free country without vaccination in 2020. The model for controlling and eradicating FMD is now applied to other major swine diseases, such as CSF and PR in Taiwan.

References

(1) Chen et al., 2011. Vet Microbio 195, 57-63.

(2) Aucouturier J et al., 2001. Vaccine 19, 2666-2672.

Keynote Lectures

Advanced Strategies for Developing and Diagnostic Tools for African Swine Fever Virus with Nanobiotechnology

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African swine fever (ASF) is one of the most lethal infectious diseases affecting domestic pigs and wild boars of all ages. Over a span of 100 years, ASF has continued to spread over continents and adversely affects the global pig industry. To date, no vaccine or treatment has been approved. The complex genome structure and diverse variants facilitate the immune evasion of the ASF virus. Recently, advanced technologies have been used to design various potential vaccine candidates and effective diagnostic tools. This review updates vaccine platforms that are currently being used worldwide, with a focus on genetically modified live attenuated vaccines, including an under-standing of their potential efficacy and limitations of safety and stability. Differentiating between infection and vaccination should also be considered in the diagnosis. Furthermore, advanced ASFV detection technologies are presented that discuss and incorporate the challenges that re-main to be addressed for conventional detection methods. We also highlight the nano-bio-based system that enhances sensitivity and specificity. A combination of prophylactic vaccines and point-of-care diagnostics can help effectively control the spread of ASFV.

The Evolution of PRRSV in Pigs - A Beautiful Example of the Darwinian Law of the Survival of the Fittest

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Porcine reproductive and respiratory syndrome virus (PRRSV) is an old virus that has been circulating for quite a long time in pigs. Two viruses with a low homology have been described: PRRSV-1 (European type) and PRRSV-2 (American type) with only 60% nucleotide homology. In Western Europe, the virus was absent in the 1980s and only after the Fall of the Iron Curtain in 1989, the virus that was present in the former states of the Eastern Communistic bloc, invaded. Antibodies against PRRSV-2 have been detected in the US in the 1980s without reports of disease, demonstrating that a low pathogenic virus was already present at that time. At the end of the 1980s, beginning of the 1990s, the virus became more virulent and pathogenic, resulting in a disease characterized by reproductive failure and respiratory problems. With time, the virulence and pathogenicity of PRRSV increased, with a peak in 2006, with the appearance of the hypervirulent PRRSV-1 subtype 3 strains (prototype: Lena strain) in Belarus and PRRSV-2 high fever strains in Asia and more recently also in the US. This evolution is a beautiful example of the Darwinian law of the survival of the fittest, where PRRSV is continuously expanding its replication in different subsets of differentiated macrophages. PRRSV has a restricted tropism for CD163-positive differentiated macrophages. The binding and internalization are mediated by sialic acid on the GP5/M complex and cellular sialic acid binding molecules such as Siglecs and DC-Sign as receptors. In the old days, before the appearance of clinical signs, the number of PRRSV-infected macrophages had to be very restricted and did not result in pathology. Receptors such as Siglec-10 and/or DC-Sign fit in this context (arche-receptor-type). An additional use of Siglec-1 in the late 1980s resulted in (i) the replication of the virus in lung, placental and lymphoid macrophages, (ii) pathology in lungs, placenta and lymphoid organs and (iii) reproductive and respiratory clinical signs. In contrast to PRRSV-2, PRRSV-1 did not replicate very well in the upper respiratory tract and spread slowly from pig-to-pig at that time. This changed in the 2000s. In Europe, PRRSV strains emerged which adapted to the replication in the macrophages in the nasal mucosa (prototype: Flanders 13 strain). This gave more power to the virus; it was shed to higher levels leading to a very efficient transmission of the virus in the nurseries. The sialic acid binding protein that is helping the virus to replicate in the nasal macrophages is not known yet (different from Siglec-1 and -10). More recently, the virus infected an additional population of macrophages: the ones that are surrounding the veins. Infection of these macrophages are causing extremely high virus titers in the blood, with a high fever as a result, and leakage of the blood vessels. The sialic acid binding receptor on the vein macrophages that is used by these strains is also not known. In conclusion, we can state that the virus is infecting more and more subpopulations of macrophages by sialic acid-binding receptor-hopping, leading to more and more aggressive strains.

What We Learned from Porcine Epidemic Diarrhoea Vaccine Development

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Porcine epidemic diarrhea (PED) caused by the porcine epidemic diarrhea virus (PEDV) is characterized by acute diarrhea and vomiting resulting in high mortality in pigs less than two weeks of age. Vaccination strategies for the porcine enteric pathogen should achieve the effectiveness, technical consistency, cost-effectiveness, and minimal labour-intensiveness.
Several potential PEDV vaccine immunogens were investigated, including live attenuated virus (LAV), inactive viral particles, trimeric spike (S) glycoprotein, PEDV virus-like particles (VLP) carrying S, membrane, and envelop proteins, and the viral vectored vaccine-recombinant baculovirus displaying S proteins (S-Bac). Although oral administration of a live attenuated PEDVPT52 P96 strain could elicit sterile immunity in pigs, potential inhibition of attenuated virus replication by the pre-existing mucosal antibodies in PEDV pre-exposed animals as well as the risk of virulence reversion might restrict the oral application of the LAV regimen. Alternatively, parental vaccinations of the LAV, inactive viral particles, trimeric S glycoprotein, PEDV VLP, and S-Bac with/without conventional adjuvants were conducted in pigs. While substantial PEDV specific systemic IgG could be successfully induced by the parental immunization with these immunogens, minimal mucosal IgA, and variable protectivity after the virulent PEDV challenge were demonstrated. Chemokines adjuvants incorporated with PEDV antigens were demonstrated to elicit and enhance PEDV specific systemic and mucosal mucosal immunity via IM administration. Importantly, most of chemokines adjuvanted VLP and inactivate vaccine immunized pigs were protected from PED. The correlation of PEDV immune responses in sows with the protectivity in their neonatal piglets and the duration of protective immunity against PEDV will be discussed.

Regional Surveillance: Can it be Timely, Effective, and Affordable?

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We usually think of regional surveillance in terms of individual farms, i.e., test pigs to determine whether a farm is negative (or positive) and when all the individual farms in a region test negative, the region is negative. As an alternative, we explored a surveillance design based on testing a few samples from many farms. Specifically, farm personnel collect samples from unthrifty pigs (n = 10) for testing and the regional status is determined by the aggregate testing result. The cost analysis assumed that 10 samples per farm would be pooled into two samples (5 pigs each) for antibody or PCR testing.

Public domain software[1] was used to simulate the introduction/spread of a pathogen in a population of 17,521 farms in a region of 1,615,246 km^2. Using the simulated spread data, the probability of detecting ≥ 1 positive farms in the region was estimated as a function of farm-level detection probability (10%, 20%, 30%, 40%, 50%), farm participation (20%, 40%, 60%, 80%, 100%), and farm-level prevalence. The approach was highly sensitive. For example, ≥ 90% probability of detection at 0.1% prevalence (18 positive farms among 17,521 farms) was achieved with producer participation ≥ 40% and farm-level detection probability ≥ 30%. Assuming PCR and ELISA costs of USD $25.00 and $7.50, the cost per pig in the region was $0.03 and $0.02 per pig, respectively.

Key points:
(1) The goal is to determine the status of the region … not individual farms.
(2) Samples are collected from unthrifty pigs because "targeted" sampling is more efficient than random sampling at low prevalence.
(3) "Alternative" specimens, e.g., blood swabs, oral swabs, etc. are easy and cheap to collect.
(4) Farm personnel collect samples because they are most aware of which pigs to target.

References

1. Harvey et al. 2007. The North American Animal Disease Spread Model: A simulation model to assist decision making in evaluating animal disease incursions. Prev Vet Med 82:176-197.

PRRSV Diagnosis, Epidemiology, Prevention and Control in the USA

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Despite advances in diagnostic techniques and improvements in control and elimination strategies, porcine reproductive and respiratory syndrome (PRRS), caused by the PRRS virus (PRRSV), has remained one of the most devastating disease affecting the global swine industry. PRRSV is an enveloped, single-stranded positive-sense RNA virus in the family *Arteriviridae* and is classified into two distinct species: *Betaarterivirus suid 1* (PRRSV-1, also known as European type), and *Betaarterivirus suid 2* (PRRSV-2, also known as North American type). Here, PRRSV diagnosis, epidemiology, prevention and control in the USA will be briefly discussed.

Specimens for PRRSV testing. Conventionally, individual serum and tissue samples are commonly used for PRRSV testing. However, population-based specimens such as processing fluid (collected during testicle castration and tail docking), family oral fluid (from both sows and piglets), and regular oral fluid have been increasingly used for PRRSV surveillance testing in the past over 10 years. Recently, tongue tips from dead animals have been used for PRRSV detection and surveillance.

Laboratory testing methods for PRRSV. Various laboratory methods can be used to detect and characterize PRRSV. For example, virus isolation (VI) in cell culture or bioassay for detecting a live virus, RNA detection by RT-PCR and sequencing, viral antigen detection by immunofluorescence staining and/or immunohistochemistry staining, antibody detection using different assays including indirect fluorescent antibody assay (IFA), ELISA, fluorescent focus neutralization (FFN) and others. Here, we will focus on PRRSV VI, PCR, and sequencing.

PRRSV can be isolated in porcine alveolar macrophages (PAMs), sublines (CL-2621, MARC-145) of the African monkey kidney cell line MA-104, or genetically engineered cell lines expressing PRRSV receptors, but the most commonly used cells for PRRSV VI are PAMs and MARC-145. However, PAMs are primary cells requiring periodical preparations from pigs and there are quality variations between batches of PAMs. MARC-145 is still the most commonly used cell line for PRRSV VI, propagation and titration although the success rate of VI from clinical samples is low. Our group has recently demonstrated that ZMAC cell line, an immortalized cell line derived from fetal lung lavage fluids, has better success rate of isolating PRRSV-1 and PRRSV-2 from serum and lung samples than MARC-145 cell line. However, PRRSV VI success rates are overall low in processing fluid and oral fluid samples regardless of using ZMAC or MARC-145 cells. RT-PCR cycle threshold (C_T) value can significantly affect the success rate of VI and it is generally recommended to conduct PRRSV VI from serum and lung samples with C_T<30. VI success rate in ZMAC cells is significantly higher for PRRSV-2 in lineages L1 and L8 but similar for PRRSV-2 in L5 when compared to MARC-145 cells. In addition, when a clinical sample contains ≥2 PRRSV-2 strains, ZMAC is superior to MARC-145 cell line to isolate the predominant virus strain from the sample.

The common strategy for the detection of PRRSV in our laboratory is to test samples first using PRRSV screening real-time RT-PCR that targets the conserved genomic regions. Some commercial PRRSV screening PCR assays contain multiple combinations of forward and reverse primers and probes to achieve sufficient coverage. In most scenarios, PRRSV screening PCR results are correct. However, it is possible that a screening PCR could miss detecting a PRRSV strain due to mismatching of primers and/or probes. Hence, it is a good practice to have an alternative PRRSV screening PCR as a backup assay. Commercial PRRSV screening PCR assays could detect and differentiate PRRSV-1 and PRRSV-2 species but are not designed to further characterize the virus, such as differentiating vaccine viruses from wild-type viruses. Additional molecular testing such as vaccine-like PCRs, Sanger sequencing, and next-generation sequencing (NGS) can be conducted to further characterize the virus detected by PRRSV screening PCR.

In our laboratory, we have developed and validated vaccine-like real-time RT-PCR assays against the six PRRSV-2 commercial vaccine viruses available in the USA (Ingelvac PRRS MLV, Ingelvac PRRS ATP, Fostera PRRS, Prime Pac PRRS, Prevacent PRRS, and PRRSGard) to specifically detect the presence of vaccine-like viruses. Due to its high genetic variability, ORF5 sequencing via Sanger method followed by sequence identity matrix and phylogenetic analysis has been commonly used to determine genetic relatedness and diversity of PRRSV-1 and PRRSV-2 isolates. ORF5-based genetic classification systems have been developed or proposed to help analyze ORF5 sequences. We have developed a web-based tool ISU *PRRSView* (https://prrsv.vdl.iastate.edu/) to help analyze PRRSV-2 ORF5 sequences. When performing the PRRSV ORF5 sequencing on a sample containing more than one PRRSV strain, the virus present in higher concentration is likely the one that is detected. Also, for a sample containing co-infections with a vaccine virus and a wild-type virus, ORF5 sequencing via Sanger method may preferably detect the vaccine virus when they have similar concentrations. To overcome this, CLAMP technology can be used to inhibit amplification of the vaccine virus and hence increase the possibility of sequencing the wild-type virus. Whole genome sequencing (WGS) can reveal sequences in genomic regions besides ORF5, thus providing more information for comparison. Next-generation sequencing (NGS) technology makes it possible to determine PRRSV WGS faster and at lower cost. Different NGS platforms such as MiSeq and Nanopore platforms have been described to determine PRRSV WGS. NGS can detect multiple PRRSV strains present in the sample and also help detect recombinant strains in the sample.

Molecular epidemiology of PRRSV in the USA. Based on testing 785,891 cases in the USA from 2010-2022, the PRRSV screening PCR-positive rates fluctuated between 19.25% and 26.31% when all age of pigs were taken into consideration. PRRSV was detected throughout the whole year but there was seasonality to some degree, with relative lower positive rate in summer. Based on analyzing 106,392 PRRSV screening PCR-positive cases in the USA from 2007-2018, the percentages of PRRSV-2, PRRSV-1, PRRSV-2 & PRRSV-1 positive cases were 95%, 2%, and 3%, respectively. In the USA, PRRSV-2 is still the predominant species.

Based on analysis of 82,237 global ORF5 sequences reported during 1989–2021, we classified PRRSV-2 into eleven genetic lineages (L1–L11) and 21 sublineages (L1A–L1F, L1H–L1J, L5A–L5B, L8A–L8E, and L9A–L9E). To meet the epidemiological investigation needs, the sublineage L1C was further classified into five groups (L1C.1–L1C.5) with L1C.5 corresponding to recently emerged L1C variant strains. Comparison between restriction fragment length polymorphism (RFLP) typing and phylogenetic classification revealed the inaccuracy of using RFLP to determine PRRSV-2 genetic relatedness in most scenarios. The PRRSV-2 ORF5 sequences collected in the USA during 1989-2021 belong to four major lineages L1, L5, L8 and L9 and a few minor lineages L2, L6, and L7. PRRSV-2 L1 has been the dominant virus strain circulating in the USA since 2005. However, the frequency of sublineage sequences within L1 have changed in the USA over time, including a sharp increase of 1-7-4 L1A strains since 2014 and the emergence of L1C.5 (L1C variant) strains in 2020. It is also noteworthy that sublineage L1H rose from 1.69% in 2013 to over 20% during 2019-2021 in the USA. Lineage L5 was reported roughly in a range of 8.4%-16.6% during 2002-2009 and rose to ~20% during 2010-2021. Most of the detected L5 sequences were Ingelvac PRRS MLV vaccine-like viruses. Lineage L8 sequences were at ~10% during 2002-2010 and in the range of 4.6% to 10% during 2011-2021 in the USA. Lineage L9 was a dominant lineage before 2004 representing >30% of sequences and declined to ~20% during 2005-2009 and to less than 1% after 2013 in the USA.

Prevention and control of PRRS. For PRRSV prevention and control, biosecurity, air filtration, inducing immunity, and other approaches are practiced depending on the production systems. Induction of immunity is generally achieved through the use of MLV vaccines and/or exposure to farm-specific wild-type virus via inoculation with killed virus isolate (autogenous vaccine) or inoculation with serum or other clinical samples containing live virus. Currently, in the USA, there are six commercial PRRSV-2 MLV vaccines (Ingelvac PRRS MLV, Ingelvac PRRS ATP, Fostera PRRS, Prime Pac PRRS, Prevacent PRRS, and PRRSGard) derived from different genetic lineages/sublineages but there are no guidelines to help choose appropriate vaccines for use on each occasion. During an acute PRRS outbreak, it may be helpful to administer anti-inflammatories to control fever and antibiotics to mitigate secondary bacterial infections. Regarding the effectiveness of some antiviral treatments, it remains to be investigated. Despite improved biosecurity and other control efforts, PRRSV continues to circulate and evolve and remains a great threat to the U.S. swine industry. Genetically modified pigs resistant to PRRSV infection could be a solution in the future.

Swine Mycoplasma Vaccines Development in Taiwan

Dr. Jiunn-Horng Lin[1]
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Swine enzootic pneumonia is a chronic and clinically mild disease caused by *Mycoplasma hyopneumoniae* and *Mycoplasma hyorhinis* infection; the latter can also induce arthritis and polyserositis. This highly prevalent disease renders considerable economic losses due to poor productive performance, also predisposes pigs to secondary infections, and by all means, vaccination is the most effective approach for disease control.

The speaker has devoted to mycoplasma research for more than 25 years and successfully developed a number of products covering *M. hyopneumoniae* inactivated vaccine, *M. hyorhinis* inactivated vaccine, *M. hyopneumoniae* microsphere oral vaccine, *M. hyopneumoniae* cocktail vaccine, and *M. hyorhinis* cocktail vaccine. Several bivalent and trivalent mycoplasma vaccines have as well produced including mycoplasma *M. hyopneumoniae*, *M. hyorhinis*, PRRS and PCV2.

The subunit vaccines which speaker’s team developed have been made by using reverse vaccinology and is the process of antigen finding from the genome information since 1990s. The reverse vaccinology has been successfully applied to develop a Neisseria meningitides group B subunit vaccine for humans, and by modifying it, Agricultural Technology Research Institute started in 2006 to apply the technology to generate swine *Mycoplasma hyopneumoniae* and *M. hyorhinis* subunit vaccines. In this presentation, the development approaches, efficacy, and safety tests of these mycoplasma vaccines will be discussed.

African Swine Fever (ASF) in Asia; No pig No life

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Asia 2018-2023 is reviewed for prevention, detection, response, what worked and did not work, technical and non-technical issues.

ASF spread through 17 Asian countries quickly since its emergence in China 2018 causing severe damage in pig production and is now endemic in most countries. ASF outbreaks were detected mainly in small producers with limited biosecurity. There were cases detected in large commercial farms showing the difficulty of maintaining the biosecurity at high level. It is often difficult to identify the source and route of virus introduction, which makes the response difficult. The efficiency of virus transmission depending the route of introduction was discussed based on the results of experimental infection. A simple and economical method of disinfection using lime is explained.

ASF virus monitoring system using meat juice collected at markets is proposed to detect the virus circulation in the absence of active reporting.
ASF vaccine seems to be very close to field application. The information of current vaccine candidates is updated, compared, and discussed.

Control of the Infections Caused by Streptococcus Suis

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Restrictions in the use of antibiotics brought, among other consequences, an increase of clinical disease in post-weaned piglets. Among those pathogens affecting nursery piglets, *S. suis* is probably the important bacterial pathogen present worldwide. Controlling stress and predisposing factors (mainly concomitant infections, environmental and management factors) may help to reduce disease, but not always, especially in the presence of virulent strains. *S. suis* is also a zoonotic agent of main concern in some countries in Asia.

In the presence of virulent strains, a logical alternative is the use of vaccines. However, so far, there is no commercial vaccine able to protect against all serotypes/strains of *S. suis*. Indeed, the only alternative practitioners have in hands is the use of autogenous vaccines (mostly bacterins) based on the predominant strain(s) recovered from diseased pigs in the affected farm and produced by accredited laboratories. One of the most important challenge is how to identify virulent strains: this is relatively simple for serotypes 1, 2 and 14…but much less evident for other serotypes. I will address different approaches to identify virulent strains affecting a herd.

In theory, producing a killed bacterin is not complicated. However, there are so many ways to produce such a vaccine that it is literally impossible to compare autogenous vaccines produced by different laboratories. For example, the conditions of bacterial growth (medium, oxygen, etc.), bacterial concentration, methods used to kill bacteria, the use of culture supernatant, the presence of different strains in the same vaccine and the use of different adjuvants will vary depending on the producing laboratory. Once the autogenous vaccine is produced, the next decision is if we need to vaccinate sows or piglets…or both. In this talk, I will address some of these concerns and present unpublished results helping practitioners to take decisions in the field.

Reproductive Problems in Gilts and Sows in Tropical Environment

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Swine breeding herds in today's agriculture often aim for a production goal of 30 to 40 pigs weaned per sow per year. This objective can be met by enhancing the reproductive management of gilts and sows, maximizing the number of piglets born alive, minimizing pre-weaning mortality, and mitigating the effects of heat stress. However, selecting genetically superior sows with high fertility remains a significant hurdle in managing swine reproduction effectively. Within a tropical climate, modern hyperprolific sows have been documented to achieve impressive figures in terms of the average total number of piglets born and born alive per litter, reaching as high as 17.2 and 15.1 piglets, respectively. Additionally, the average total number of piglets born per litter exhibits variability depending on the sow's parity, ranging from 15.5 to 19.3. The swine industry continues to face significant welfare and economic challenges due to piglet mortality during the suckling period. In tropical regions, the average pre-weaning mortality falls within the range of 10% to 20%, displaying considerable variation among different herds.

Specifically, litters consisting of 13 to 16 piglets experience higher pre-weaning mortality (20.8%) compared to litters with 11 to 12 piglets (11.2%). Heat stress can have a long-lasting impact on pig production, particularly during the warmer seasons. However, the environmental temperature can also have an immediate and severe impact on pig farming, especially during occurrences of "heat waves". Sows experience a state of thermal comfort within a temperature range of 18 to 20 °C. However, when temperatures exceed 25°C, they can be categorized as being under heat stress. In tropical countries such as Thailand, the impact of seasonal heat stress on the reproductive performance of gilts and sows shares common characteristics with temperate regions. These include reduced feed intake, changes in follicular development and timing of ovulation in relation to estrus detection, decreased estrus expression, prolonged interval between weaning and first service, reduced luteal size, compromised embryo development, lower oocyte quality and impaired maternal recognition of pregnancy. The reproductive efficiency of gilts plays a vital role in determining the reproductive performance and longevity of sows within swine herds. In tropical countries, the sow replacement rate varied across different herds, ranging from 38.9% to 60.1%. Several significant factors contributed to the culling of gilts, including 44.0% due to anoestrus, 20.5% due to vaginal discharge, 15.5% due to repeat breeding, 10.0% due to non-pregnancy, and 10.0% due to miscellaneous causes.

Among the gilts that were culled due to a lack of estrus, approximately 52% were identified as pre-pubertal. On average, gilts were culled at 321 days of age, with a range of 211 to 504 days, and the average interval from entry to culling was 97 days, ranging from 6 to 273 days.

Effectively managing the reproduction of gilts and sows in tropical regions primarily revolves around two key aspects: ensuring adequate feed intake during lactation and mitigating the adverse impacts of heat stress.

Keywords: anestrus, heat stress, piglet mortality, reproduction, swine

Virus-Host Interactions, Diagnosis, and Control of Classical Swine Fever Virus and Other Porcine Pestiviruses

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Classical swine fever (CSF) is one of the most important viral diseases in pigs worldwide. In many parts of the world, great efforts are being made to reduce the economic losses caused by CSF or to eradicate the disease. A harmonized strategy for the diagnosis, control and eradication of CSF has been applied in the member states of the European Union (EU). The success of the common strategy is reflected in the fact that CSF has not occurred in EU member states for many years.

Nevertheless, CSF remains a constant threat to European pig and wild boar populations. The main control measurements implicated in the EU and recent activities of the EU & WOAH Reference Laboratory for CSF will be summarized. These activities include (i) the organization and quality assurance of CSF diagnosis in the EU, (ii) the development of new diagnostic tools, (iii) the updating of the CSFV sequence database, and (iv) the surveillance, identification and characterization of related pestiviruses and their differentiation from CSFV. In addition, the development and characterization of improved live attenuated CSF marker vaccine candidates based on the concept of chimeric pestiviruses, and recently obtained insights into virus-host interactions of CSFV and other porcine pestiviruses will be reported.

In contrast to rare or even unique infections of pigs with BVDV, BDV, Bungowannah Virus and Linda virus, infections with the distantly related Atypical porcine pestivirus (APPV), which is a major cause of congenital tremor in newborn piglets, occur frequently. The successful establishment of assays to detect APPV genomes and APPV-specific antibodies will allow implementation of strategies to control APPV infections.

Antibiotic Resistance in Pigs, the Environment, and Humans

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Antibiotic resistance is currently a critical concern in both human and veterinary medicine worldwide. With the increasing human population, there is growing pressure to enhance meat productivity. Consequently, it becomes crucial to prevent and treat infections in food-producing animals in order to maintain sustainable productivity and ensure the well-being of farmed animals raised for meat. Many bacterial infections in humans and animals are currently challenging to treat due to the presence of multiple mechanisms involved in bacterial resistance to commonly used antimicrobial agents. Among the numerous global health issues, antibiotic resistance exemplifies the "One Health" approach, which involves collaborative efforts across multiple disciplines to provide solutions for human, animal, and environmental health. The key issues to be addressed include antibiotic consumption in food-producing animals, the role of farm animals in the transmission of antibiotic resistance to humans and the environment, and the growing importance of veterinary antimicrobial stewardship.

Bacterial Infections: An Emerging Frontier in Swine Health

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Bacterial infections are common in swine herds and pose a major threat to swine health. They are the primary cause of economically important diseases such as mycoplasma-induced pneumonia and post-weaning diarrhea caused by *E. coli*. Additionally, bacteria are often the secondary pathogens for viral infections, augmenting the severity and mortality of viral diseases and complicating prevention and treatment. Furthermore, swine also harbor foodborne and zoonotic pathogens that may be transmitted to humans via the food chain and other routes. Antibiotics have played a key role in the control of swine bacterial infections, but many of the pathogens have developed resistance to commonly used antibiotics, compromising the effectiveness of antibiotic treatment. Additionally, the availability of antibiotics in swine production is increasingly limited due to the concern with antibiotic resistance and heightened regulation. Added on top of these challenge, new pathogenic species and variants constantly emerge and re-emerge as a result of adaption and fitness, causing large disease outbreaks and economic losses. In this presentation, the importance of bacterial infections to the swine industry, examples of emerging disease threats, and antibiotic resistance trend will be discussed. The advance in genome epidemiology and how it can be utilized to facilitate disease diagnosis and surveillance will be highlighted. Additionally, the need for developing more and effective bacterial vaccines will also be emphasized as bacterial vaccines have largely lagged behind the effort on viral vaccines.

Biosecurity: Fundamentals and Practice

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Introduction

Prevention and control of swine diseases have to be comprehensive approach. The objective of this paper is to focus on the importance of biosecurity in particular.

Fundamentals of Biosecurity

Biosecurity has to be:
1) Science based
2) Practically feasible (simple, organized)
3) Effective (cost vs. benefit)
4) Committed to continue (execution)
5) Measurable

Practices of Biosecurity

Components of biosecurity include as follows:
1)Internal biosecurity (within-farm): To minimize the transmission of pathogens that already exist within a farm
2) External biosecurity (Between-farms): To prevent new introduction of pathogens into a farm
3) Monitoring, auditing, and education

Paradigm shift of biosecurity concept:
1) Bio-exclusion
2) Bio-containment

Transboundary risk of swine disease transmission: Feed risk

Most recently, Dee et. al. have developed a transboundary model to prove the risk of transmission of certain swine pathogens such as PEDV, PRRSV, Seneca Valley virus (as a surrogate of FMDV) and ASFV via selected feed ingredients (1, 2).

Area regional approach and global collaboration

In some regions, economical significance of particular swine diseases such as PRRS and PED has let producers and veterinarians to initiate area regional approach in order to control or eliminate such diseases. Recent research has shown that a risk of transmission of certain swine pathogens is transboundary (1, 2). Global collaboration is required for sustainable success of biosecurity in each country.

Conclusions & summary

Biosecurity is an only way of the "true" proactive approach of disease prevention. Biosecurity should be comprehensive approach. Execution is the key of prevention/control of swine diseases through successful biosecurity. Because the risk of swine disease transmission is transboundary, sustainable success of biosecurity requires area regional approach and global collaboration.

References

(1) Dee. et. al. (2018) PLoS ONE 13(3): e0194509. https://doi.org/10.1371/journal.pone.0194509
(2) Dee, et. al. The risk of viral transmission in feed: What do we know, what do we do?. *Transbound Emerg Dis.* 2020,001-7. Willeyonlinelibrary.com/journal/feed

Intestinal Health and Mucosal Microbiota for the Growth of Nursery Pigs

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Introduction

In modern pig production, pigs are weaned at their early life when the gastrointestinal tract is not fully mature to handle typical feedstuffs. Upon weaning, pigs receive external stressors from feeds and environmental challenges impairing their intestinal health. Dietary stressors come from typical feedstuffs used in the diet including allergens, antigens, mycotoxins, endotoxins, pathogens, numerous anti-nutritional compounds, and undigested nutrients. These dietary stressors directly causes immune responses to intestinal epithelium or indirectly through the changes of mucosa-associated microbiota in the small intestine (1, 2). The jejunum is the major site within the gastrointestinal tract for digestion and absorption of nutrients and for immune protection from these external stressors. Host intestinal epithelium recognizes the changes of mucosa-associated microbiota and the dietary stressors through various receptors including toll-like receptors (TLRs), nucleotide-binding oligomerization domains (NODs), cluster of differentiation (CDs), etc (3, 4). Recognition by host intestinal epithelium causes inflammatory responses by intestinal immune cells and intestinal enterocytes which are related to the mass production of reactive oxygen species leading to oxidative damages to intestinal epithelium and destruction of villi. With intestinal inflammation and oxidative damages, pigs have reduced capability of nutrient digestion and absorption due to functional limitation of villi (5). Another aspect of intestinal health challenge is increased activation of B cells with elevated immunoglobulin levels in the jejunal mucosa. To recover from these intestinal damages, stem cells in the jejunal crypts are newly proliferated into enterocytes. As a consequence of intestinal health challenge and recovery process, growth efficiency of nursery pigs are impaired with reduced growth. Kim Lab at NC State University has conducted over 55 individual animal studies investigating dietary challenges or interventions influencing intestinal health of nursery pigs. From these studies, over 4,000 nursery pigs were used for the collection of jejunal mucosa, jejunal tissue, and ileal digesta after the measurement of their growth efficiency. These samples were used to measure changes in the relative abundance and diversity of mucosa-associated microbiota, production of pro-inflammatory cytokines in the jejunal mucosa, production of oxidative damage products in the jejunal mucosa, production of immunoglobulins from intestinal immune cells in the jejunal mucosa, structure of villi and crypts, crypt stem cell proliferation, ileal digestibility of nutrients, and growth of nursery pigs contributed the jejunal samples. Results from individual studies have been published and these results were further evaluated by meta-analysis. Increase in the amount of TNF-a, IL6, IL8, IgG, malondialdehyde, and protein carbonyls in the jejunal mucosa reduced average daily gain of nursery pigs. Increase in the relative abundance of prevotellaceae and Bifidobacteriaceae was related to reduced TNF-a and increased average daily gain of nursery pigs. Multiple correlations from meta-analysis allow the prediction of growth performance of nursery pigs based on their intestinal health parameters which can be a tool for animal health monitoring in commercial farms

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References

1. Kim SW, Duarte ME. 2021. Understanding intestinal health in nursery pigs and the relevant nutritional strategies. Anim Biosci 34:338-344.
2. Zheng L, Duarte ME, Sevarolli Loftus A, Kim SW. 2021. Intestinal health of pigs upon weaning: challenges and nutritional intervention. Frontiers Vet Sci 8:628258.
3. Duarte ME, Kim SW. 2022. Significance of mucosa-associated microbiota and its impacts on intestinal health of pigs challenged with F18+ E. coli. Pathogens 11:589.
4. Duarte ME, Kim SW. 2022. Intestinal microbiota and its interaction to intestinal health in nursery pigs. Anim Nutr 8:169-184.
5. Jang KB, Kim SW. 2022. Role of milk carbohydrates in intestinal health of nursery pigs: a review. J Anim Sci Biotechnol 13:1-14.

Sharpen the Knife: Recent Progress in ASF Vaccine Research

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African swine fever (ASF) virus (ASFV) is the etiological agent of a devastating disease of Suidae species and currently circulating in Asian countries except Japan, Taiwan and Sri Lanka. The virus shows a high mortality rate in pigs and imposes huge economic impacts on human societies. Consequently, there is a pressing need for an effective vaccine to control the disease. However, despite decades of effort, no reliable ASF vaccines became available until recently.

In a notable breakthrough, Vietnam approved the world's first attenuated live vaccine (ASF-G-DMGF) in 2022. Since then, additional types of attenuated vaccines receive authorization from the government. These vaccines are now ready for use by local pork producers in affected areas.

Developing effective ASF vaccines is a formidable task due to several challenges. ASFV belongs to a unique group of viruses called “Nucleocytoplasmic Large DNA Viruses” and shares only limited genetic similarity with other "normal" viruses. Consequently, deciphering the functions of molecules encoded in its lengthy DNA genome, which spans over 170 kilobase pairs, is exceptionally difficult. The complex nature of the virus and the absence of a sustainable cell line capable of supporting the growth of ASFV and suitable animal models for evaluating vaccine efficacy further hinder the development process.

To aim at successful development of ASF vaccines, we established a porcine macrophage-derived cell line IPKM and developed in vitro technique to manipulate ASFV genome as planned. In addition, we developed rapid and sensitive diagnostic methods to monitor both ASFV and its specific antibodies. Employing all these technologies, we established several variants of ASFV, which may possess attenuated virulence to host animals.

Lastly, in my presentation, I would like to draw attention to an important issue that will raise awareness of potential risks and promote safer use of this “newly-hatched” technologies.

Porcine Circoviruses: What Is New?

Tanja Opriessnig[1]

[1] *The Moredun Institute and Iowa State University*

Circoviruses (CV) include some of the smallest viruses known. They obtained their name from their circularly arranged single-stranded DNA genome with a gene encoding and a conserved replicase protein on the sense strand. In pigs, four different CVs have been identified and named with consecutive numbers based on the order of their discovery: Porcine circovirus 1 (PCV1, 1974), porcine circovirus 2 (PCV2, 1997), porcine circovirus 3 (PCV3, 2015) and most recently porcine circovirus 4 (PCV4, 2019). PCVs are ubiquitous in the global pig populations and uninfected herds are rarely found. It is generally accepted that PCV1 is non-pathogenic. In contrast, PCV2 is considered an important, economically challenging pathogen on a global scale with comprehensive vaccination schemes in place. Since its discovery, PCV2 has been associated with several disease syndromes in pigs commonly summarized as porcine circovirus associated disease (PCVD). Just like PCV2, PCV3 appears to be widespread in the global pig population and can be readily identified in healthy and clinically affected pigs. The overall role of PCV3 is still controversial several years after its discovery but connections with reproductive failure in sows have been confirmed. Due to often high viral load in infected pigs, overall long shedding of PCVs, associated environmental contamination, and their high stability and resistance against inactivation, PCV2 and PCV3 can be readily identified on pig premises but also in many other animals. For example, there are reports of PCV3 being present for prolonged periods of time in dogs and other species. PCV4 has only recently been discovered and further information on this virus is required to understand its potential impact on pigs. At this point, PCV4 has been identified in China, South Korea, Thailand, and Vietnam. This presentation summarizes current knowledge on PCVs in pigs and aims to contrast and compare known facts.

Early Monitoring and Control Strategies of ASFV

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Strengths and Challenges in Pig Welfare Farming System

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The farming system and pig welfare have become growing concerns in the public. There is an increasing public demand for more natural and species-appropriate housing systems. This societal expectation encompasses sufficient space, outdoor access or enrichment for pig rearing.

Currently, the majority of pigs are kept in intensive indoor production systems. Such systems may limit the natural behaviour of pigs and lead to abnormal behaviours, aggression and further impacts on tail biting, eventually affecting the performance and welfare. On the other hands, pigs rear in alternative systems, such as outdoor housing system, group housing system, deep-bedded or straw-bedded system, are reported to show more species-specific behaviour. These behaviours include rooting, social interaction, nesting, which may improve overall pig welfare. Moreover, adherence to international welfare standards can improve the industry's reputation and broaden its access to global markets. Most importantly, this alignment may meet the expectations of ethically conscious consumers.

However, transitioning to alternative housing systems comes with its challenges. Implementing biosecurity measures in these systems can be more difficult. Outdoor access for pigs may lengthen the wean-to-estrus interval for sows, potentially impacting reproductive efficiency. Furthermore, transitioning from conventional to alternative systems often requires significant initial investment, potentially dissuading some farmers due to upfront costs.

Despite these challenges, the commitment to improving pig welfare remains crucial. A focus on innovative solutions, collaboration between stakeholders, and further research into cost-effective methods and biosecurity measures can help to address these issues. By investing in education and research, improving infrastructure, and adopting proactive measures against emerging diseases, the swine industry can move towards sustainable and welfare-oriented practices that benefit animals, producers, and consumers.

Classical Swine Fever in Japan

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Classical swine fever is one of the most contagious viral infections in pigs. In September 2018, the outbreak was reported for the first time in 26 years in Japan at Gifu prefecture. As of July 17, 2023, more than 356,000 pigs were culled in 86 cases. Besides, more than 6,100 wild boars have been detected. Preventing the spread of viruses in wild boars is the key to eradicating this disease, and we are promoting population reduction, oral vaccines, and sanitary measures for dead wild boars. However, since it takes time to control classical swine fever virus in wild boars, it is necessary to continue high biosecurity measures in pig farms. One year has passed since the first outbreak, and biosecurity measures alone could not prevent the outbreak due to the contaminated wild boars. Finally, vaccination against domestic pigs was resumed in the areas where wild boars were infected in 2019. In addition to the latest status of classical swine fever outbreaks in Japan, recent research activities about classical swine fever virus in our laboratory will be introduced in this presentation.

Workshop Abstracts

Saving time on farms – people utilization

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Time is a valuable resource which need to be understood by the swine veterinarian. How can time be saved, and, enhance the welfare of the pigs, and, the profitability of the farm, and, keep farm staff motivated and yet still get the job done. The job is feeding 8 billion (and increasing) people excellent healthy pork every day.

This paper discusses some of the successful measure which have saved considerable time with no significant negative impacts on health and production.

Teeth clipping. Stopping teeth clipping has no negative impact on production despite being resistantly held on my many farms.

Batching. This allows for much better time management specialty groups of staff to emerge. Batching allows for better health management.

Mixing pigs in the farrowing house. Reduces the time to wean and enhanced post-weaning feed intakes.

Boar semen numbers and collections. Semen numbers are too high in semen doses reducing the effectiveness of each excellent boar reducing enhancement of FCR and Carbon footprint management.

Heat checking. Weaned sows could have no boar exposure until day 4 postweaning. This enhances litter size and reduces the time required to achieve pregnancy.

Single serving. Reduces the time taken to breed sows. Will also reduce FCR when applied to a review of genetics.

Single serve gestation sows. Feeding pregnant sows once a day in the afternoon reduces the heat stress in the gestating sows and feeding once a day takes less time.

Gestation parking. Methods should be explored to reduce the need to move pregnant sows. Group housing conditions are also going to become more common.

Wean to finish. Wean to finish reduces the number of times a pig is moved and therefore, reduced the need to clean the building to only twice a year.

The Effect of Cold Environment on Pig Respiratory Diseases and Ways to Solve It

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There are two major common diseases in the pig industry, GI tract diseases, and respiratory tract diseases. Respiratory tract diseases can cause big economic losses for pigs, high mortality rates, and low growth rates. The cold definition includes low temperature, high moisture, high draught, and high temperature difference. Here it needs to explain the character of some bacteria and viral. What is the optimum environmental temperature and pH for them? It seems that bacteria and viral prefer low temperature and high pH (＞6). According to a physicist report the lower the temperature the higher the pH. Consequently, the lower temperature not only causes lower immunity but also causes higher pH. Both situations induce the outbreak of pig diseases, especially GI tract and respiratory tract diseases. When a pig continuously inhales cold air, that is, the respiratory tract will become extremely cold. As we said before the cold condition will cause lower immunity and higher pH. Both conditions cause higher bacteria and viral which causes respiratory diseases to break out. There are several methods to solve these problems: 1. to increase the insulation value of the roof and wall of the pig house; 2. to avoid water cleaning or too much urine accumulation inside the room or over ventilation (to avoid reaching dew point of the wall inside the house); 3. to avoid cold air blow inside of the house, especially piglet house; 4. to be very careful to avoid higher temperature difference in spring, fall, and winter. It is necessary to provide hot air for pigs during this season during ventilation. All these operations should consider your financial condition to do it. The most important basic principle is to keep the pig house dry and warm. However, if you plan to build a new house you should consider at least you can use it for at least 25 years according to your local environmental temperature. If your pig house standard cannot reach a very high standard, then you need to provide enough feed for pigs. This is because the second method to keep pigs warm is to feed pigs to eat more feed. Keeping pigs warm is the most important way to avoid respiratory diseases.
Weaning and nutrition related to piglet management

Weaning and nutrition related to piglet management

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Introduction

In modern pig production, pigs are weaned at their early life when the gastrointestinal tract is not fully mature to handle typical feedstuffs. Increased prolificacy of sows gives increased number of pigs at birth but with limited capacity of milk production resulting in low weaning weight and increased number of runts (1, 2). In the US, typical weaning is done at 17 to 25 days of age that is similar to 6 to 15 months of age for human. At weaning, pigs weigh between 6 to 7 kg and the function of the small intestine is not fully mature. Feedstuffs derived from plants often bring intestinal health challenges resulting in diarrhea, feed refusal, and weight loss to these newly weaned pigs (3). Consideration of feedstuffs from animals or with reduced allergens can reduce weaning stress to the pigs. Feeding newly weaned pigs until 8 kg body weight is critically sensitive. There can be three possible approaches to tackle these problems. (1) Provide creep feeds with allergens prior to weaning, (2) Wean pigs early near 6 to 7 kg body weight and fed diets with reduced allergens, and (3) Wean pigs late near 8 kg body weight when the intestine can handle allergens better. Improper handling of weaning causes intestinal health challenges with increased inflammation and villus destruction resulting in reduced nutrient utilization and growth reduction. Providing creep feeds has been implemented in the past. Typical practice is to provide creep feed the best feedstuffs without allergens. However, when pigs are protected from the sow with the milk, this could be the best time to train the intestine of these nursing pigs with feed allergens prior to the weaning. Upon early weaning, typical early weaner feeds are with high inclusion of feedstuffs derived from animals such as blood plasma, meat meal (poultry meal and fish meal), and milk co-products to reduce the use of feedstuffs derived from plants potentially with high level of allergens. Blood plasma contains high levels of immunoglobulins (about 20 to 30%) providing protections against feed allergens. Meat meals including poultry meal and fish meal do not contribute allergens and thus ideal protein supplements. Milk co-products do not contain feed allergens and great sources of highly digestible carbohydrates, lactose, for the newly weaned pigs. In general feedstuffs derived from plants contains allergens and anti-nutritional compounds causing intestinal health challenges and diarrhea. However, some of selected processing of these plant feedstuffs can greatly reduce these concerns including physical (heating), chemical (acidification and alkalization), and biological (fermentation) processes. Excess nutrients (exceeding requirements) in feeds can be a source of intestinal challenge as well. Increased undigested protein in the intestine causes the shift of intestinal bacterial population by increasing proteobacteria and reducing firmicute and actinobacteria (4). These changes cause intestinal inflammation leading to villus destruction. Weaning pigs at older age when they reach near 8 kg body weight can be another practical approach to handle weaning stress to newly weaned pigs. However, nutritional management of these old weaned pigs should be different from pigs with similar body weights that were weaned at early age and fed early weaner feeds (5). Smart nutritional management starts from understanding the mechanisms of interactions among feed allergens, intestinal bacteria, and intestinal health that are clearly related to growth performance of newly weaned pigs.

Acknowledgement

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References

1. Guo JY, Sun Y, DeDecker AE, Coffey MT, Kim SW. 2019. Effect of suckling intensity of primiparous sows on production performance during current and subsequent parities. J Anim Sci 97:4845–4854.
2. Kim SW, Easter RA. 2003. Amino acid utilization for reproduction in sows. In: (ed. J. P. F. D’Mello) Amino Acids in Animal Nutrition (ISBN: 085199654X). CABI Publishing. pp 203-222.
3. Kim SW, Duarte ME. 2021. Understanding intestinal health in nursery pigs and the relevant nutritional strategies. Anim Biosci 34:338-344.
4. Duarte ME, Kim SW. 2022. Intestinal microbiota and its interaction to intestinal health in nursery pigs. Anim Nutr 8:169-184.
5. Jang KB, Purvis JM, Kim SW. 2021. Impacts of weaning age on dietary needs of whey permeate for pigs at 7 to 11 kg body weight. J Anim Sci Biotechnol 12:111.

Oral Abstracts

O-T-001

COMPARING COMPETITIVE FITNESS OF DIFFERENT GENOTYPES OF CLASSICAL SWINE FEVER VIRUSES

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Introduction
Classical swine fever (CSF) caused by classical swine fever virus (CSFV), is a highly contagious disease in pigs. In Taiwan, the attenuated genotype 1.1 (G1.1 CSFV) lapinized Philippine Coronel vaccine has been used to protect pigs against CSFV since 1958. There were two genotypes of CSFVs reported in Taiwan. Before 1993, the historical genotype 3.4 (G3.4 CSFV) was predominant in Taiwan. However, the emerging genotype 2.1 (G2.1 CSFV) caused sporadic outbreaks in 1994 had become the major genotype affecting pigs after 1996[1]. A shift in virus genotypes was also observed in several CSFV endemic countries[2]. The previous studies have shown that the G2.1 CSFV had a higher secreted(S)/cell-associated (C) ratio than the G3.4 CSFV in porcine cells and the G2.1 CSFV might secret and replicate more efficiently than that of G3.4 CSFV[3]. In the present study, to further investigate the differences of viral replication and transmission of G2.1 and G3.4 CSFV, two groups of SPF pigs were inoculated with G2.1 or G3.4 CSFV and cohabitated with SPF pigs. Investigation of the mechanism responsible for the genotype switching would be important to design interventions for the controlling and eradicating the disease.

Materials and methods
Ten six-week-old SPF pigs were used in this study. Three pigs were individually inoculated with G2.1 or G3.4 CSFV and cohabitated with four pigs. The pigs were monitored for body temperature and clinical scores, and the blood, serum, oral swab, and fecal swab were collected for further analysis. Genotype-specific primers and probes were used to detect the viral load[4].

Results
The results showed that the blood viral load, peak viremia, and oral and fecal viral shedding of G2.1 CSFV were higher than those of G3.4 CSFV in the single inoculation group. In cohabitation groups, coinfection of both genotypes of CSFV was detected in 3/4 pigs and the rest of the pig was only infected by G2.1 CSFV. The viral shedding and transmission of G2.1 CSFV were also higher than G3.4 CSFV in the cohabitation group. In addition, in the blood and fecal swab detection of the single inoculation group, no detectable virus other than the original inoculum was detected in both groups, showing the phenomenon of superinfection exclusion.

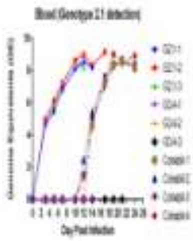
Conclusions
The present study demonstrates that the replication fitness and transmissibility of G2.1 CSFV are more efficient than those of 3.4 CSFV in pigs.

References
1. Deng, M.-C., et al., Phylogenetic analysis of classical swine fever virus isolated from Taiwan. Veterinary microbiology, 2005. 106(3-4): p. 187-193.

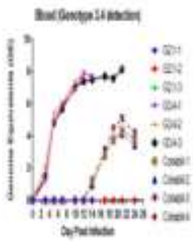
2. Shivaraj, D., et al., Genetic clustering of recent classical swine fever virus isolates from Karnataka, India revealed the emergence of subtype 2.2 replacing subtype 1.1. VirusDisease, 2015. 26(3): p. 170-179.
3. Huang, Y.-L., et al., Competitive replication kinetics and pathogenicity in pigs co-infected with historical and newly invading classical swine fever viruses. Virus research, 2017. 228: p. 39-45.
4. Huang, Y.-L., et al., Development of a reverse transcription multiplex real-time PCR for the detection and genotyping of classical swine fever virus. Journal of Virological Methods, 2009. 160(1-2): p. 111-118.

Figure
Detection of viremia by real-time PCR using the genotype-specific primers and probes (A: G2.1 CSFV, B: G3.4 CSFV). Three pigs were individually inoculated with G2.1 or G3.4 CSFV and cohabitated with four pigs.

Graphs or Images 1



Graphs or Images 2



O-T-002

CROSS-PROTECTION BY VIRUS NEUTRALIZATION TEST OF O1 CAMPOS (BIOGENESIS BAGO) FMD VACCINE STRAIN AGAINST CIRCULATING O/ME-SA/IND-2001 FMD VIRUSES WORLDWIDE

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Introduction
Foot-and-Mouth Disease (FMD) is one of the most important diseases of livestock worldwide and is endemic in several countries in Asia. O/ME-SA/Ind-2001 lineage of FMD virus (FMDV) was first detected in India in 2001 and started to spread in other regions, with the first report in Southeast Asia in 2015 due to FMD outbreaks from Vietnam [1]. Most recently, Indonesia, a country free of FMD since 1986, had an incursion of FMD on April 2022 and the lineage involved was confirmed to be O/ME-SA/Ind-2001 as well. [2]
O1 Campos Biogenesis Bago (BB), a well-known FMD vaccine strain with demonstrated broad-cross protection, is widely used in South America and Asia and has shown excellent efficacy in controlling the disease in these regions. Here we report satisfactory cross-protection of an FMD vaccine formulated with O1 Campos (BB) through virus neutralization titer (VNT) test against O/ME-SA/Ind-2001 field isolates circulating worldwide.

Materials and Methods
A monovalent water-in-oil single emulsion (>6PD50) vaccine containing highly purified O1 Campos antigen (Aftogen Oleo) was used in this study, manufactured by Biogenesis Bago in compliance with Good Manufacturing Practices (GMP). After single vaccination of cattle and pigs, serum samples were collected and assessed against O/ME-SA/Ind-2001 isolates (n = 41) coming from different pools isolated from 2013 to 2022. VNT were performed at The Pirbright Institute, World Reference Laboratory for FMD (WRLFMD), and at SENASA, (National Service for Agrifood Health and Quality), the WOAH reference laboratory for Foot-and-Mouth Disease in Argentina VNT titers greater or equal to 1.5log10 were considered as an indicator of minimum heterologous cross-protection [3-4].

Results
The results showed that VNT titers of O1 Campos (BB) against all O/ME-SA/Ind-2001 field viruses were greater than 1.5 (log10); even more, for most of them, titers were greater than 1.9 (Log10). With regards to the Indonesian isolate (O/ISA/3/2022) a highly potent O1 Campos vaccine showed a good match with VNT titer of 2.46 (log10) (Figure 1).

Conclusions and Discussions
These results indicate its adequacy to protect infections from O/Ind-2001 viruses, as reported. Based on these results, Biogenesis Bago world-class vaccines (Bioaftogen and Aftogen Oleo) containing O1 Campos FMD vaccine strain have proven to achieve high levels of neutralizing antibodies against O/ME-SA/Ind-2001 field isolates in the region as well as with the most recent isolates. Therefore, the use of these vaccines is an effective tool in controlling FMD outbreaks from O/ME-SA/Ind-2001 lineage.
References
[1]. Vu LT, Long NT, Brito B, Stenfeldt C,Phuong NT, Hoang BH, et al. (2017) First detection of foot-and-mouth disease virus O/Ind-2001d in Vietnam. PLoS ONE 12(6), 2017

[2] WRLFMD. Reference Laboratory Reports
Quarterly Report July-September 2022.
[3] Hammond, J.M., Maulidi, B., Henning, N. Targeted FMD Vaccines for Eastern Africa: The AgResults Foot and Mouth Disease Vaccine Challenge Project. Viruses
13(9), 2021
[4] Karabassova AS, et al. Toward the calibration of serological assays using sera collected from cattle and sheep following a single dose of foot-and-mouth disease vaccine. Vet World. 2022 Feb;15(2):524-530. doi: 10.14202/vetworld.2022.524-530. Epub 2022 Feb 28. PMID: 35400944; PMCID: PMC8980403.

Graphs or Images 1

Figure 1. Vaccine matching studies for O field isolates **O/Ind2001** lineage collected during 2016-2022 (n=27). VNT was performed at Pirbright Institute (WRL for FMD) or SENASA employing O1 Campos **bovine or pig sera** (21-28 dpv) from Biogenesis Bago vaccine. VNT titers = > 1.5 log10 was considered as an indicator of minimum heterologous cross-protection, as previously proposed

O-T-003

THE 1ST ASF VIRUS CELL PASSAGE & SELECTING MASTER SEED CANDIDATES, USING NON-PRIMARY CELL LINE IN KOREA & VIETNAM

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- Introduction
African swine fever (ASF) is currently causing a pandemic in major swine countries, resulting in devastating losses to the industry. Therefore, a safe and effective vaccine seems to be a reasonable demand for the prevention and control of ASF. Many experimental ASF vaccines, so far, mainly rely on the production of vaccine with primary swine macrophages, which is not practically possible for the commercial production.

- Materials and Methods
The African swine fever virus (ASFV) of Korea and Vietnam isolate were derived from CA_CAS_01_A cell line. Titration of ASFV from freeze-thawed samples was performed using primary swine macrophage cell cultures in 96-well plates. When CA_CAS_01_A cells were used for virus titrations, the presence of virus-infected cells was detected by alkaline phosphatase assay using an anti-ASFV p30 monoclonal antibody. Titers were calculated using the method of Reed and Muench and expressed as 50% hemadsorption doses (HAD50)/ml. and Next-generation sequencing (NGS) of ASFV genomes.

- Results
We report here the genotypic analysis of viruses obtained at different passages during the process of adaptation of a virulent ASFV field isolate from the Korea and Vietnam to grow in cultured cell lines. ASF viruses were successively passaged about 20 times in CA_CAS-01_A cells. Viruses obtained in Korea and Vietnam were evaluated in vitro for the ability to replicate in CA_CAS-01_A cells. Replication of ASF viruses in in CA_CAS-01_A cells increased with successive passages. Full-length sequence analysis of each of these viruses revealed significant deletions that gradually accumulated in multi-areas at the right and left variable ends of the genome. Mutations that result in amino acid substitutions and frameshift mutations were also observed, though in a rather limited number of genes. The potential importance of these genetic changes in virus adaptation/attenuation is discussed.

- Conclusions and Discussion
Here, we report vaccine candidates for ASFV with a deletion in the variable region. This deletion allows for growth in stable cell cultures while altering the virulence of the parental vaccine strain. This discovery provides valuable information that can be used to further the understanding of ASFV gene function, virus attenuation, and protection against infection.

O-T-004

TRANSMISSION OF CLASSICAL SWINE FEVER VIRUS IN COHABITATION PIGLETS OF VARIOUS IMMUNE STATUSES WITH ATTENUATED LIVE VACCINE

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[1]Animal Health Research Institute, Council Of Agriculture, Taiwan, [2]School of Veterinary Medicine, National Taiwan University, Taiwan

Introduction
Classical swine fever (CSF) is a systemic hemorrhagic disease that affects domestic pigs and wild boars. The modified live vaccine induces quick and solid protection for pigs against CSF virus (CSFV) infection. Maternal-derived antibodies could interfere with the modified live vaccine (MLV) efficacy, leading to the incomplete protection of pigs against CSFV infection [1-4]. This study aims to understand the transmission of CSFV in between experimental piglets with various MLV vaccination immune statuses.

Materials and Methods
A total of nineteen 4-week-old piglets, including 1 SPF piglet without anti-CSFV neutralizing antibodies (NAs) (Group 1) and 18 healthy piglets (Groups 2–5) from the LPC-vaccinated sows of a CSFV-free herd were used in this experiment. The 18 healthy piglets were randomly separated into four groups (Groups 2–5). This SPF piglet (Group 1) was inoculated oral-nasally with the 5 × 105 TCID50 TD/96 strain at 7 DPE. Group 2 (N = 6) was vaccinated with one dose of LPC vaccine at 0 DPE. Group 3 (N = 3) was not vaccinated with the LPC vaccine, The Group 1 cohabited with Groups 2 and 3 for 10 days (from 7 to 17 DPE). Group 3 to Room 4 to cohabit them with Group 5 (N = 3). The clinical signs, CSFV antigen, and anti-CSFV neutralizing antibody (NA) were examined.

Results
1. Clinical signs

The CSFV-associated clinical signs in Group 1, 3, and 5 were found respectively at 12-18, 23-36, and 27-39 DPE, respectively and only piglet 8138 was survive. Both group 2 and 4 was healthy without CSFV-associated clinical signs during the experimental stage.

2. CSFV Viremia

TD/96 viremia in the piglet of group 1 was detected between 10 and 17 DPE (Figure 1). In Group 2, TD/96 viremia was detected in only one piglet during 21 to 35 DPE. In Group 3, TD/96 viremia was all detected after 24 DPE. However, the TD/96 viremia of Piglet 8138 turned negative by 28–35 DPI (Figure 2). In Group 4, TD/96 viremia was not detected during the experiment. In Group 5, all the piglets were positive for TD/96 viremia 31–35 DPE.

3. Anti-CSFV NA

The anti-CSFV NA was not detected in the CSFV donor of Group 1 between 7 and 17 DPE (Figure 2). The sera of Groups 2–5 at 0 DPE showed titers that ranged between 5 and 7 log2. The average titer of Group 2 did not decline between 0 and 28 DPE but was significantly increased between DPE 31 and 35. In Group 3 piglets, the average titer gradually decreased between 0 and 28 DPE but reversely increased between 31 and 35 DPE. The average titers of Groups 4 and 5 decreased over time.

Conclusions

The currently used MDAs followed by the MLV vaccination policy can induce sufficient immunity. Although a few MLV-vaccinated individuals had transient low-level viremia, CSFV transmission to the third party could be blocked by MLV vaccination.

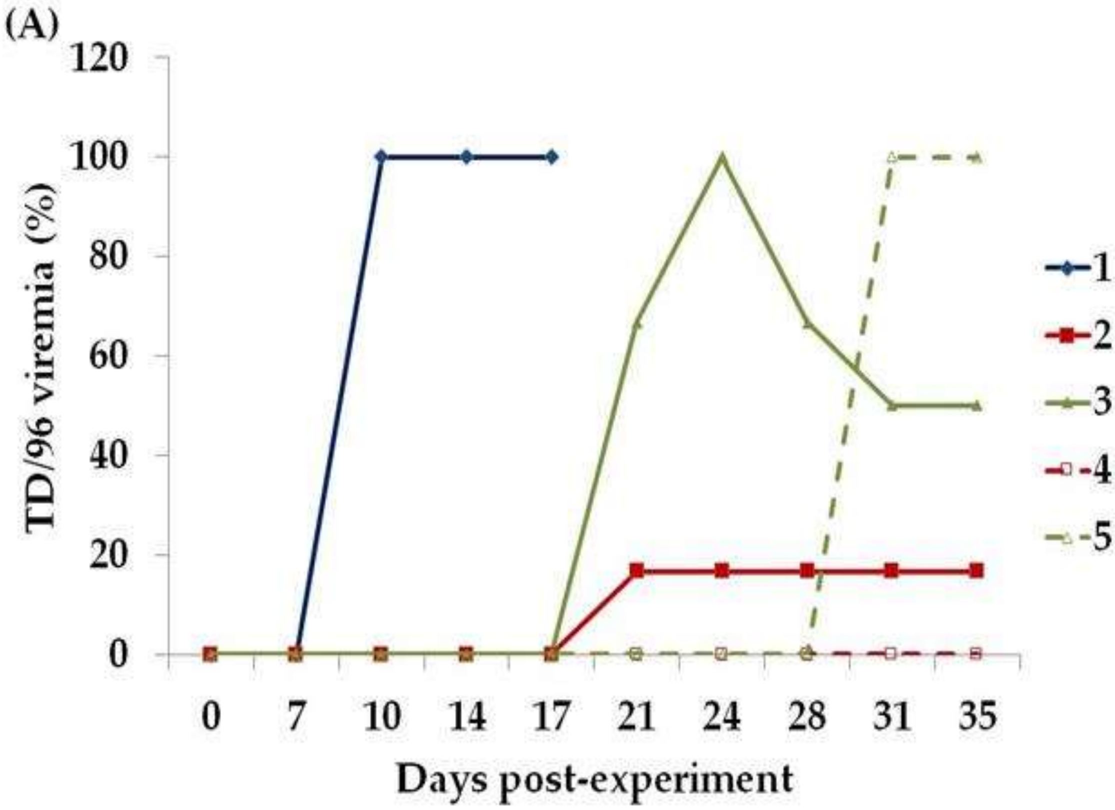
Acknowledgement
We especially thank our colleagues in the Division of Classical Swine Fever Research for their assistance in the animal experiments.

References

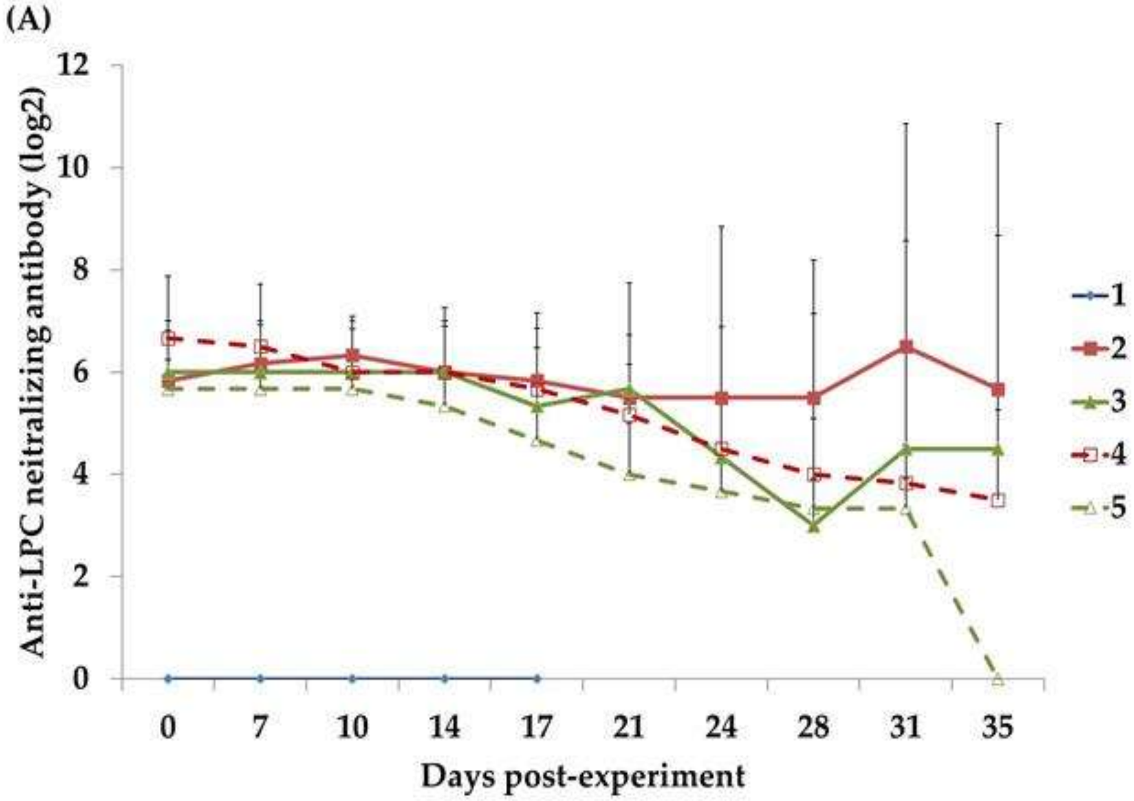
1. Huang, Y.L. et al. Virus Res. 2014, 179, 1–11.
2. Suradhat, S. et al. Vet. Microbiol. 2007, 119, 1–9.
3. van Oirschot, J.T. Vet. Microbiol. 2003, 96, 367–384.
4. Terpstra, C. et al. Vet. Microbiol. 1988, 16, 123-128.

Figure 1. The percentage of piglets positive for TD/96 detected by QRRT-PCR in blood.
Figure 2. The anti-LPC neutralizing antibodies in sera of piglets in each group during the experimental period.

Graphs or Images 1



Graphs or Images 2


(A)
Anti-LPC neitralizing antibody (log2)
12
10
8
6
4
2
0
0
7
10
14
17
21
24
28
31
35
Days post-experiment
1
2
3
4
5

O-T-005

TEMPORAL AND SPATIAL PATTERNS OF AFRICAN SWINE FEVER SPREAD IN THE PHILIPPINES, 2019-2022

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Introduction
African swine fever (ASF) is an acute hemorrhagic disease of swine that causes high mortalities in domestic pigs and wild boar. The recent spread of genotype II ASF virus in Asia caused substantial economic loss and raised public health concerns in affected countries, such as the Philippines. The objective of the spatial-temporal analysis here was to identify the pattern of ASF spread in the country between August 2019 to July 2022, with the ultimate objective of supporting disease control.

Materials and Methods
The Philippines confirmed the first ASF outbreak in Central Manila in July 2019. Data on reported outbreaks between August 16, 2019, and July 20, 2022, were made available to us by the International Training Center on Pig Husbandry (ITCPH), Agricultural Training Institute, Department of Agriculture of the Philippines. Recorded data, for each farm-outbreak, included event ID, administrative level (region, province, municipality, and barangay), and date of reporting. Outbreak location was approximated using the centroid (latitude and longitude) of the barangay in which the affected farm was located. Data were organized in a generic database and descriptive statistics were computed using the R software, version 4.2.0. The global Moran'I test, implemented using ArcGIS Pro (ESRI Inc., Redlands, CA, USA), was computed to assess whether outbreaks were geographically aggregated. Local space-time clusters of disease were identified using the space-time permutation model of the scan statistic, implemented in the SaTScan software v10.0.2 (Kulldorff, Cambridge, MA, USA) [1]. Directional tests were performed using ClusterSeer software (Biomedware Inc., Ann Arbor, MI, USA) to determine whether the direction of spread of ASF outbreaks within each time-space cluster identified by the scan statistic. Significance ($p < 0.05$) of results was assessed for all statistical tests using Monte Carlo simulation.

Results
A total of 19,697 farm-level ASF outbreaks were reported in domestic pigs from 2019 to 2022. The highest number of outbreaks was reported between July and December 2020 (27.85%, 5486/19,697) compared to any other 6-month intervals of all study periods. Region III (Central Luzon) accumulated the highest number of outbreaks (24.66%, 4857/19,697). Results of the Global Moran' I autocorrelation test suggested that ASF was significantly clustered ($p<0.05$) in the Philippines. Five significant ($p<0.05$) time-space clusters were identified with a maximum temporal window of 4 months. The first three clusters (#1 to #3, Figure 1) were consecutive and located in Luzon island, whereas the fourth cluster centered in East Visayan island (Region VIII). The fifth cluster returned back to the Northern Luzon island. The direction tests in the five clusters were all statistically significant ($p < 0.01$) and indicated different directions of spread at the local level.

Conclusions and Discussion

Space, time, and space-time analyses presented here provided quantitative metrics that help to characterize the spread of ASF in the Philippines between 2019 and 2022. The three largest detected clusters, in terms of number of outbreaks included, occurred over time periods of approximately 3 months and during the second half of the year annually, which may be associated with cultural practices and environmental conditions. Results here contribute to the epidemiological characterization of ASF spread with the ultimate objective of supporting the design and implementation of disease control strategies in the Philippines and other affected countries in the region.

Acknowledgements
We thank the Bureau of Animal Industry and International Training Center on Pig Husbandry of the Philippines (Dr. Ruth Miclat-Sonaco) for facilitating the data analyzed here.

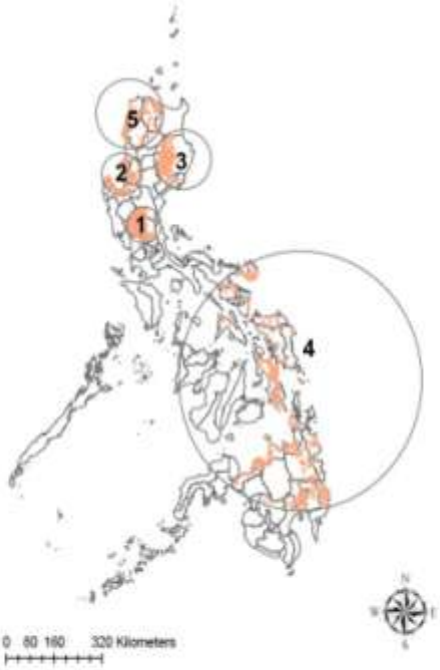
References

1. Kulldorff et al. (2005) PLoS med, 2(3), e59.

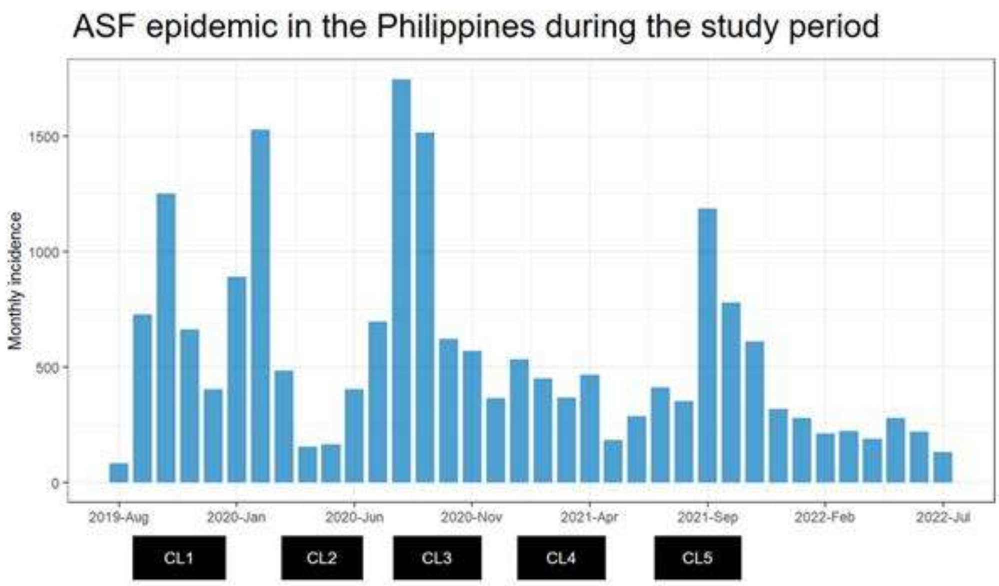
Table 1

Space-time clusters of ASF epidemic in the Philippines							
Cluster	Radius (km)	Start Date	End Date	OBSERVED	EXPECTED	ODE	p value
1	55.86	2019/9/1	2019/12/31	2850	633.98	4.50	<0.00001
2	64.90	2020/3/1	2020/6/30	494	89.58	5.51	<0.00001
3	91.67	2020/8/1	2020/10/31	1496	361.67	4.14	<0.00001
4	399.64	2021/1/1	2021/4/30	1261	310.01	4.07	<0.00001
5	107.00	2021/7/1	2021/10/31	1776	295.63	6.01	<0.00001

Graphs or Images 1



Graphs or Images 2



O-V-001

A CASE STUDY OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME （PRRS） ERADICATION ON A FINISHING FARM USING AN INACTIVATED VACCINE IN JAPAN

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[1]*Value Farm Consulting Co., Ltd.*, [2]*Nisseiken Co., Ltd.*

【Introduction】
PRRS is one of the most important diseases related to PRDC. It has been reported that eradication of PRRS virus (PRRSV) from a farm can be achieved by the uniform PRRSV exposure to the all-breeding pigs and the farm closure for a certain period, followed by the partial depopulation and the strict all-in all-out (AIAO) rearing. However, in farrow to finish farms where the production and pig introduction cannot be interrupted, this approach is difficult to be implemented. In this study, we tried to eradicate PRRSV from a PRRSV-positive finishing farm by using the PRRSV inactivated vaccine.

【Materials and Methods】
The test farms were finishing farms, which received groups of 9 weeks old piglets weekly from a 450 sows farm. The sow farm was PRRSV negative, while the finishing farms were found to be PRRSV positive after May 2019. The piglets were inoculated with PRRS modified live vaccine on the arrival to the finishing farms. From June 2020, for the further improvement, Nisseiken PRRS Inactivated Vaccine ME (Nisseiken Co., Ltd., Japan) started to be inoculated three weeks after the live PRRS vaccination on the farm arrival. In October 2020, two new finishing buildings were completed, and the pig flow was changed to completely AIAO rearing and the PRRS vaccine program also was changed to a program where piglets are vaccinated with two doses of the inactivated vaccines on the arrival to the finishing farms and three weeks after that.

【Results】
Blood viral tests by PCR showed that PRRSV was still detected in many pig buildings after the start of test. When all pigs were replaced by the pigs vaccinated only with PRRS inactivated vaccines and in the second round of pig herds, all PCR results were negative, and no viremia was observed in the simultaneous blood samplings. The mortality in the growing and finishing buildings showed a significant ($p<0.01$) decrease after the start of PRRS live vaccine vaccination. A year-on-year comparison of the six-month period, when PRRSV was negative, showed a significant improvement of -2.4 % in mortality, +0.6 kg/head in carcass weight, and -0.23 in carcass weight based whole herd FCR. Using a carcass price of 450 yen/kg as a rough estimate, the improvement was approximately 1,974 yen per pig.

【Discussion】
It has been reported that vaccine-derived viruses can be detected by PCR in sera for at least 8 weeks or longer. It would be extremely difficult to eradicate PRRSV under such circumstances. In this study, PRRS eradication was successful even though the introduction of piglets was continued. The reason was thought to be the shortening of the viremia period due to the PRRS inactivated vaccine. Genetic analysis showed that the field strain of PRRSV

on this farm belonged to cluster Ⅲ with RFLP type 1-30-2. Further research is needed to determine whether the fact that the farm and vaccine strains belonged to the same cluster had a positive effect on the results. This finishing farm actively worked to improve the pig flow and to ensure that staff members took several quarantine measures. Considering the high transmission ability of PRRSV, these efforts were also considered a major factor in the elimination of PRRSV. It was also suggested that these efforts are essential to further maintain the cleanliness on the farm.

【References】

NIAH, JASV (2009) PRRS Control Technology Collection, p89-99.

Locke A． Karriker（2005） PRRSV Eradication: Depopulation and Roll-over Techniques. George A Young Swine Conference.

K.Kure, Y.Yahara(2009) Serological and viral response of neonatal piglets inoculated PRRS live vaccine in a commercial farm. APVS Japan.

Table 1

Table 1: Summary of PRRS virus test results in each pig building

	Jul. 2020	Oct.	Dec.	Mar. 2021	Jun.*
Positive	5	4	1	0	0
Negative	8	8	4	12	15
Total	13	12	5	12	15

*：Examination using oral fluid

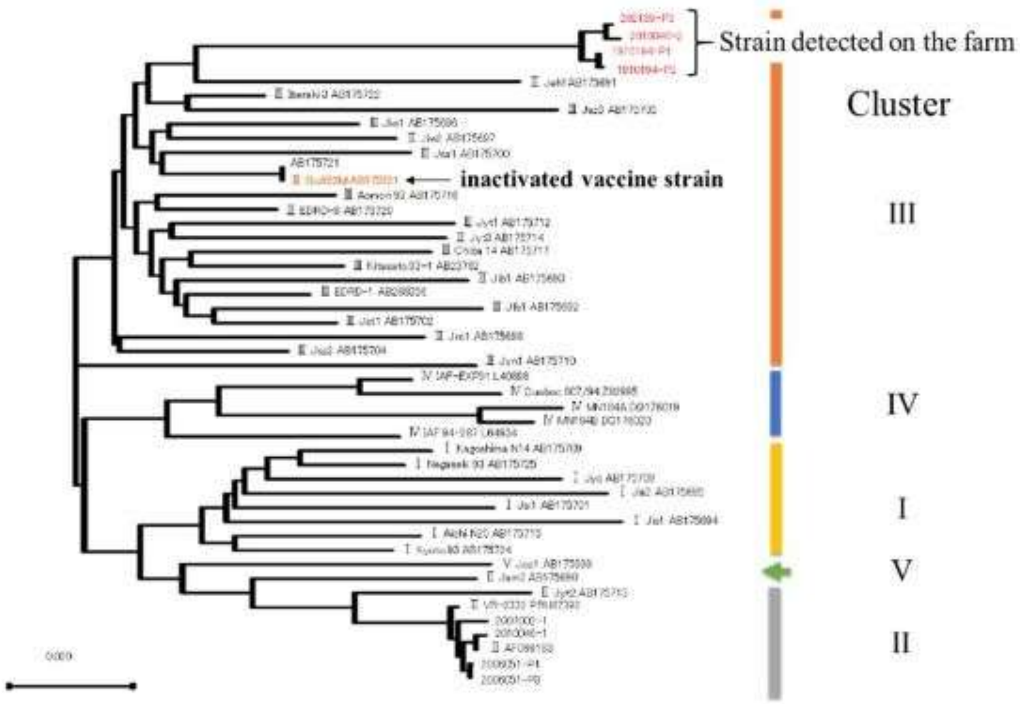
Table 2

Table 2: Comparison of productivity results before and after PRRSV eradication

	Feb-Jul 2020 (pre-eradication)	Feb-Jul 2021 (post-eradication)	post-pre difference
Number of sows	446	442	-4
Mortality on the finishing farm	6.0%	3.6%	-2.4%
Number of pigs marketed	5653	6311	+658
Average carcass weight (kg)	76.7	77.3	+0.6
FCR live wt. basis	2.81	2.66	-0.15
FCR carcasses wt. basis	4.17	3.94	-0.23

Graphs or Images 1

The phylogenetic tree of the farm



O-V-002

A DRAMATICALLY INCREASED OF SECRESTORY-IMMUNOGLOBULIN A LEVEL IN GILT SERUM FOLLOWING MULTIPLE INJECTION OF PORCINE EPIDEMIC DIARRHEA LIVE VACCINE AND ITS CONSEQUENT LEVEL IN COLOSTRUM AND MILK

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Introduction
Porcine epidemic diarrhea (PED) virus infection is one of important causes of diarrhea in sows and piglets. Particularly in piglets, this porcine coronavirus causes severe damage of small intestine by decreasing the proportion of crypt and villi from 1:7 to 1:3, subsequently malabsorption of nutrients and electrolytes, which is the primarily cause of high piglet mortality rate (i.e. 20-50%) and growth retardation in piglets found in infected herd and lead to economic loss in pig industry worldwide. Nowadays, the most practical method is to provide the passive immune to the piglet via colostrum from the immunized gilts/sows. It is well documented that there are two techniques to immunize the gilts/sows, first by vaccination them just before farrowing and second is to feedback fresh small intestine of PED sick piglets (1,2). With these techniques, the sow colostrum contains plenty of passive immune, especially IgG and IgA that specific to PEDV (3). However, during the past decade, it has been shown that intramuscular injection with inactivated or live vaccine did not show compromise immunity, particular the level of IgA (4).

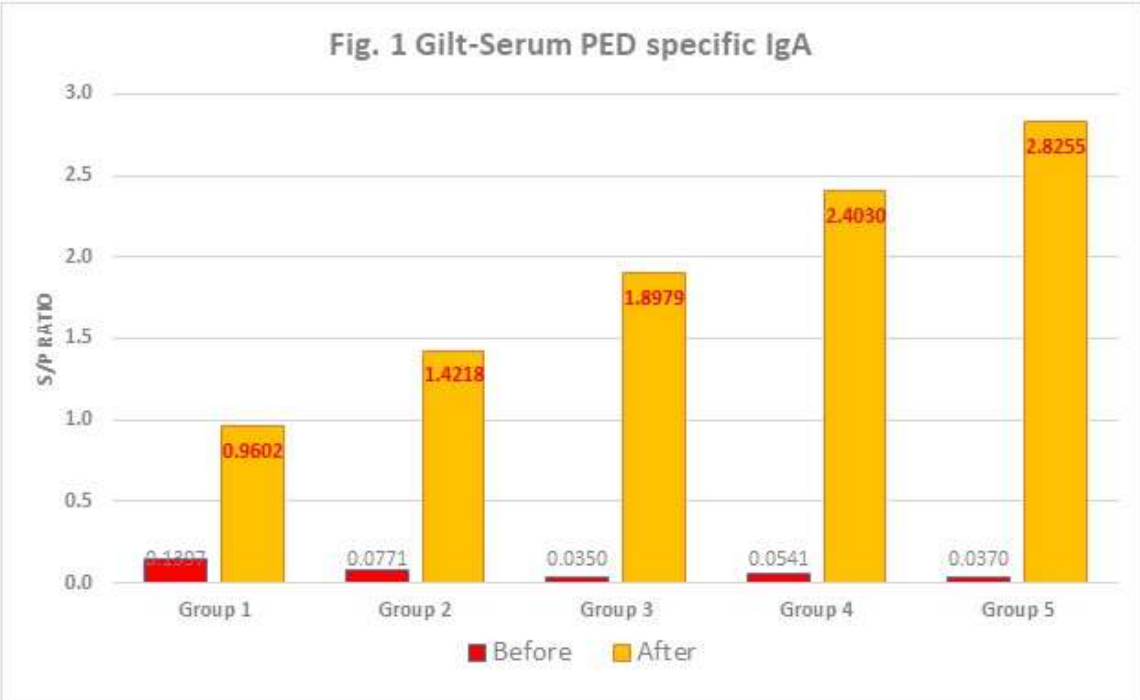
Materials and Methods
Altogether 50 gilts were allocated into 5 different groups as follows: Group A (control, n=10), injected with sterile water 2 times at 20 and 22 wk of age; Group B (n=10), injected with Porcine Epidemic Diarrhea Live vaccine (PEDL, Nisseiken, Japan), 2 times at 20 and 22 wk of age; Group C (n=10), injected with PEDL, 4 times at 20, 22, 24 and 26 wk; Group D (n=10), injected with PEDL, 6 times at 20, 22, 24, 26, 28 and 30 wk and Group E (n=10), injected with PEDL, 8 times at 18,20, 22, 24, 26, 28, 30 and 32 wk. All the gilts at 35 wk of age were inseminated twice at oestrus, they were tested for pregnant at 4 weeks after artificial insemination by using ultrasound. Feeding was performed by using the farm protocol. Blood samples of gilts in both groups were collected for serum before and after vaccination, and kept frozen for analysis of pig PED specific IgA levels by using ELISA (IDEXX Laboratories Co., Ltd., Beijing, China).

Results and Discussion
The S/P value of PED specific IgA in each group is shown in Figure 1 (serum). The S/P values of PED specific IgA are correspondent to the number of vaccinations in each group. A higher significant serum level of PED specific IgA in groups 2-5 was found when compared with control (group 1). This indicated that multiple intramuscular injection with PED Live vaccine (PEDL) in gilt can provide a high compromise immunity for themselves and their coming piglets via the colostrum intake. To our knowledge, this is the first study demonstrated that

multiple intramuscular injection with PED live vaccine is able to stimulate extremely high level of serum IgA.

References
1. Constable et al. (2011). Veterinary Medicine. 11th ed. Elsevier Health Sci, 353.
2. Lee (2015). Virol J, 12(1), 1-16.
3. Markowska-Daniel et al. (2010). Pol J Vet Sci, 13(1), 21-27.
4. Liu, Q. and Wang, H.Y. (2021). Vet Res Commun, 45(2-3), 75-86.

Graphs or Images 1



O-V-003

COMPARISON OF THE EFFICACY OF 2 COMMERCIAL VACCINES AGAINST PORCINE CIRCOVIRUS TYPE 2 AND MYCOPLASMA HYOPNEUMONIAE OF PIGLETS IN THE FACE OF MATERNALLY DERIVED ANTIBODY

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[1]Zoetis Japan, [2]For Pig Nasu

Introduction
Interference by maternally derived antibody (MDA) is a common concern for the vaccination of young pigs against Porcine circovirus type 2 (PCV2) 1,2. Recently, a commercially available PCV2 bivalent vaccine containing two genotypes, PCV2a and PCV2b, has shown to provide broad cross-protection against all major circulating PCV2 genotypes, not only PCV2a and PCV2b but also to PCV2d3. In the present study, we compared the efficacy of a bivalent PCV2a2b vaccine with a monovalent PCV2a vaccine in piglets with high MDA in a farm diagnosed with subclinical PCV2 infection and poor performance 4.

Materials and methods
A total of 1,192 weaned piglets from sows vaccinated with PCV2 vaccine of an integrated production farm in the Kanto region of Japan were included in the study. The sows were vaccinated with a different PCV2 vaccine than the one used for piglets three weeks prior to farrowing. Two years ago prior to the study, PCV2d was clinically detected with increased mortality, The situation at present has improved with no significant herd death loss patterns and no observable clinical signs of disease. Growth performance and feed efficiency, however, are still affected while lacking of overt PCV2d viremia detection. .PRRS virus (PRRSV), Mycoplasma hyopneumoniae (Mhp), IAV-S and App were presence. Piglets were divided into 2 groups; T1: 567 heads received a bivalent PCV2a2b/Mhp combination vaccine and T2: 585 heads received monovalent PCV2a/Mhp combination vaccine. Each vaccine was administered at weaning (~21 days old). Blood samples were randomly taken every 30 days from 30 to 150 days of age from 20 heads in T1 and 5 heads in T2, and antibody titers for PCV2, PRRSV, Mhp, IAV-S, and App were measured by ELISA. PCV2 and PRRSV were also tested by PCR. The mortality rate of each group from wean to finishing was also investigated.

Results
No PCV2 virus was detected from blood samples in both groups. PCV2 ELISA titer were 1,610 in T1 and 1,335 in T2 at 30 days of age which were considered high titers. The ELISA titer decreased gradually thereafter, but increased during the fattening period (Figure 1). The PRRSV was positive at 60 days of age in T1, and ELISA titer increased after 60 days of age in both T1 and T2. ELISA titers for Mhp and App increased during the fattening period for both T1 and T2, while IAV-S titer increased at 60 days of age and during the fattening period. The mortality rate was 3.53% in T1 and 8.38% in T2 ($p<0.01$).

Discussion

The mortality rate was significantly lower in T1 group ($p<0.01$) even though PRRSV was detected at 60 days of age. Since PCV2d had been detected on this farm in the past, the bivalent PCV2a2b vaccine, which has been reported to cover broader PCV2 genotypes, appeared to be effective. The situation of other disease, i.e. Mhp, IAV-S and App, did not differ significantly between the two groups based on the results of ELISA tests. It seemed the pigs in T2 group got a greater exposure than T1 prior 90 days of age. In conclusion, the bivalent PCV2a2b vaccine performed well even in high- maternally derived antibody piglets for this farm.

References

1) Franzo, G., et al. Pathogens. 2020. 9(12):1049.
2) Poulsen Nautrup, B., et al. Vaccines. 2021.9(8):923.
3) Bandrick, M., et al.,Vet Immunol Immunopathol. 2020. 223:110034.
4) Alarcon, P., et al. Prev Vet Med. 2013. 110(2):88-102.

Graphs or Images 1

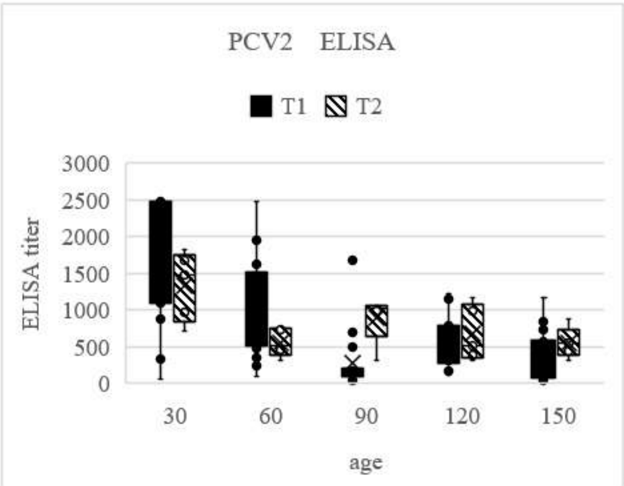

Figure 1. The alteration of PCV2 ELISA titers from 30 to 150 days of age.

O-V-004

Introduction and Efficacy of a New Commercial Modified-Live PRRSV-2 Vaccine (IMMUNIS-PRRS-NA)

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) is a threatening viral disease in the swine industry for over three decades. Previous studies have shown that PRRSV induces a variety of pathogenic in pigs due to its genetic diversity and high mortality rate. In South Korea, in the case of PRRSV2 (NA type) Lineage 1, Lineage 5, and country-specific lineages (Lin Kor A, B, and C) were prevalent. Among them, the frequency of Lineage 1 occurrence was noteworthily increased. Currently, there are two commercially available PRRSV2 modified-live vaccines (MLV) in the Korea market. However, MLV vaccine strains were belonging to Lineage 5 and Lineage 8, respectively, Therefore, the goal of this study was conducted to develop a PRRSV2 lineage 1 MLV vaccine, isolated in South Korea.

Materials and Methods

Three different conventional pig farms that were reported as PRRS-positive farms were selected. One hundred Twenty, 3-week-old pigs have been distributed in two groups (20 pigs/group, respectively) and inoculated with PRRSV2 Lineage 1 vaccine candidate (IMMUNISᴏR PRRS-NA). Negative groups were kept as non-vaccinated pigs. Vaccination was conducted to intramuscular (I.M) in pigs and the animal experiment was carried out until the pigs were commercialized. Blood samples were collected on designated days for quantification of serum antibodies, viremia, and virus-neutralizing test (VNT).

Results

During an animal experiment, pig death was observed, however, the cause of death was confirmed to be due to the pathogen present on the farm, not the side effects of vaccination. After vaccination, the serum antibody was significantly ($p<0.005$) elevated, and interferon-gamma (IFN-γ) secreting cell populations were also increased ($p<0.01$). In addition, serum viremia and lung gross lesion were significantly decreased ($p<0.0005$, $p<0.005$) compared to the negative groups. Consequently, PRRS-positive reaction was significantly decreased in lung gross lesion and immunohistochemistry (IHC).

Conclusions and Discussion

Taken together, the PRRSV2 vaccine candidate (IMMUNISᴏR PRRS-NA) displayed remarkable vaccine efficacy, including increased ADWG (Average daily weight gain), antibody, and IFN-γ secretions. This vaccine candidate will be contributed to the swine industry suffering from PRRSV2 lineage 1 strains in South Korea.

Reference

1. SC Kim, CG Jeong, GS Park, et al. Temporal lineage dynamics of the ORF5 gene of porcine reproductive and respiratory syndrome virus in Korea in 2014-2019
2. TW Oh, SJ Park, HJ Cho, et al. Comparative evaluation of 4 commercial modified-live porcine reproductive and respiratory syndrome virus (PRRSV) vaccines against heterologous dual Korean PRRSV-1 and PRRSV-2 challenge

Acknowledgment
This work was carried out with the support of the "BioGreen21 Agri-Tech Innovation Program" Rural Development Administration, Republic of Korea (Project No. PJ01561102).

O-V-005

DEVELOPMENT OF SWINE FC-CONJUGATED SEROTYPE O FOOT-AND-MOUTH DISEASE VIRUS-LIKE PARTICLES AND IMMUNOLOGICAL EVALUATION IN SWINE

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[1]*Chungnam National University*

- Introduction:

Many advancements have been made including the development of new-generation vaccines such as peptide vaccines, recombinant vaccines, DNA vaccines, and VLP vaccines. In this investigation, we developed the Ad5 FMDV VLP-sFc vaccine, a novel and distinctive Ad5 FMDV VLP vaccine conjugated with swine IgG Fc. Additionally, we demonstrated that our novel Ad5 FMDV VLP-sFc vaccine was highly immunogenic and more effective than currently available vaccines.

- Materials and Methods:

Production and characterization of FMDV VLPs, Atomic force microscopy (AFM) analysis, Transmission electron microscopy (TEM) analysis, Animal experiments, Serum neutralization (SN) test

- Results:

As a result, we modified the FMDV VLP, which is coupled with swine IgG Fc, and projected it onto the VLP surface. It is well-studied that the 3C protease cleaves into the FMDV P12A precursor, which then self-assembles into empty capsid particles.
This study demonstrated the efficacy of an FMD vaccine developed from swine immunized with FMDV VLPs. Immunization results clearly showed that commercial vaccines, Ad5 FMDV VLP vaccines, and Ad5 FMDV VLP-sFc vaccination significantly induced FMDV-specific IgG antibodies (Figure 6B). Even though the three vaccines induced similar levels of antibodies against FMDV, most importantly, the SN titers were still greatly variable. The SN titer in Ad5 FMDV VLP-sFc vaccinated sera was significantly higher compared to that in commercial and Ad5 FMDV VLP vaccines (Figure 6C). SN antibodies have been considered the most relevant parameter for protection against pathogens. Not only neutralizing activities but also Ad5 FMDV VLP-sFc demonstrated the highest level of all cytokines that we examined. Natural killer (NK) and NK T cells, as well as macrophages, have important roles in the immune cells that are activated by IFN-γ in antiviral and antitumor processes. Compared to the nonvaccination and commercial vaccine groups, the production of TNF and IL-4 was significantly higher in Ad5 FMDV VLPs and Ad5 FMDV VLP-sFc, but there was no significant difference between VLPs (Figures 6F and 6G). Despite significantly increased IL-4 and IFN-γ, Ad5 FMDV VLP-sFc eventually induced the highest neutralizing activities. These results suggest that conjugated sFc was able to successfully interact with IgG gamma receptors, which increased the immune response.

- Conclusions and Discussion:

In conclusion, our Ad5 FMDV VLP-sFc proved its potential as an FMD vaccine, and sFc-conjugated VLPs enhanced immunological reactions with higher neutralizing activities and cytokine production. Cytokine production and neutralizing activity are critical evaluation parameters for not only the FMD vaccine but also all viral vaccines. In this study, we found that compared to newly created FMDV VLP-sFc vaccines, commercial FMD vaccines exhibited reduced cytokine production and neutralizing effects. We also found that vaccinations with the Fc molecule specifically showed high levels of cytokines and, particularly, neutralizing activity. Although the Ad5 FMDV VLP-sFc transduction rate was lower than that of Ad5 FMDV VLPs, its efficacy as a vaccine was better than that of Ad5 FMDV VLPs. Therefore, our newly developed FMDV VLPs is able to address those problems and have better immunological effects. Furthermore, for protection, studies with a challenge are needed along with the subject matter of subsequent studies on other serotypes.

- Acknowledgement and References: This study supported by a grant from Korean government.

Table 1

Table 1
Park *et al.*

Group	No. swine	VN titer	Average	SD
Negative	#1	0	0	0
	#2	0		
	#3	0		
	#4	0		
Commercial vaccine	#1	64	65.75	18.46
	#2	90		
	#3	64		
	#4	45		
Ad5 FMDV VLP	#1	64	51.25	15.85
	#2	32		
	#3	64		
	#4	45		
Ad5 FMDV VLP-aFc	#1	181	213	102.43
	#2	362		
	#3	181		
	#4	128		

Graphs or Images 1

목록

O-V-006

EFFECT OF STORAGE TEMPERATURE BY TIME ON PRRSV DETECTION BY RT-QPCR

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[1]Iowa State University, [2]Pig Improvement Company Latinoamerica, [3]IDEXX Laboratories, Inc.

Introduction
PCR targets in diagnostic samples are subjected to a variety of adverse conditions (e.g., range of temperatures over time) in the course of collection, storage, transport, and processing before being tested, but the effect of these conditions on PCR results is largely unexplored. Herein, the effect of storage temperature by time on the detection of PRRSV RNA and a pig-specific internal sample control (ISC) inherent to all pig-derived specimens, was evaluated in serum, oral fluids, and fecal samples by RT-qPCR.

Materials and Methods
Serum samples (n = 5) used in the study were from pigs experimentally inoculated with wild-type PRRSV. Oral fluids (n = 5), and fecal samples (n = 5) were from individually housed pigs vaccinated with a PRRSV MLV (Ingelvac® PRRS MLV).

Each sample was divided into 28 aliquots (500 uL) and each aliquot was subjected to one combination of (temperature by time), i.e., 4, 10, 20, or 30°C by 24, 48, 72, 96, 120, 144, or 168 hours. After completing all treatments, samples were tested using a commercial RT-qPCR that detected both PRRSV and the ISC simultaneously (IDEXX Laboratories, Inc.). The Cq values from the RT-qPCR testing were re-expressed as "efficiency standardized Cqs (ECqs)", Thereafter, ECqs were cube root transformed for analysis, and the effect of storage temperature by time was analyzed by a mixed-effects regression (MRM) model using R 4.1.0 (R core team, 2020).

Results
In serum, PRRSV was stable at 4, 10, and 20°C ($p > 0.05$) but not at 30°C ($p < 0.05$), with an estimated decrease of 0.11 ECqs every 24 h. For the ISC, an ECq loss of 0.07 and 0.10 was observed at 20 and 30°C respectively. In oral fluids, a decrease in PRRSV and ISC was observed at all temperatures ($p < 0.05$), the estimated ECq decrease every 24 h at 4, 10, 20, and 30°C was 0.03, 0.05, 0.07, and 0.08 for PRRSV, and 0.02, 0.04, 0.05, and 0.05 for ISC, respectively. In fecal samples a significant ($p < 0.05$) daily decline in PRRSV ECq of 0.05 and 0.06 was observed at 4°C or 10°C and 0.05 for 20 or 30°C. For ISC, the ECqs declined by 0.06 at 4°C or 10°C and 0.10, 0.08 for 20 or 30°C, respectively.

Conclusions and Discussion
Overall, the results showed that storage of serum at 4, 10, and 20°C for up to 168 h had little impact on the detection of PRRSV, but it was affected at 30°C. The ISC in serum was stable at 4 and 10°C, but a discernible effect was observed at 20 and 30°C. In oral fluids and feces, a significant decrease in PRRSV and ISC detection was observed when stored at either 4, 10, 20, or 30°C. Based on this data, we recommend storing serum for PRRSV RT-qPCR testing at ≤ 20°C. In contrast, oral fluid and fecal samples should be stored frozen to avoid the loss of detectable PRRSV RNA.

Acknowledgements
PRRSV RT-qPCR testing was partially supported by donations from IDEXX Laboratories, Inc.

O-V-007

ESTIMATING THE HIDDEN COST OF VACCINATION CAUSED BY CHANGES IN THE GROWTH RATES AND MORTALITY OF WEANED PIGS IN FOUR FIELD STUDIES OF CHINESE PIGS RECEIVING DIFFERENT VACCINES

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Introduction
A particular challenge facing swine producers is balancing the benefit of disease prevention and control with the cost of achieving those goals in order to maintain profitability. In the case of vaccination, the cost per head can be much higher than the purchase price of the vaccine alone. Failure to account for these hidden costs can lead to undesired economic outcomes.
Changes in growth rates are one of the most significant hidden costs of vaccination, followed by mortality. We determine what these costs are from four separate studies conducted on Chinese farms comparing the impact that several vaccines (PCV2 vaccines and PCV2 & M.hyopneumoniae combination vaccines) had on the production performance of natural challenged wean to finish pigs. Individual records were kept on mortality and pig weights from weaning, the time of vaccination administration, to final marketing. From these datasets we estimate the effect and total cost of each vaccine.

Materials and Methods
A growth curve was estimated for each pig using a Gompertz function, a distribution was then fit to the individual growth curves for each vaccination group. Using these distributions, we use a grow-finish marketing model to simulate the growth and marketing of 10 groups of 1,000 animals on their profit maximum day1. Profitability is determined by taking the sale price of each animal minus the cost of feed, vaccination, and mortality. Feed and hog sale prices were taken from distributions of 10 years of Chinese feed and hog prices. A packer matrix was used to assign each pig a premium or penalty to the base hog price based on weight. The model maximized the annual profitability of each vaccine group to ensure an optimal balance was achieved between market group weight and number of groups marketed in a year. The economic results of each vaccination group were compared within each study. Model inputs were also standardized to control for idiosyncratic factors such as genetics and farm technology. This allowed the results of each vaccination group to be compared across studies to identify the impacts of variation and mortality.

Results
We demonstrate that there is a range of hidden economic costs when vaccinating weaned pigs. These costs are due to decreasing the average growth rate and by increasing the variation in growth rates, and by mortality, both by the opportunity cost of lost sales and the cost of feed prior to death. The relevant production performance of each group is shown on Table 1. Table 2 shows the within study group total economic cost of the vaccine, separated by dose cost, costs incurred from changes in growth rate, and mortality costs. Table 3 shows the results of the across study analysis where inputs were standardized, with

the goal of providing results not specific to the farms involved in the studies. The key drivers of increased costs are lightweight animals in conjunction with an increased number of days to market (which can decrease total throughput by reducing the number of groups marketed in a year), and mortality. When accounting for these factors, the total cost per head of vaccines can far exceed the purchase price of the vaccine alone. Which can lead us to conclude that vaccine choice is an important factor in a farm's overall profitability.

References

1. Calderón Díaz JA et al. Financial Analysis of Herd Status and Vaccination Practices for PRRS virus, Swine Influenza Virus, and Mycoplasma hyopneumoniae in Farrow-to-Finish Pig Farms Using a Bio-Economic Simulation Model. Frontiers in Veterinary Science. 2020;7.

Table 1

		ADG (g)		Mortality	
Study	Vaccine	Mean	StDev	Loss	Mean Age at Death
Study 1	FLEXCombo	726.3	90.6	5.3%	39.9
	Vaccine A	712.5	93.0	7.8%	60.3
Study 2	FLEXCombo	659.4	52.3	5.4%	48.8
	Vaccine B	643.3	45.2	5.4%	48.8
Study 3	FLEXCombo	853.9	90.3	5.4%	48.8
	Vaccine B	834.7	105.0	6.3%	53.2
Study 4	CircoFLEX	894.2	86.3	3.3%	65.4
	Vaccine C	875.8	86.7	3.2%	68.7

Note:

FLEXCombo - Fleshly mixed combination of Ingelvac CircoFLEX® and Ingelvac MycoFLEX®
CircoFLEX - Ingelvac CircoFLEX®
A - Chinese ready-to-use PCV2/Mycoplasma hyopneumoniae vaccine.
B - Multinational recombinant inactivated chimeric ready-to-use PCV2/Mycoplasma hyopneumoniae vaccine
C - Multinational inactivated whole PCV2

Table 2

	Study 1		Study 2		Study 3		Study 4	
	FLEX Combo	Vaccine A	FLEX Combo	Vaccine B	FLEX Combo	Vaccine B	Circo FLEX	Vaccine C
Dose Cost	¥35.90	¥18.90	¥35.90	¥23.80	¥35.90	¥23.80	¥28.90	¥21.00
Slowed Growth Cost	¥0.00	¥40.65	¥0.00	¥46.71	¥0.00	¥46.40	¥0.00	¥46.61
Mortality Cost	¥0.00	¥94.80	¥0.00	¥0.00	¥0.00	¥24.03	¥0.00	¥1.21
Total Cost per Head	**¥35.90**	**¥154.34**	**¥35.90**	**¥70.51**	**¥35.90**	**¥94.24**	**¥28.90**	**¥68.82**

Table 3

PCV2 & M. hyo Vaccines				
	Dose Cost	Slowed Growth Cost	Mortality Cost	Total Cost per Head
FLEXCombo	¥35.90	¥0.00	¥0.00	¥35.90
Vaccine A	¥18.90	¥40.98	¥88.44	¥148.32
Vaccine B	¥23.80	¥55.05	¥17.77	¥96.62
PCV2 Vaccines				
	Dose Cost	Slowed Growth Cost	Mortality Cost	Total Cost per Head
CircoFLEX	¥28.90	¥0.00	¥0.00	¥28.90
Vaccine C	¥21.00	¥39.06	¥3.92	¥63.98

O-V-008

EVALUATING THE RELATIONSHIP BETWEEN RESPIRATORY HEALTH STATUS (REHS) AND PCR CT VALUES FROM TESTING OF ORAL FLUIDS AND AIR SAMPLES FOLLOWING MULTIPLE RESPIRATORY DISEASE CHALLENGES

Dr. Dale Polson[1], Dr. Carmen Alonso[2]
[1]*Boehringer Ingelheim,* [2]*Boehringer Ingelheim*

Introduction
An experimental study was conducted to evaluate the relationship between detectable respiratory disease episodes using SoundTalks technology and multiple PCR testing of weekly oral fluid samples (every pen) as well as air samples (every zone).

Materials and Methods
Eleven week old pigs (n=1655) were allocated to 72 pens across two rooms, with three SoundTalks zones per room, each zone covering 12 pens. In every pen, three randomly selected seeder pigs were challenged seven days apart with Mycoplasma hyopneumoniae (MHP) and PRRS virus (PRRSv). All oral fluid and air samples were tested by PCR for Mycoplasma hyopneumoniae, PRRS virus and swine influenza virus (IAV-S). Ct (cycle threshold) values were obtained as well as the Ct values for the positive-negative (PN) cutoff for each PCR test. Ct delta values (positive-negative cutoff minus test result) were calculated for each PCR result and the delta values for all three agents were summed for each test date. These PCR delta data for both oral fluid and air samples were then compared to each other as well as each to the SoundTalks ReHS data. PCR deltas for a range of oral fluid sample sizes (1, 2, 3, 4, 5 or 6 per zone) were compared to air sample Ct delta values using 1,000 iterations of a stochastic model to assess comparability of the two sample types for tracking the progression of infection levels.

Results
The sum of the oral fluid Ct delta values for the three respiratory disease tests was highly correlated with the corresponding air sample Ct delta sum values ($r=-0.794$, $p=0.0012$). ReHS was highly correlated with both the sum of the air sample Ct delta values for the three respiratory disease PCR tests ($r=-0.809$, $p=0.0008$) and the sum of the oral fluid sample Ct delta values for the three respiratory disease PCR tests ($r=-0.909$, $p<0.0001$).

The stochastic model showed no difference in early diagnostic detection by PCR between oral fluids and air samples for PRRS or IAV-S. However, air samples were shown to detect MHP at least one week earlier than oral fluids at all oral fluid sample sizes modeled, with detection probabilities ranging from 21.2% to 87.3% for 1 to 6 oral fluids per zone in the week that MHP was first detected in air samples.

Conclusions and Discussion
PCR Ct delta values from oral fluid and air samples are useful to diagnostically characterize the combined clinical effect of multiple respiratory disease agents, as continuously measured by SoundTalks ReHS. This approach can be useful for characterizing clinical episode patterns driven by one or multiple swine respiratory disease agents.

These results suggest that while both oral fluid and air sample PCR testing are useful for the early diagnostic detection of PRRS and IAV-S, air samples may be a marginally better sample type for MHP clinical disease episodes detected by SoundTalks ReHS.

O-V-009

GENERATION OF PORCINE EPIDEMIC DIARRHEA VIRUS SPIKE MRNA-CONTAINING LIPID NANOPARTICLES

Mr. Chun-Han Wu[1], Dr. Hui-wen Chang[1]
[1]*National Taiwan University*

Introduction:
Porcine epidemic diarrhea (PED) characterized by severe watery diarrhea has caused high morbidity and mortality in nursing piglets leading to a great economic loss in the swine industry. The cause of PED is porcine epidemic diarrhea virus (PEDV) which is a positive single strained RNA virus belonging to Alphacoronavirus, Coronaviridae. Two major genotypes (G) of PEDVs have been identified. Traditional PEDV G1 vaccines couldn't control global PEDV G2 outbreaks since 2013. Rapid development of a robust and safe PEDV vaccine capable of eliciting both humoral and cellular immune responses and protection is urgent needed. In the past, development of vaccines is time-consuming and might not be able to prevent and control currently-circulating viruses, especially RNA viruses, in time. During the COVID-19 pandemic, the successful application of mRNA vaccines against COVID-19 has opened the new field of mRNA vaccines against animal infectious diseases. The mRNA encoding the immunogens encapsulated in a lipid shell could enter the cytoplasm of target cells. The host cell machinery directs the translation of the antigen proteins that induce effective and long-lasting immune responses. Compared to conventional vaccines, mRNA vaccines have the potential for high potency, low-cost manufacture, rapid development, and safe delivery. Therefore, mRNA vaccine againt PEDV will be a new way for controlling PED.

Materials and Methods:
Two plasmids containing spike (S) 1 domain and full-length ectodomain of PEDV S gene, namely pcDNA3.1-PEDV S1-639-V5-His and pcDNA3.1-PEDV S-V5-His, were constructed and in vitro transcribed with 5' Capping for mRNA synthesis. The systhesized mRNA was mixed and encapsulated in lipid nanoparticles (LNP). The size of PEDV S1 and S mRNA-LNP particles was determined by Zetasizer Nano ZS instrument and the encapsulation efficiency was determined by the ribogreen assay. To detect the expression and the stability of PEDV S1 and S mRNA-LNP stored for 0, 7, 14, and 28 days at 4 °C, the expression of S1 and S proteins in PEDV S1 and S mRNA-LNP treated HEK-293 cells was analyzed by immunocytochemistry (ICC) and western blot (WB).

Results:
The PEDV S1 and S mRNA were successfully synthesized and able to produce PEDV S1 and S proteins in transfected HEK-293 cells determined by ICC and WB. The PEDV S1 and S mRNA-LNP in size about 100 to 110 nm were successfully encapsulated with six of the molar N/P ratios (ionizable cationic lipid over phosphate) and the efficiency is about 50%. Using WB and ICC, the PEDV S1 and S mRNA-LNP were able to successfully transfect the mRNA into cells to express PEDV S1 and S proteins. Interestingly, the transfection efficiency of the mRNA-LNP was better than those using the commercial mRNA transfection reagent. Importantly, both PEDV S1 and S mRNA-LNP were proved stable at 4 °C for 28 days.

Conclusions and Discussion:

Two PEDV mRNA-LNP vaccine candidates have been developed in the present study. Further animal experiments need to perform to confirm the immunogenicity and protectivity of these PEDV mRNA-LNP vaccines.

Acknowledgment and References

1. Le, Ting, et al. "mRNA Vaccine Development for Emerging Animal and Zoonotic Diseases." Viruses 14.2 (2022): 401.
2. Kiaie, Seyed Hossein, et al. "Recent advances in mRNA-LNP therapeutics: immunological and pharmacological aspects." Journal of Nanobiotechnology 20.1 (2022): 1-20.
3. Schoenmaker, Linde, et al. "mRNA-lipid nanoparticle COVID-19 vaccines: Structure and stability." International Journal of Pharmaceutics 601 (2021): 120586.
4. Chang CY, Cheng IC, Chang YC, Tsai PS, Lai SY, Huang YL, Jeng CR, Pang VF, Chang HW (2019, Feb). Identification of Neutralizing Monoclonal Antibodies Targeting Novel Conformational Epitopes of the Porcine Epidemic Diarrhoea Virus Spike Protein. Scientific Reports , (9): 2529.

O-V-010

PARENTERAL ADMINISTRATION OF AN ATTENUATED PORCINE EPIDEMIC DIARRHEA VIRUS TO INDUCE IMMUNOPROTECTIVITY IN PIGS

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Introduction
Porcine epidemic diarrhea virus (PEDV) belongs to Alphacoronavirus causing porcine epidemic diarrhea (PED) that leads to a high mortality in neonatal piglets and great economic loss in the swine industry. Traditional PEDV genotype 1 (G1)-based vaccines failed to control the outbreaks of high virulent genotype 2 (G2b) occurring since 2013 and several attempts to develop PEDV G2b-based inactive or subunit vaccines via parental routes have been demonstrated unsuccessfully if without mucosal adjuvants. Recently, evidences suggest that PEDV could infect dendritic cells and peripheral blood T cells and transfer the virus to intestinal epithelium to establish infection and immune responses. The findings suggest that intramuscular administration of an attenuated PEDV might be able to induce systemic and mucosal immunity and serve as a potential vaccine candidate.

Material and methods
Total number of 9 four-week-old pigs were intramuscularly administrated with an attenuated PEDV PEDV-PT P96/100 strain or PBS two times with three-week interval. Two weeks after the second immunization, both groups of animals were orally challenged with the high virulent P5 PEDV-PT strain. During the period of immunization program and after orally challenging, daily fecal viral shedding was detected by reverse transcription-quantitive PCR, systemic and mucosal PEDV specific IgG/ IgA were detected by ELISA, and PEDV specific IFN-γ secreting cells were detected by ELISpot. After challenging, vaccine protectivity was evaluated by detecting clinical signs, fecal scores, and fecal viral shedding.

Results
While no clinical signs and any viral shedding were detected after the attenuated viral inoculation, prominent systemic immunity and slight mucosal immunity and cellular immunity specific to PEDV-PT were detected after the boost immunization. After orally challenging, pigs administrated with the P96/100 PEDV-PT showed milder symptoms and lower viral shedding than those of the control group.

Conclusions and Discussion
The results reveal that intramuscular administration of the attenuated P96/100 PEDV-PT is safe based on no clinical sign and viral shedding were detected; and the attenuated strain also elicited prominent and immunoreactivity to partially protect piglets from high virulent PEDV-PT challenging and reduce the severity of clinical signs and shedding titers. These results indicated that intramuscular administration of the attenuated P96/100 PEDV could be a potential vaccine candidate against PEDV.
Acknowledgements and References
1. Chang, Y.-C., Kao, C.-F., Chang, C.-Y., Jeng, C.-R., Tsai, P.-S., Pang, V. F., Chiou, H.-Y., Peng, J.-Y., Cheng, I.-C., & Chang, H.-W. (2017). Evaluation and Comparison of the Pathogenicity and Host Immune Responses Induced by a G2b Taiwan Porcine Epidemic Diarrhea Virus

(Strain Pintung 52) and Its Highly Cell-Culture Passaged Strain in Conventional 5-Week-Old Pigs. Viruses, 9(121). https://doi.org/10.3390/v9050121
2. Chiou, H. Y., Huang, Y. L., Deng, M. C., Chang, C. Y., Jeng, C. R., Tsai, P. S., Yang, C., Pang, V. F., & Chang, H. W. (2017). Phylogenetic Analysis of the Spike (S) Gene of the New Variants of Porcine Epidemic Diarrhoea Virus in Taiwan. Transbound Emerg Dis, 64(1), 157-166. https://doi.org/10.1111/tbed.12357
3. Hsu, C. W., Chang, M. H., Chang, H. W., Wu, T. Y., & Chang, Y. C. (2020). Parenterally Administered Porcine Epidemic Diarrhea Virus-Like Particle-Based Vaccine Formulated with CCL25/28 Chemokines Induces Systemic and Mucosal Immune Protectivity in Pigs. Viruses, 12(10). https://doi.org/10.3390/v12101122
4. Hsueh, F. C., Chang, Y. C., Kao, C. F., Hsu, C. W., & Chang, H. W. (2020). Intramuscular Immunization with Chemokine-Adjuvanted Inactive Porcine Epidemic Diarrhea Virus Induces Substantial Protection in Pigs. Vaccines (Basel), 8(1). https://doi.org/10.3390/vaccines8010102
5. Li, Y., Wu, Q., Huang, L., Yuan, C., Wang, J., & Yang, Q. (2018). An alternative pathway of enteric PEDV dissemination from nasal cavity to intestinal mucosa in swine. Nat Commun, 9(1), 3811. https://doi.org/10.1038/s41467-018-06056-w

O-V-011

PRRSV-2 LINEAGE 8 MODIFIED LIVE VIRUS VACCINE IS AS EFFECTIVE AGAINST WILD-TYPE PRRSV LINEAGE 1 AS A PRRSV-2 LINEAGE 1 MODIFIED LIVE VIRUS VACCINE

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[1]*Zoetis*

Introduction:
Porcine reproductive and respiratory syndrome (PRRS) is one of the most important diseases in the swine industry worldwide and despite that current modified live virus (MLV) vaccines confer partial cross protection against porcine reproductive and respiratory virus (PRRSV), they have demonstrated to be efficacious mitigating clinical and economic impact in both, reproductive and respiratory presentations (1). One of the drawbacks of current PRRS MLV vaccines is the unpredictable effectiveness level against PRRSV (1). Prediction of PRRS MLV vaccine efficacy based on genetic homology between the vaccine and field virus is an inadequate practice (1,2). Fostera PRRS®, a lineage 8 MLV vaccine, has showed cross protection against a genetically diverse field viruses including lineage 1, 8 and 9, and PRRSV-2 (2). The objective of this study was to compare two MLV vaccines (PRRSV-2 lineage 8 MLV and a PRRSV-2 lineage 1 MLV) efficacy when challenge with PRRSV-2 lineage 1 (RFLP 1-7-4).

Materials and Methods:
At 2 weeks of age (day 0 of the study), pigs were weaned, and administered either PRRSV-2 lineage 8 MLV (n = 52; single 2 mL dose), PRRSV-2 lineage 1 MLV (n = 50; single 1 mL dose), or sterile water (n = 47; single 2 mL dose) and placed in separate nursery rooms. On day 28, all pigs received a PRRSV 1-7-4 challenge (TCID50: 1 x 104 per 4 mL dose; 2 mL i.n. and 2 mL i.m.) and treatment groups were co-mingled. Blood samples were collected immediately before challenge and 3, 6 and 12 days later to determine viremia via qPCR in serum. On day 40 (12 days post-challenge) pigs were weighed and the percentage of lung lesions was determined.

Results:
Non-vaccinated pigs had the greatest lung lesions ($p<0.001$) compared to pigs vaccinated with either vaccine, which did not differ each other ($p>0.05$). Similarly, average daily gain (ADG) from day 0 (start of trial and vaccination) to day 40 (necropsy) was not different between the vaccinated pigs ($p>0.05$), but it was greater compared to non-vaccinated pigs ($p<0.001$). Table 1. On days 3 and 6 post-challenge, pigs vaccinated with PRRSV-2 lineage 8 MLV had the lowest ($p<0.05$) percentage change in viremia compared to pigs vaccinated with PRRSV-2 lineage 1 MLV and non-vaccinated control group, followed by PRRSV-2 lineage 1 MLV which had lower ($p<0.05$) change in viremia compared to non-vaccinated pigs. By day 12 post-challenge, non-vaccinated pigs had the greatest ($p<0.05$) percentage change in viremia, where both vaccinated groups were similar (Chart 1).

Discussion and Conclusions:
Pigs vaccinated with either PRRSV-2 lineage 8 MLV or PRRSV-2 lineage 1 MLV showed similar protection against PRRSV lineage 1 strain, with reduce lung lesions and greater ADG compared to non-vaccinated pigs. These results add to an extensive database demonstrating cross-protection of Fostera PRRS to different PRRSV strains, not just the lineage from which

it was derived and confirms the lack of correlation of homology of vaccine and cross protection against field viruses.

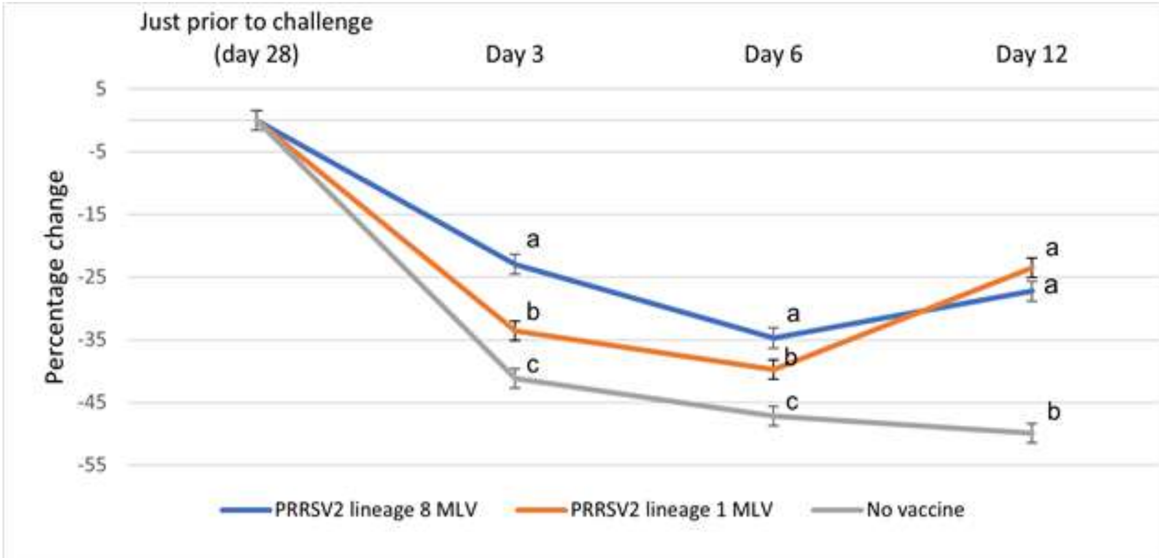
References
1 Chae C. Vaccines. 2021. Feb 22;9(2); 185.
2 Rawal G. et al. Research in Vet Science. Vol 150, 2022, Pg. 170-178

Table 1

	PRRSV-2 lineage 8 (n=52)	PRRSV-2 lineage 1 MLV (n=50)	Control (n=47)	P value
*Lung lesions%	2.55 ± 0.82 [a]	1.60 ± 0.66 [a]	20.03 ± 2.16 [b]	< 0.01
**ADG	0.33 ± 0.05 [a]	0.34 ± 0.05 [a]	0.27 ± 0.06 [b]	< 0.01

*GMeans (SEM) presented. **LSMeans (SEM) presented. Fixed effects of treatment, sex and the interaction of treatment x sex, and the random effects of room, pen (room) and the residual error

Table 2



O-V-012

T CELL EPITOPE COMPARISON OF GLOBAL PCV2 STRAINS FROM 2017-2021 CONFIRMS RELEVANCE OF A BIVALENT VACCINE

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[1]Zoetis Inc, [2]EpiVax Inc, [3]Zoetis Inc, [4]University of Georgia

Introduction
Of the 9 proposed PCV2 genotypes, PCV2a-I, PCV2a/b/d are clinically relevant. The economic impact of PCV2 and associated disease is substantial; PCV2 vaccination is routine. Until recently all vaccines were based on PCV2a, reflecting the predominant genotype when they were developed. Whether current vaccines will remain effective is obviously a concern given PCV2's high capacity for genetic change, potentially including evolution of strains less susceptible to vaccine-induced immunity. The non-specific nature of PCV2's impact on health and productivity, may make it difficult to determine whether vaccine-induced protection is optimal.
In silico tools can be used to predict the T cell epitope content of the PCV2 capsid in vaccines and field strains, providing information on T cell epitopes held in common. Further, PCV2 T cell epitopes predicted to bind putative class I and class II Swine Leucocyte Antigen (SLA) can be expressed as an Epitope Content Comparison (EpiCC) score, reflecting the degree of antigenic relatedness of those PCV2 sequences which might actually be recognized by the pig (1). Previous work demonstrated EpiCC scores for specific monovalent PCV2 vaccines were highest when compared to field strains of the same genotype (2) with the more complete match suggesting that vaccine efficacy may be preserved or enhanced. A bivalent PCV2a/PCV2b vaccine gave biologically superior protection to PCV2a or PCV2b monovalent vaccines in a series of experimental challenge studies (3). In this study, we use EpiCC to compare putative T-cell epitope content of 4 PCV2 vaccines to a larger and more recent population of field strains, all associated with herds affected by PCVAD and analyzed by geographic origin.

Materials and Methods
PCV2 ORF2 nucleotide sequences (n=746) from diagnostic submissions dating 2017-2021 were analyzed. These comprised of 36% sequences from Europe, 32% from North America, 25% from Asia and 8% from South America, 17% being PCV2a, 15% PCV2b and 68% PCV2d. DNA sequences were translated to amino acid sequences, screened for class I and II T cell epitopes, and using the EpiCC algorithm, compared to those of 4 vaccines. The non-parametric Wilcoxon test for paired samples was used to determine whether the EpiCC score for each PCV2 genotype was significantly different between pairs of vaccines. P-values <0.05 were considered significant.

Results
PCV2d predominated globally with co-circulation of PCV2a and PCV2b. PCV2a showed the greatest between-region variation; variation was smallest for PCV2d. EpiCC scores for the bivalent vaccine were significantly higher than for the monovalents for all genotypes in all regions, showing global relevance of the bivalent. Of most practical relevance, given that most commercial vaccines are based on PCV2a, the addition of PCV2b to PCV2a increased T cell epitope coverage by 2.1%, 33% and 21% for PCV2a, PCV2b and PCV2d respectively.

Conclusions and Discussion

The addition of PCV2b to PCV2a in a single vaccine enhanced T cell epitope coverage against a, b and d field isolates, suggesting that the bivalent vaccine provides value in countering genetic diversity within as well as between genotypes. The impact was consistent across regions, and taken together with recent publications (3,4), suggests a bivalent vaccine may result in enhanced and longer-term efficacy in the face of continuing virus evolution.

References

1. Gutiérrez, et al., 2017. Influenza Resp Viruses
2. Bandrick, et al., 2020. Vet Immunol Immunopathol
3. Bandrick, et al., 2022. Vet Res
4. Um, et al., 2021. Vaccines

O-B-001

COMPARISON OF THE EFFICACY OF SWINE ATROPHIC RHINITIS VACCINES IN JAPAN BY DISTRIBUTION OF NASAL TURBINATE LESION SCORES

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Introduction
Swine atrophic rhinitis (AR) is caused by Bordetella bronchiseptica (Non-Progressive Atrophic Rhinitis) and its coinfection with toxigenic strains of Pasteurella multocida type D (Progressive Atrophic Rhinitis). AR is a respiratory disease associated with nasal turbinate hypoplasia and atrophy, often leading to coinfection with other pathogens [1]. As of 2022, a total of seven kinds of AR vaccines from five different companies are available in Japan and widely used in Japanese farms. Although the AR vaccine administration rate in Japan is very high at about 80%, there isn’t enough data comparing the efficacy among vaccines.
The purpose of this study was to investigate the distribution of nasal turbinate lesion scores and to compare the efficacy among AR vaccines in Japan.

Material and Methods
A total of 257 animals were randomly selected from a total of 26 farms; 23 farms were vaccinated with AR vaccine, whereas 3 farms were not. Five kinds of vaccines were used on the farms. Nasal turbinate cut at the slaughterhouse was evaluated using AI DIAGNOS tool [2] to score nasal turbinate lesions.

Results
The nasal turbinate lesion scores in the 257 animals showed a normal distribution with a peak at the score of 4 equivalent to mild lesion (Figure 1). Turbinate lesion scores were the highest for Vaccine C (mean 9) and the lowest for Vaccine D (mean 3.15), respectively (Fig.2). Vaccine D significantly reduced the nasal lesion score compared to those of the other vaccines, but there was no significant difference in the presence or absence of nasal turbinate lesions.

Discussion
The AR vaccine penetration rate is very high in Japan and the number of farms with PAR clinical symptoms is low, therefore, the awareness of AR countermeasures has declined. In this study, however, it was confirmed that AR lesions still exist in a certain proportion in most of pigs and farms.
The importance of AR countermeasures should be reaffirmed because the damage to the nasal turbinate caused by AR facilitates coinfection with other important pathogens such as Streptococcus suis [3]. On the other hand, the results of this study suggested that there were differences in the degree of nasal turbinate lesions among the five kinds of AR vaccines used in this study. Therefore, it is recommended to select a vaccine suitable for each of the farms considering the management style, AR contamination status in the farm, etc.

Reference

[1]Bordetella bronchiseptica and respiratory disease in swine: an overview. Hau, S.J., Brockmeier, S. 2019. The Pig Site. Review Article. p.1-21

[2] Jordà, R. Et al. IPVS 2022, Oral presentation.

[3] Vötsch, et al. Virulence 2020, Vol. 12, No. 1, 84–95

Graphs or Images 1

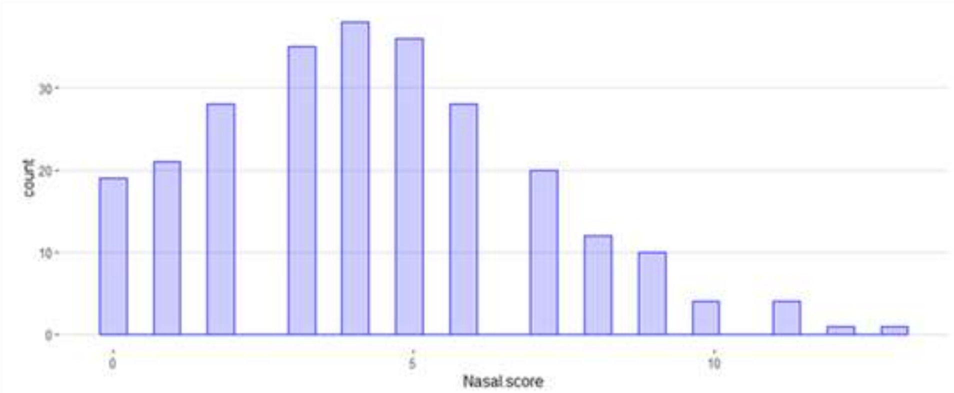

Figure 1. Distribution of nasal turbinate lesion score

*Mild: Nasal turbinate lesion score from 0 to 4 on the European Pharmacopoeia score, moderate: nasal turbinate lesion score from 5 to 8, and severe: higher than 8

Graphs or Images 2

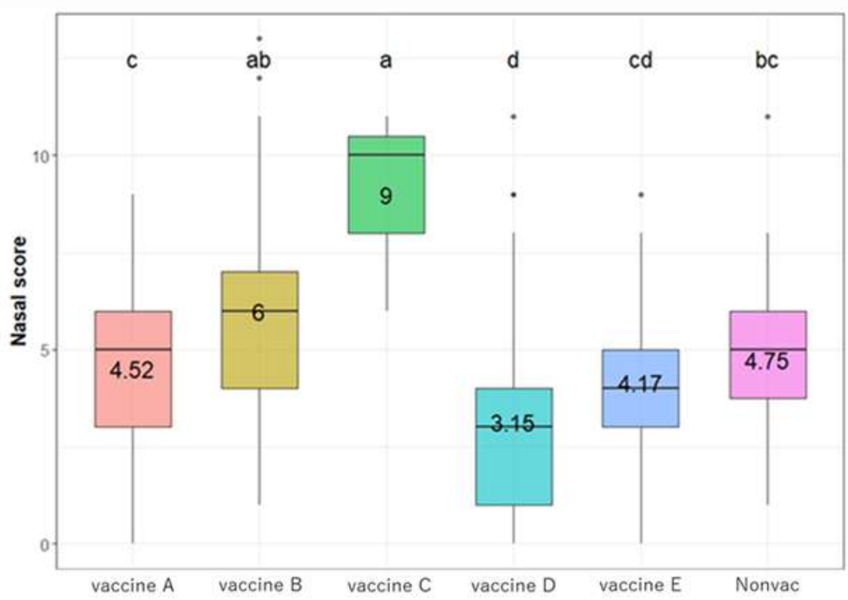

Figure 2. Nasal Turbinate Lesion Score comparison by vaccine

O-B-002

DYNAMICS OF LAWSONIA INTRACELLULARIS INFECTION IN PIG FARMS IN SOUTHERN VIETNAM

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Introduction:
Ileitis (Porcine Proliferative Enteritis) is a group of conditions relating to pathological changes in the small intestine in pigs caused by Lawsonia intracellularis, an anaerobic obligate intracellular bacterium, and having negative impacts on production. Despite high prevalence of the disease worldwide, the success of prevention has been reported to be variable with the use of many methods (vaccination, antibiotic, management change, etc.). To have a better prevention program, the understanding of the infection time is the key point, especially in the situation of Vietnam where information on this disease is very limited.

Materials and methods:
A cross-sectional study about infection of L. intracellularis in pigs of different ages was conducted from June to September 2022 at 10 commercial farms without Ileitis vaccination in the South of Vietnam. At each farm, five categories of pigs including sows, nursing piglets, weaners, growers, and finishers were selected and about 10 blood samples from animals of each category were randomly taken. These blood samples were used to detect Lawsonia-specific antibodies by blocking ELISA (Svanovir L. intracellularis/Ileitis-Ab, Svanova, Uppsala, Sweden) from which the PI (percentage inhibition) values were used to determine the positivity of L. intracellularis infection (cut-off was 30%) and the level of antibody in these samples. Herd prevalence and within-herd prevalence of L. intracellularis infection were calculated. Mixed linear models with PI value as independent variable, week age of pig as dependent variable, and farm identification as cluster variable were built to understand the dynamic of L. intracellularis infection.

Result:
With a total of 381 study animals, the result illustrated that 10 farms (100%) were positive for L. intracellularis and the within-herd prevalence was 43.3% (range: 20.0% - 68.8%). Among categories of pigs, the sow and the finisher were the two most positive categories, 73.9% and 66.7% (Fig. 1), respectively. The most explainable mixed linear model showed that there was a cubic relationship between the week age of pig and PI value as illustrated in Fig. 2. From this model, it was obvious that maternal immunity seemed to be very limited and pigs tended to have seroconverted around week-age 13 (that means the infection might occur 1-2 weeks before) and reach the highest level of antibody at 21 week age, then decline slightly with age.

Conclusions and Discussion:
This finding means that L. intracellularis infection is ubiquitous issue in Vietnam as in other countries (Arnold et al., 2019). Lawsonia-specific antibodies were found in all pig ages. After the natural decay of Lawsonia-specific antibodies in the finishing period, the phenomenon of increasing seropositive in the gilt/sow had been reported in many previous studies, and it was hypothesized that there was reinfection in the gilt or parity-1 sow followed by another

seroconversion (Jacobson et al., 2010). In conclusion, with the very high prevalence of L. intracellularis infection in Vietnam pig farms, pig producers have to seriously take into account this disease and the potential invasion time of this bacteria into pigs might be around the transition time to grower (~ 10-12 weeks) when control measure should be applied.

Acknowledgement and References:
We acknowledge Elanco Vietnam Company Limited and the staff, the farm owners and technicians who are the sponsors and participants in the project.

1. Arnold, M., Crienen, A., Swam, H., von Berg, S., Jolie, R., & Nathues, H. (2019). Prevalence of Lawsonia intracellularis in pig herds in different European countries. Porcine Health Management, 5(1), 31. https://doi.org/10.1186/s40813-019-0137-6
2. Jacobson, M., Aspan, A., Nordengrahn, A., Lindberg, M., & Wallgren, P. (2010). Monitoring of Lawsonia intracellularis in breeding herd gilts. Veterinary Microbiology, 142(3–4), 317–322. https://doi.org/10.1016/j.vetmic.2009.09.034

Graphs or Images 1

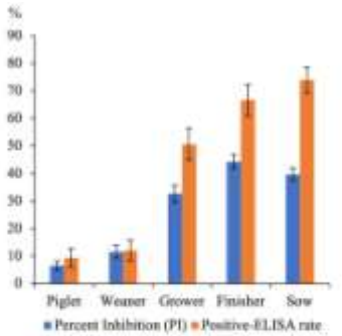

Figure 1. Percent Inhibition and positive rate (with standard error) stratified by pig categories

Graphs or Images 2

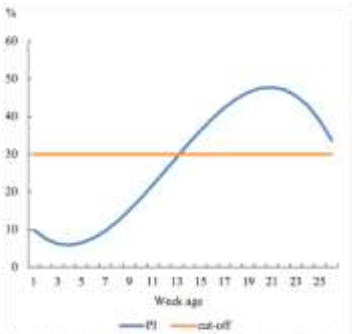

Figure 2. Estimation of PI as antibody level with week age of pigs in a farm) from Mixed linear model

O-B-003

EVALUATION OF POLYMER AND EMULSION ADJUVANTS FOR STREPTOCOCCUS SUIS VACCINATION

Mr. Regis Louis Robert Vialle[1], Ms. Aisa Kong[1], Ms. Aude Puget[1], Dr. Juliette Ben Arous[1], Dr. Nicolas Versille[1]
[1]*Seppic*

Introduction:
Streptococcus suis is a major swine pathogen causing considerable economic losses in the porcine industry. S. suis is an emerging zoonotic worldwide bacteria colonizing the respiratory and digestive tracts. In pigs and humans, S. suis causes meningitis, septicaemia, endocarditis, arthritis, and septic shock with a high mortality. Developing a safe and effective vaccine is the key to preventing and treating S. suis. Inactivated vaccines remain the best strategy today to fight against the disease but their effectiveness is controversial and potent adjuvants are needed.

Materials and Methods
In this study, Montanide™ Gel 02 and Montanide™ ISA 201 VG adjuvants formulated with a trivalent (serotype 1 strain Z1, serotype 2 strain Z2, serotype 7 strain S7) inactivated S. suis antigens are assessed in pig trials.
The safety of the vaccine groups tested was first evaluated by vaccinating pigs intramuscularly with double dose (4 ml) of the trivalent inactivated S. suis vaccine. In this aim, the safety profile induced by vaccines formulated with Montanide™ Gel 02 (polymer) and Montanide™ ISA 201 VG (water-in-oil-in-water emulsion) is compared respectively to alum and a homemade water-in-oil (W/O) emulsion vaccines.

Results
After injection, a very weak increase of the body temperature (less than 1°C at 24 hours) is observed in all groups except in the alum group. The local reactions at slaughter 14 days after vaccination showed that Montanide™ ISA 201 VG provided an improved safety profile compared to W/O emulsion and that Montanide™ Gel 02 demonstrated an excellent safety profile comparable to Alum.
Then, to assess efficacy, pigs were vaccinated with a classical dose of vaccine i.e. 2 ml twice 3 weeks apart. Two weeks after the boost, a challenge is performed by injection of one lethal dose of S. suis (serotype 1 Z1 or serotype 2 Z2). The Montanide™ ISA 201 based vaccine provided highest efficacy among all adjuvants tested, with 100% protection against both challenged S. suis serotypes. The Montanide™ Gel 02 group also showed 100% protection to serotype 2 and a similar protection rate to serotype 1 compared to the homemade W/O emulsion.

Conclusions and Discussion
Taken together, these results showed that Montanide™ ISA 201 VG adjuvant is well suited for formulating highly efficacious S.suis inactivated vaccines, providing high protection and balanced efficacy/safety profile. In addition, the aqueous adjuvant Montanide™ Gel 02 is presenting a high efficacy and an excellent safety profile constituting a good alternative to alum adjuvant.

Acknowlegment and References

Trial in collaboration with Pr Zhao Zhanqin - Henan Science and Technology University, China

Table 1

Table 3
Protection results to challenge test of S. Suis Serotypes 1 and 2 of trivalent inactivated Swine S. suis vaccine candidates formulated with various adjuvants (Montanide ISA 201 VG, Traditional W/O adjuvant, Montanide GEL 02 PR and Alum)

Vaccine groups	Serotype 2			Serotype 1		
	Sick pigs	Mortality*	Protection rate	Sick pigs	Mortality*	Protection Rate
Homemade W/O	2/5	1/5	3/5	1/5	0/5	4/5
ISA 201	0/5	0/5	5/5	0/5	0/5	5/5
Alum	3/5	0/5	2/5	2/5	0/5	3/5
GEL 02	0/5	0/5	5/5	1/5	0/5	4/5
Control	5/5	2/5	0/5	4/5	3/5	1/5

* Pigs of the control group started to die 48 hours after challenge

O-B-004

EVALUATION OF THE PATHOGENIC RESERVOIR IN THE SWINE CECAL MICROBIOME USING SHOTGUN METAGENOMIC APPROACH

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- Introduction
Swine are considered important food animals in Thailand, with the Northern region having the third highest proportion of swine raised after the Eastern and Western regions. Previously, the prevalence of pathogens from swine farms was determined using a traditional method that did not account for the entire range of microorganisms in the intestinal tract. Shotgun metagenomic sequencing, which extracts genetic information directly from a single environment and yields more microbial data, is currently used. This technique can also be used to assess the pathogen reservoir in the swine gut microbiome that can be transmitted to humans. In this study, swine cecal samples were collected from swine raised on commercial farms in Northern Thailand to determine the pathogen burden considering to the public health problem.

- Materials and Methods
Five swine cecal samples were chosen from four commercial farms in Thailand's northern region, including Lampang, Chiang Mai, Chiang Rai, and Uttaradit. (Fig. 1) The intestinal scraping method was used to collect the cecal tissue and contents, and genomic DNA was extracted. The Illumina platform was used for shotgun metagenomic analysis. Following data quality control and de novo assembly, taxonomic classification was conducted using Kraken2 with Bracken. The balloon plot was created in R using the ggpubr package.

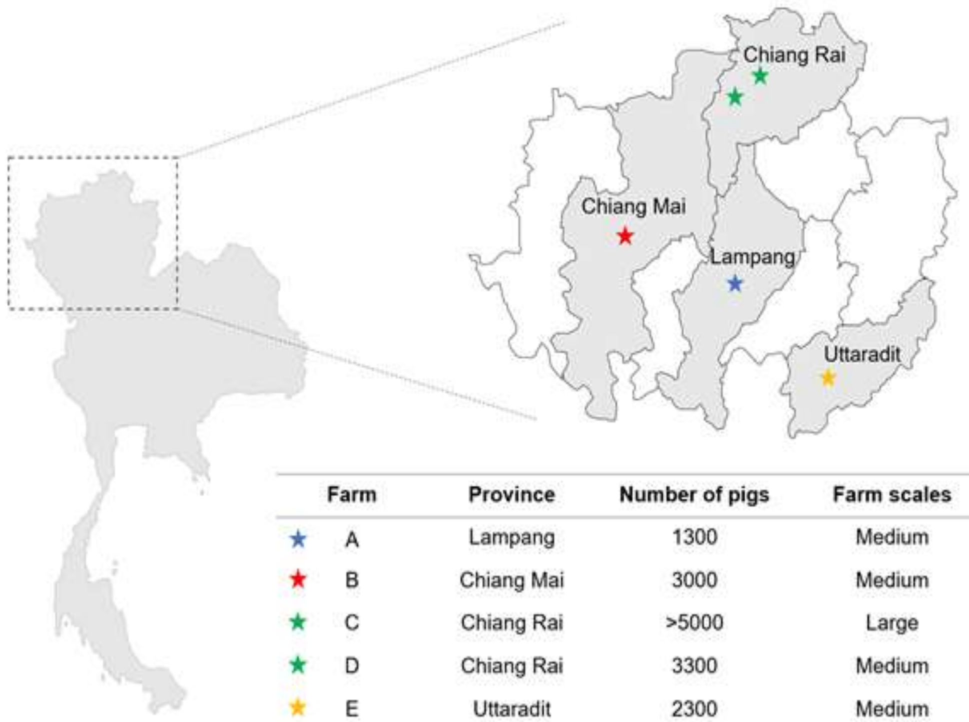
- Results
Each farm contains a diverse range of pathogenic bacteria. Regardless of the province in which each farm was located, Escherichia coli and Clostridium botulinum were the most common pathogenic species on all farms. In addition, pathogenic bacteria were found on each farm, including Corynebacterium species in Farm A and Staphylococcus aureus in Farm B. Even though Farm D contained only one pathogenic bacterium (Escherichia coli), the abundance of this species was greater than that of other species on the farm. (Fig. 2)

-Conclusions and Discussion
This finding revealed several pathogenic bacteria that cause disease in both humans and animals, including Escherichia coli, which was the primary cause of post-weaning diarrhea and edema disease in swine and an important cause of foodborne illness in humans. (Tseng et al., 2014) Furthermore, Clostridium botulinum was also found to be prevalent on every farm. These bacteria were commonly found in the environment and it was considered a food safety hazard. (Myllykoski et al., 2006) Furthermore, Corynebacterium and Staphylococcus, both important opportunistic pathogens, were found in this study. Corynebacterium was commonly found in agricultural animal manure. This pathogen was the most common nosocomial pathogen in patients. (Song et al., 2021; Salem et al., 2015) In addition, previous studies have shown that Staphylococcus aureus, which is found in swine farms environment,

can be transmitted to farm workers and cause illness. (Islam et al., 2020) All of the pathogenic pathogens discovered in this study indicated that swine were the critical pathogenic reservoir and could cause disease in both humans and animals. This approach yielded data that reflected this critical issue and was employed to reinforce the swine farm's standardized sanitation.

- Acknowledgements and References
We would like to express our appreciation to our colleagues at Chiang Mai University. This research project was supported by National Research Council of Thailand (NRCT): NRCT5-RGJ63004-073.
Tseng et al., 2014. Anim Health Res Rev. 15(1): 63-75.
Myllykoski et al., 2006. Int J Food Microbiol. 110(2): 172-177.
Salem et al., 2015. Infect Drug Resist. 21: 129-145.
Song et al., 2021. Environ Sci Eur. 33(1): 1-13.
Islam et al., 2020. Plos one. 15(4): p.e0232456.

Graphs or Images 1



	Farm	Province	Number of pigs	Farm scales
★	A	Lampang	1300	Medium
★	B	Chiang Mai	3000	Medium
★	C	Chiang Rai	>5000	Large
★	D	Chiang Rai	3300	Medium
★	E	Uttaradit	2300	Medium

Figure 1 Geographical distribution and the details of 5 commercial swine farms in this study

Graphs or Images 2

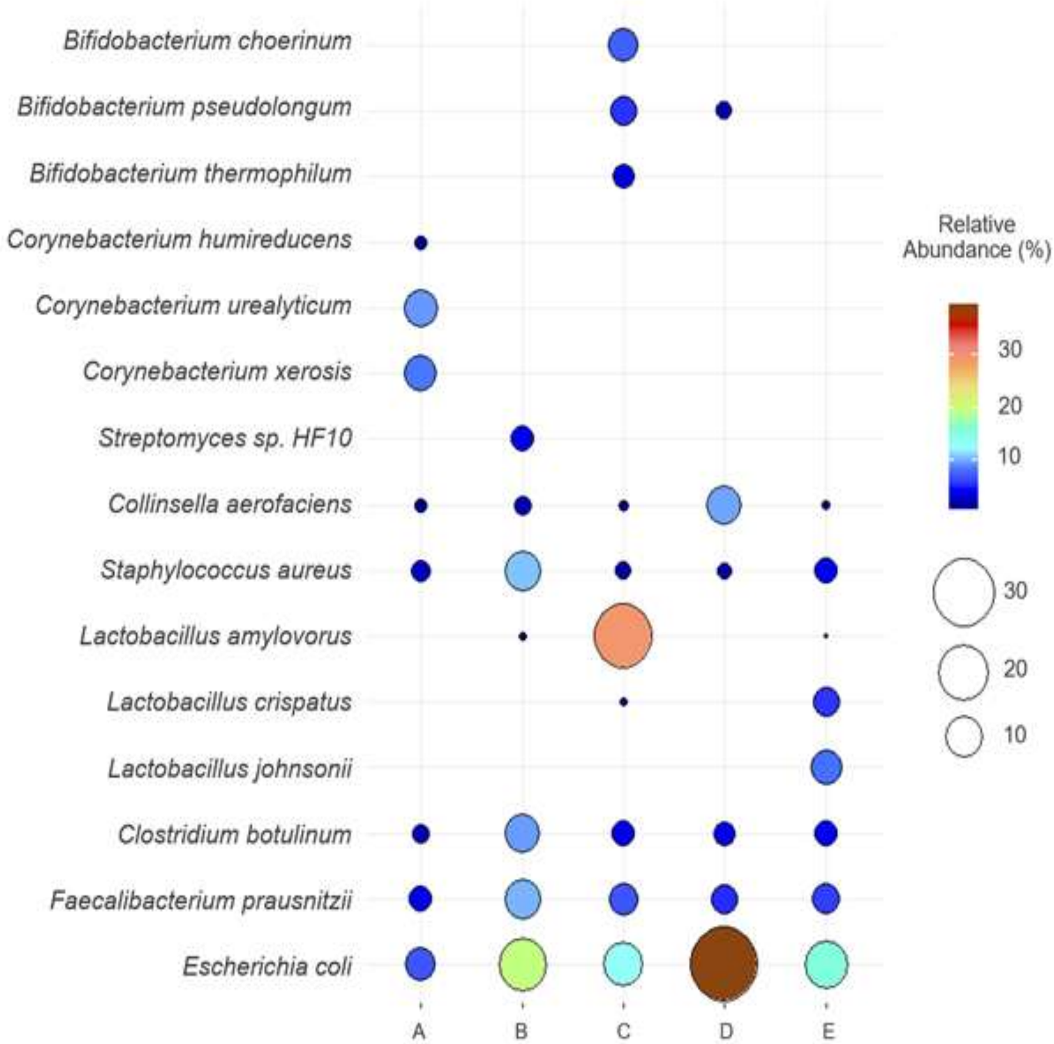


Figure 2 The bacterial taxonomic balloon plot of the relative abundance and diversity of the swine cecal microbiome at species level distributed in each commercial farms.

O-B-005

IDENTIFICATION OF ACTINOBACILLUS PLEUROPNEUMONIAE SEROVARS IN SWINE PLEUROPNEUMONIA OUTBREAKS IN TAIWANESE SWINE HERDS

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Background and Objectives
Pleuropneumonia remains an important challenge to swine production worldwide [1]. Country A. pleuropneumoniae (App) prevalence can change over time. In a 2002-07 slaughterhouse investigation, Yang et al. (2011) identified serovars 1=65.4%, 2=34.1%, and 5=0.5% by slide agglutination in Taiwan [2]. The objective of this study was to identify the acute clinically relevant serovars of App currently present in Taiwan.

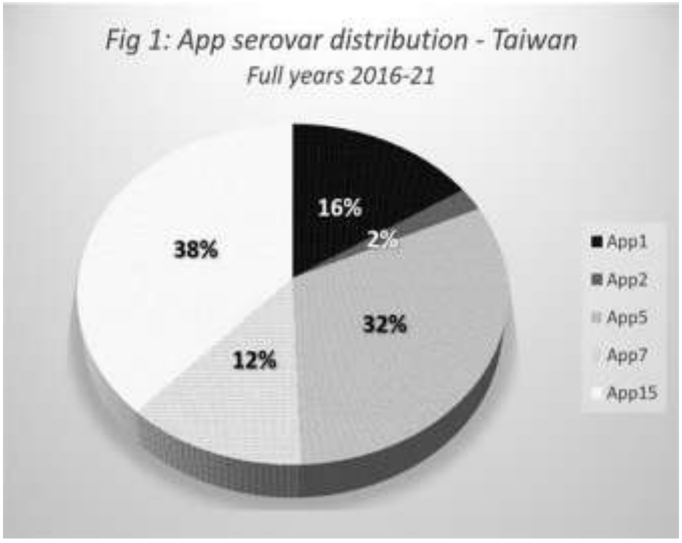
Material and Methods
Ninety-seven samples of lung lesions typical of App infection were collected from 74 pig herds during clinical outbreaks of swine pleuropneumonia in pigs between mid-2015 and February 2022. The distribution of samples/herds per year were: 6/4, 13/10, 15/10, 20/12, 14/12, 15/13, 12/10, and 5/3 respectively, without apparent farm clustering. App isolation was performed at the Animal Disease Diagnostic Center, National Pingtung University of Science and Technology, transferred to FTA-cards, and shipped to Imperial College London. Serotyping, based on capsular loci, was carried out by multiplex-PCR as described by Stringer et al. (2021) [3].

Results
One hundred isolates were recovered from the 97 samples as three were dual-serovar infected: App1+5, App15+7, and App5+7. Five serovars were detected from the 100 App isolates (2015-2022): Serovar 1 (15=15.0%), 2 (4=4.0%), 5 (29=29.0%), 7 (12=12.0%), and 15 (40=40.0%). Looking at 89 isolates of the full years only (2016-2021) distribution is a bit different (Fig 1).
The annual distribution varies greatly, without an obvious tendency, both in terms of serovars and numbers of samples (Fig 2).
One could speculate that two serovars are no longer present in Taiwan: App2 since 2017, and App1 since 2021, but the annual number of samples and farms represented do not allow for such a definitive conclusion.

Discussion and Conclusion
During 2002-07 App1 was predominant followed by App2, and App5. In contrast, during 2015-22 App15, was predominant followed by App5, App1, App7 and App2. As sampling subjects and identification methodologies are different, not all serovars were identified, and annual numbers were relatively small during 2015-22, this does not necessarily reflect a change in true prevalence. However, the quality of data obtained from PCR serotyping is high; at present state-of-the-art, so the identification of App serovars 1, 2, 5, 7, and 15 as causes of clinical App in Taiwan is highly reliable.

References
1. Taylor D.J., Actinobacillus pleuropneumoniae in: Disease of Swine 11th edition (2019) 349-354.
2. Yang C-Y. et al., Serotypes, Antimicrobial Susceptibility, and Minimal Inhibitory Concentrations of Actinobacillus pleuropneumoniae Isolated from Slaughter Pigs in Taiwan (2002–2007). J Vet Med Sci (2011) 205–208.
3. Stringer O.W. et al., Proposal of Actinobacillus pleuropneumoniae serovar 19, and reformulation of previous multiplex PCRs for capsule-specific typing of all known serovars. Vet Microbiol (2021).

Graphs or Images 1



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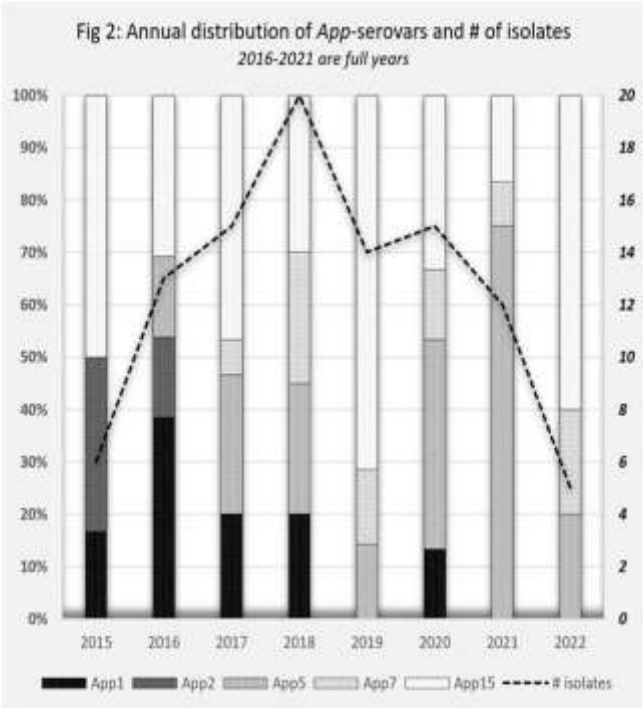

O-B-006

IDENTIFYING AND EVALUATING PATHOGENICITY OF HYBRID SHIGA TOXIN-PRODUCING AND ENTEROTOXIGENIC ESCHERICHIA COLI (STEC/ETEC) IN PIGS

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Introduction
Porcine pathogenic Escherichia coli (E. coli) is an important global recognized pathogen in pigs. Several virulence factors such as adhesins, toxins, lipopolysaccharides, and polysaccharide capsules are used for classification. The Enterotoxigenic E. coli (ETEC), causes porcine neonatal diarrhea and post weaning diarrhea, frequently carries F4 adhesin, F18 adhesin, heat-stable toxin (ST), and/or heat-labile toxin (LT). The Shiga Toxin-Producing E. coli (STEC) produces F18 adhesin and Shiga toxin type 2e (Stx2e) leading to systemic endothelial cells damage and edema disease in post-weaning pigs. In this study, eight isolates of pathogenic E. coli were characterized according to their virulence genes. The pathogenicity of a novel “Hybrid STEC/ETEC” isolate was evaluated by orally inoculation in post-weaning pigs.

Materials and Methods
Virulent genes of eight pathogenic E. coli isolates were determined by multiplex polymerase chain reaction (PCR) and conventional PCR. A Hybrid STEC/ETEC field isolate exhibiting PCR-positive for STx2e, F18, LT, STa, and STb genes was identified and orally inoculated into 4-week-old pigs. Daily fecal consistency scoring and fecal shedding of the Hybrid STEC/ETEC isolate from fecal swab were evaluated for seven days while a pig firstly appeared severe watery diarrhea (score 3) in the Hybrid STEC/ETEC inoculated group together with a pig appeared normal in the mock-inoculated group at the same time point (2 days post-inoculation; 2 DPI) were euthanized for pathological evaluation. At 7 DPI. all animals were euthanized for pathological evaluation. All experiment procedures performed on the animal were reviewed and approved by the Institutional Animal Care and Use Committee of National Taiwan University (Taipei, Taiwan, NTU-111-EL-00070).

Results
Three of eight (37.5%) hemolytic E. coli isolates were identified as hybrid STEC/ETEC. Animals inoculated with the hybrid STEC/ETEC exhibited severe watery diarrhea at 2 (n=1), 3 (n=1), or 5 (n=2) DPI but no signs of edema disease. Daily fecal shedding of the challenge strain was detected in fecal swabs by PCR targeting the virulent genes. The hybrid STEC/ETEC was successfully re-isolated from intestine of the hybrid STEC/ETEC-inoculated pigs.

Conclusions and Discussion
A high prevalence of hybrid STEC/ETEC was demonstrated in diarrheal pigs in the present study. Animal experiment has confirmed that the Hybrid STEC/ETEC was able to cause watery diarrhea in post weaning piglets. Our results suggest that the hybrid STEC/ETEC should be considered as a new target for vaccine development in controlling pathogenic colibacillosis.

References
1. Bai X, Zhang J, Ambikan A, Jernberg C, Ehricht R, Scheutz F, Xiong Y, and Matussek A. Molecular characterization and comparative genomics of clinical hybrid Shiga toxin-producing and enterotoxigenic Escherichia coli (STEC/ETEC) strains in Sweden. Scientific reports 9: 1-9, 2019.
2. Madoroba, E., Van Driessche, E., De Greve, H., Mast, J., Ncube, I., Read, J., Beeckmans, S., 2009. Prevalence of enterotoxigenic Escherichia coli virulence genes from scouring piglets in Zimbabwe. Trop Anim Health Prod 41, 1539-1547.
3. Verdonck F, Cox E, van Gog K, Van der Stede Y, Duchateau L, Deprez P, and Goddeeris B. Different kinetic of antibody responses following infection of newly weaned pigs with an F4 enterotoxigenic Escherichia coli strain or an F18 verotoxigenic Escherichia coli strain. Vaccine 20: 2995-3004, 2002.

O-B-007

MONITORING PLEURISY BY LUNG LESION SCORING IN A COMMERCIAL SWINE FARM IN THE PHILIPPINES

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INTRODUCTION

Pleurisy (or pleuritis) is a chronic lesion seen on lungs due to pathogens like Actinobacillus pleuropneumoniae and these firm adhesions may take months to develop.1 Often pleurisy lesions are seen at slaughter; lung lesion scoring (LLS) provide valuable information on the incidence and severity of swine respiratory pathogens on the farm, can be correlated to farm data and can complete the animal health program assessment. LLS gave information when monitoring changes following the implementation of the medication program. Calculation of disease cost further guided the veterinarian's decision in treatment management.

MATERIALS AND METHODS

A commercial farm in the Philippines used in-feed Pulmotil® medication to address respiratory disease in the herd. Twelve LLS activities were done across several months for a period of three years. Score system used was the Slaughterhouse Pleurisy Evaluation System or SPES.2 Cost of disease calculations were based on farm production data related to pleurisy.3 The Dorsal Pleurisy Index (DPI) was calculated and classified a specific group of swine farms into red (severe), orange (high), yellow (moderate) and green (normal) and pleurisy prevalence was also noted.

RESULTS AND DISCUSSION

Baseline LLS1 was done before adding Pulmotil® (tilmicosin) to the grower feeds at 2 kg/ton for 21 days. LLS2-4 showed the gradual decrease in the number of lungs that had pleurisy, transitioning from red to orange and lower prevalence down to 80%. The farm decided to use a generic tilmicosin and LLS5 showed a resurgence of lesions. Pulmotil® in grower was used again with LLS6-7 and it had moved to yellow DPI withlower prevalence of 30-50%. LLS 8-9 showed the DPI at orange with Pulmotil® in both grower as treatment and lactation diets for control approach. LLS10 showed a red DPI due to removal from grower and it increased further when the farm opted to use a generic tilmicosin in the grower feed during a respiratory disease break in LLS11 with a high prevalence of 90%. Pulmotil® was returned and used in grower feeds and was eventually discontinued but remained in the lactating feed with evidence of improvement with yellow DPI as seen in LLS12.
The cost of pleurisy was calculated mainly from the mortalities due to the acute cases in the farm correlating with prevalence. The effect of pleurisy on ADG, slaughter weight and slaughter age was also calculated. Disease cost was below USD5 when the farm had a yellow status, below USD10 if orange and higher than USD15 when in the red.

CONCLUSION

LLS is an effective tool for monitoring swine respiratory diseases that show pleurisy as chronic lesions. It alerted the farm veterinarians about the efficacy of their medication programs and the cost of disease incursions.

REFERENCES:

1. VanAlstine, WG. Chapter 21. Respiratory System. Diseases of Swine 10th ed.
2. Dottori, M., Evaluation of pig pleuritis at the slaughterhouse available on: Evaluation of pig pleuritis at the slaughterhouse - Articles - pig333, pig to pork community, 2013
3. Tucker A.W., McKinley T.J., Jaeger H.J., 2009: BPEX Pleurisy in Pigs: Associated risk factors and impact on health, welfare and performance. Department of Veterinary Medicine, University of Cambridge

Graphs or Images 1

Figure 1: Cost of Pleurisy

O-B-008

PHARMACOKINETIC / PHARMACODYNAMIC RELATIONSHIP OF VETMULIN® / RODOTIUM® (TIAMULIN) INJECTION FOR THE TREATMENT OF MYCOPLASMA ARTHRITIS

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Introduction
Mycoplasmal arthritis in pigs can lead to significant economic losses due to lameness, swollen joints and poor growth. Curative intervention with antibiotics is the standard measure for Mycoplasmal arthritis treatment. Arthritis treatment requires a sufficient distribution of the antibiotic into the joint fluid.
Vetmulin®/Rodotium® (tiamulin Huvepharma TM) Injectable is registered for therapy of Mycoplasmal arthritis infections. The pharmacokinetic (PK) behaviour of tiamulin after parenteral administration was investigated and the synovial fluid concentration (SFC) related to the minimum inhibitory concentration determined by the testing of Mycoplasma hyorhinis (Mhr) and Mycoplasma hyosynoviae (Mhs) isolates.

Materials and Methods
Tiamulin plasma concentrations and SFC (beside other specimen) were determined in a pharmacokinetic study with forty healthy pigs (mixed breed, equal male/female, age 3-7 days) injected once with tiamulin base at 15mg/kg bw. Plasma and synovial fluid concentrations were determined at pre-defined time points over 24 hours.
Prudent use of antibiotics requires susceptibility testing of antibiotics.
Mycoplasma-specialised laboratories worldwide conduct MIC testing of Mhr and Mhs strains to provide MIC data for antimicrobials used for arthritis treatment. The results of these Tiamulin MICs, based on testing of Mhr and Mhs strains generated globally, are used for PK/PD relationship determination. The tiamulin MIC data were published between 2004-2022.

Results
The tiamulin SFC recorded in the pharmacokinetic study after one parenteral administration of Vetmulin®/Rodotium® shows a consistent plateau concentration between 2-12 hours p.i. (average 0.6 µg/ml). A peak (Cmax) of 0.77 µg/ml was determined 4 hours p.i. Plasma tiamulin concentration was higher with a mean ratio plasma:SFC of 1.6:1.
The tiamulin MIC_{90} values determined for Mhr and Mhs strains were low with a slightly broader tiamulin MIC_{90} range for Mhr strains (0.097 – 0.39µg/ml) in comparison to Mhs strains (0.049-0.25µg/ml).
The tiamulin synovial fluid concentration exceeds the tiamulin MIC_{90} values for approximately 24h (recommended dose interval). The good distribution of tiamulin into the joint fluid and tiamulin joint fluid concentrations above the determined MICs provide a condition for an inhibitory or killing effect of tiamulin during the dosing interval.

Conclusion
Vetmulin®/Rodotium® Injectable is the antibiotic product of choice for Mycoplasmal arthritis treatment based on its excellent PK/PD profile.

Table 1

Table 1: In vitro sensitivity (µg/ml) of *M.hyorhinis* and *M.hyosynoviae* strains from different countries and regions to tiamulin (Vetmulin®/Rodotium®)

Author	*Mycoplasma spp.* strain	Country / Region (n=number of strains tested)	Vetmulin®/Rodotium® (Tiamulin) MIC_{90}
Beko et al., 2019	*M.hyorhinis*	Hungary (n=38)	0.312
Rosales et al., 2020	*M.hyorhinis*	Spain (n=48)	0.25
Makhanon et al., 2012	*M.hyorhinis*	Thailand (n=104)	0.39
Thongkamkoon et al., 2011	*M.hyorhinis*	Thailand (n=9)	0.097
Jang et al., 2016	*M.hyorhinis*	Korea (n=12)	0.25
Klein et al., 2022	*M.hyorhinis*	EU (D, H, I, Pol n=76)	0.312
Stipkovits et al. 2004	*M.hyosynoviae*	EU (n=38)	0.25
Makhanon et al. 2012	*M.hyosynoviae*	Thailand (n=8)	0.049

Graphs or Images 1

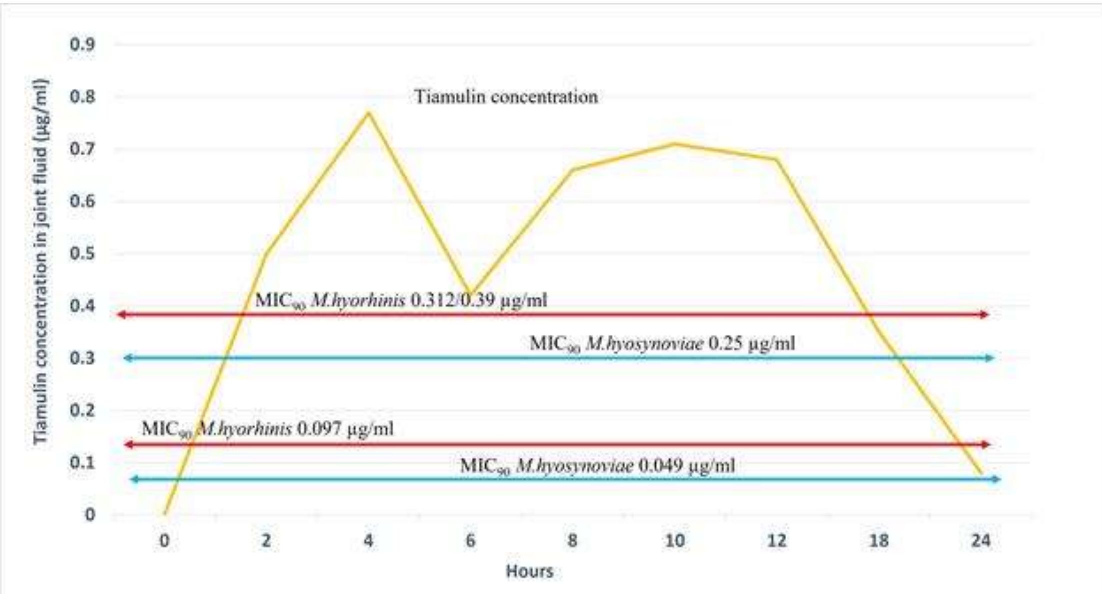


Figure: PK/PD relationship of Vetmulin®/Rodotium® tiamulin on globally generated *M.hyorhinis* and *M.hyosynoviae* strains (arthritis synovial fluid samples)

O-B-009

PLANT-BASED PREMIX OF ADDITIVE IS SOOTHING LAWSONIA INTRACELLULARIS RELATED DIARRHEA SYMPTOMS IN YOUNG FATTENING PIGS

Prof. Dr. Morakot Nuntapaitoon[1,2], Dr. Sawang Katedangsakulwut[3], Dr. Rachod Tantilertcharoen[4], Dr. Napawan Bunpapong[4], Dr. Nanthiya Iampraphat[4], Dr. Suphadtra Therarachatamongkol[5], Dr. Branislav Vejnovic[6], Dr. Marko Vasiljević[7], Dr. Jog Raj[7], Dr. Jasna Bosnjak-Neumüller[7]

[1]Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, [2]Swine Reproduction Research Unit, Faculty of Veterinary Science, Chulalongkorn University, [3]Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, [4]Veterinary Diagnostics Laboratory, Faculty of Veterinary Science, Chulalongkorn University, [5]AmcoVet Co., Ltd., [6]Department of Economics and Statistics, Faculty of Veterinary Medicine, University of Belgrade, [7]PATENT CO. DOO

- Introduction

Lawsonia intracellularis is the highly prevalent cause of diarrhea in growing-finishing pigs, especially in open housing system farms (1). The plant-based feed additive (PBFA) decreased the number of L. intracellularis in pig feces in both open and closed farm systems (2, 3). The objective of this study was to evaluate the effect of PBFA on diarrhea score in open housing system farms where pigs were naturally infected with L. intracellularis.

- Materials and Methods

The trial included 40 fattening pigs (12-week-olds) randomly allocated into two groups including a control group (n=20) and a treatment group (n=20). The pigs in each group were fed a conventional diet (Control) and the same diet supplemented with 2 kg/ton of commercial PBFA (PATENTE HERBA® PLUS, PATENT CO. DOO, Serbia), for 14 days (Treatment). The feces samples were collected and scored on days 0, 7, and 14 after supplementation. The fecal consistency. classified into 3 scores including normal feces, soft and runny, and/or watery feces. The effects of PBFA on fecal consistency were analyzed with the chi-square ($\chi 2$) test. Values with $P < 0.05$ were regarded as statistically significant.

- Results

On day 0 of the trial, there were no statistical differences in fecal consistency between groups. The PBFA show to increase in the percentage of normal feces in the treatment group on day 7 of the trial (Fig. 1). On day 14, the percentage of normal feces was higher in the treatment group (60%) in comparison to the control group (30%) the statistical difference was not observed.

- Conclusions and Discussion

In conclusion, the plant-based feed additive may be used in controlling the diarrhea of pigs associated with L. intracellularis.

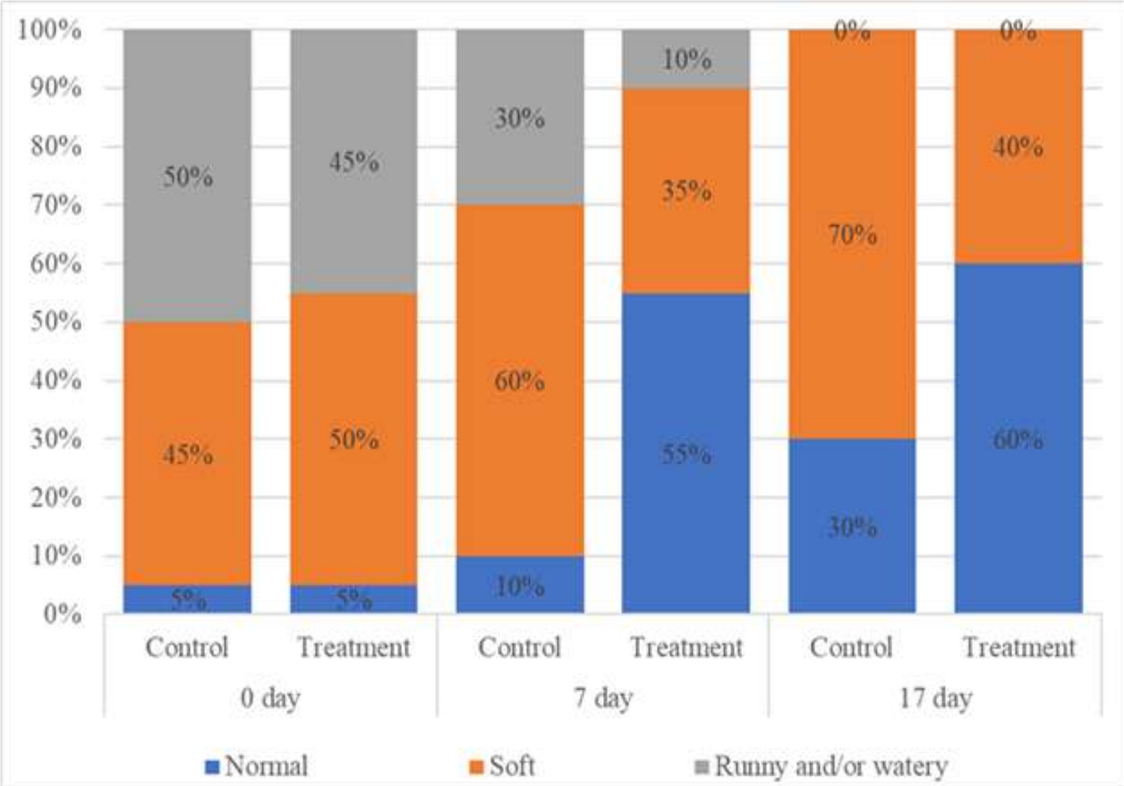
Figure 1. The percentage of feces score (i.e., normal, soft, and runny) during the trial in the control and treatment groups.

- Acknowledgment: In some countries, the premixture of feed additives PATENTE HERBA® PLUS is registered as DYSGUARD-S®.

- References:

1. Nuntapaitoon et al., 2021. Thai J. Vet. Med. 51, 715-722.
2. Nuntapaitoon et al., 2022. IPVS. P.311.
3. Draskovic et al, 2018. Prev. Vet. Med. 151, 46-51.

Graphs or Images 1



O-B-010

PREVENTION OF COCCIDIOSIS WITH TOLTRAZURIL PARENTERALLY IMPROVES INTESTINAL PERMEABILITY

Dr. Daniel Sperling[1], Dr. Francisco Murciano[2], Dr. Ana Gonzalez-Guijarro[2], Dr. Elisa Hernández-Rodríguez[2], Dr. Daniel Serrano[3], Dr. David Espigares[1], Dr. Eva Llamas[2], Prof. Guillermo Ramis[2,3]
[1]Ceva Sante Animale, France, [2]Facultad de Veterinaria. Universidad de Murcia. Spain, [3]Departamento de Sanidad Animal. Facultad de Veterinaria. Universidad de Murcia. Spain, [4]Instituto Murciano de Insvestigación Biomédica (IMIB). Murcia. Spain

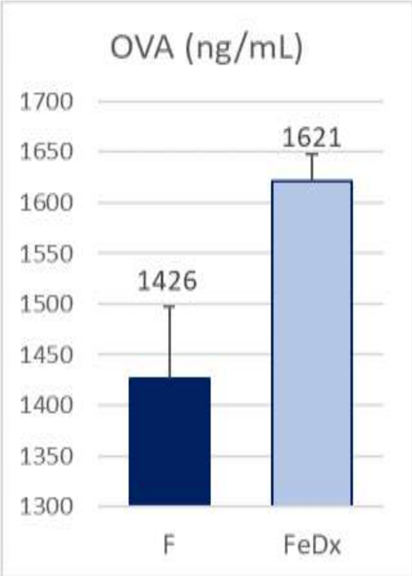
Introduction:
Coccidiosis, caused by Cystoisospora suis and Eimeria sp. in piglets remains a major concern during the neonatal period. It frequently causes diarrhoea and can lead to loss of intestinal integrity with significant variations in intestinal permeability. This can facilitate the passage through the paracellular pathway of undesirable elements such as viruses, bacteria, toxins or undigested protein. The aim of this study was to compare the intestinal integrity of two groups of piglets treated with Forceris® versus a group of piglets that did not receive coccidiostat treatment, by means of fecal samples analysis and OVA absorption test.

Material and methods:
A total of 162 Duroc piglets were randomly included in two groups: the Forceris® group (N=81) received 1.5 mL of this drug containing 45 mg toltrazuril and 200 mg iron per piglet. A control group (n=81) was set up, which received dextran iron parenterally but not coccidiostat by any route. Piglets were housed in 9-piglet pens with 4 place feeders, with feed ad libitum and water by means of a freely disposable water trough.
An ovalbumin absorption test was performed by administering 100g of OVA via a gastric tube and obtaining a blood sample at the time of administration. The amount of OVA was determined by ELISA. The OVA quantitation was compared to fecal gene expression for intestinal integrity and immune activation on samples obtained after prestarter period (PRES) and at the end of nursery (END). Fifteen biomarkers (Zonulin, claudin, occluding, IL1α, IL1β, IL4, IL6, IL8, IL10, IL12p35, IL12p40, TGFβ, IFNα, IFNγ and TNFα) were analyzed for gene expression in feces.

Results:
There was a significant difference for OVA quantification in the two groups (Figure 1), with higher uptake in the Control group than in the Forceris® group ($p=0.001$). Significant negative correlations were found between the occludin, claudin, and IL12b from PRES samples, and significant negative correlations with occludin, zonulin, IL10 and IL6 from END samples.
OVA is absorbed transcellularly, so increased absorption would indicate that there may be altered permeability due to altered TJs (tight junction) and thus abnormal absorption of OVA via the paracellular route. The negative correlation of quantification with gene expression of TJ proteins corroborates this.
Figure 1. Quantification of ovoalbumin (OVA) in blood after administration.

Conclusion:
The use of Forceris® resulted in better intestinal integrity and lower local immune activation compared to control group. The use of Toltrazuril improves notably the intestinal permeability as demonstrated by the OVA absorption test.

Table 1



O-B-011

RESISTANCE RATES TO ANTIBIOTICS OF STREPTOCOCCUS SUIS FROM 2016 TO 2022 IN CHINA

Mr. Jun Zhang, Prof. Liping Wang[2], Dr. Jinhu Huang[2], Mr. Xu Cao[1], Ms. Danielle Ye[1], Dr. Daniel Sperling[3]
[1]Ceva Sante Animale, [2]Nanjing Agricultural University, [3]Ceva Sante Animale

Introduction
Streptococcus suis was considered as an important pathogen of swine causing arthritis and lameness for suckling piglets and mastitis-metritis-agalactia (MMA) syndrome for sows post-farrowing. Clinical infection was frequently controlled by using antibiotics. Drug-resistance could lead to overuse of antibiotics, hindering the swine industry's trend of reducing antibiotic use in China.

Materials and Methods
From 2016-2022, 1,032 samples were collected from different farms with S. suis history and confirmed clinical infection in China. The strains of S. suis were isolated and identified (Fig.1). MICs were determined for 7 antibiotics, frequently used for control and therapy in China. Panel of selected antibiotics consisted of amoxicillin (Amo), penicillin (PNC), enrofloxacin (Enro), florfenicol (Flor), tetracycline (Tet), tilmicosin (Til) and lincomycin (Linco). MIC test was determined by the microbroth dilution method recommended by CLSI and the resistance rate referred to the resistance breakpoint provided by CLSI (2013).

Results
In total, 184 strains of Streptococcus suis were isolated. The MICs of the selected antibiotics were determined and of resistant isolates per individual antibiotic was established(Fig.1). Among the 7 antibiotics, amoxicillin was of the lowest resistance rate, lower than 20%. The resistance rates of tilmicosin (86.96%), tetracycline (92.62%) and lincomycin (98.91%) were very high. Among the 184 isolated strains, resistance rates of florfenicol (38.04%), penicillin (46.74%) and enrofloxacin (46.74%) needed to pay enough attention.

Conclusions and Discussion
Antibiotic resistance for S. suis was already very common and needed to be given high attention in China. Amoxicillin was the most effective antibiotic against Streptococcus suis in-vitrol, florfenicol and enrofloxacin were also effective in some degree. However European Medicines Agency (EMA) took enrofloxacin in category B as restrict, florfenicol in category C as caution. Amoxicillin was taken in category D as prudence (1), low risk, could be used as first line treatments, whenever possible. Farms could take amoxicillin as first choice of metaphylaxis for S. suis infection, enrofloxacin and flofenicol as a back-up option based on the results of MIC testing.

Acknowledgement and References
[1] Categorisation of antibiotics in the European Union, EMA, 2019, Dec.

Graphs or Images 1

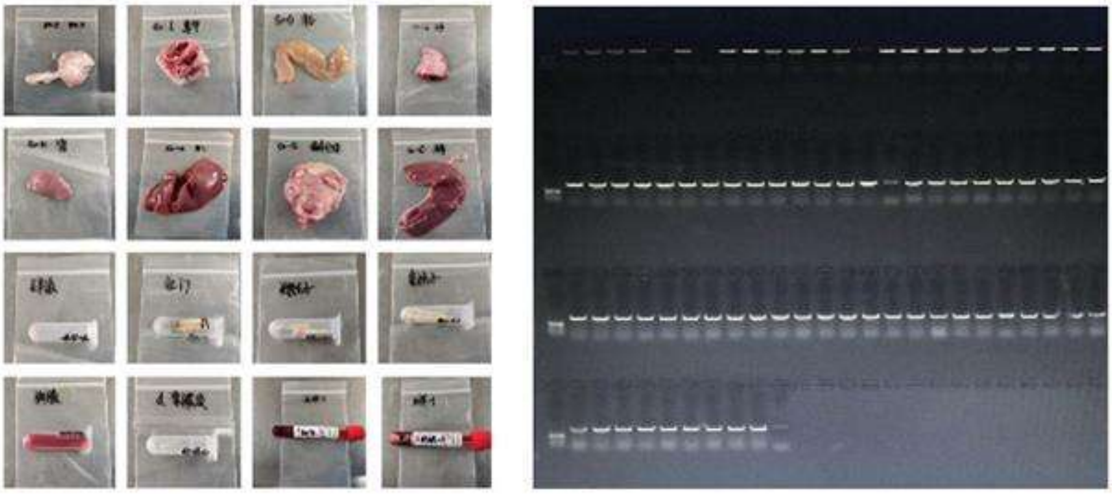


Fig. 1 *S. suis* sample collection and PCR of gdh gene

Graphs or Images 2

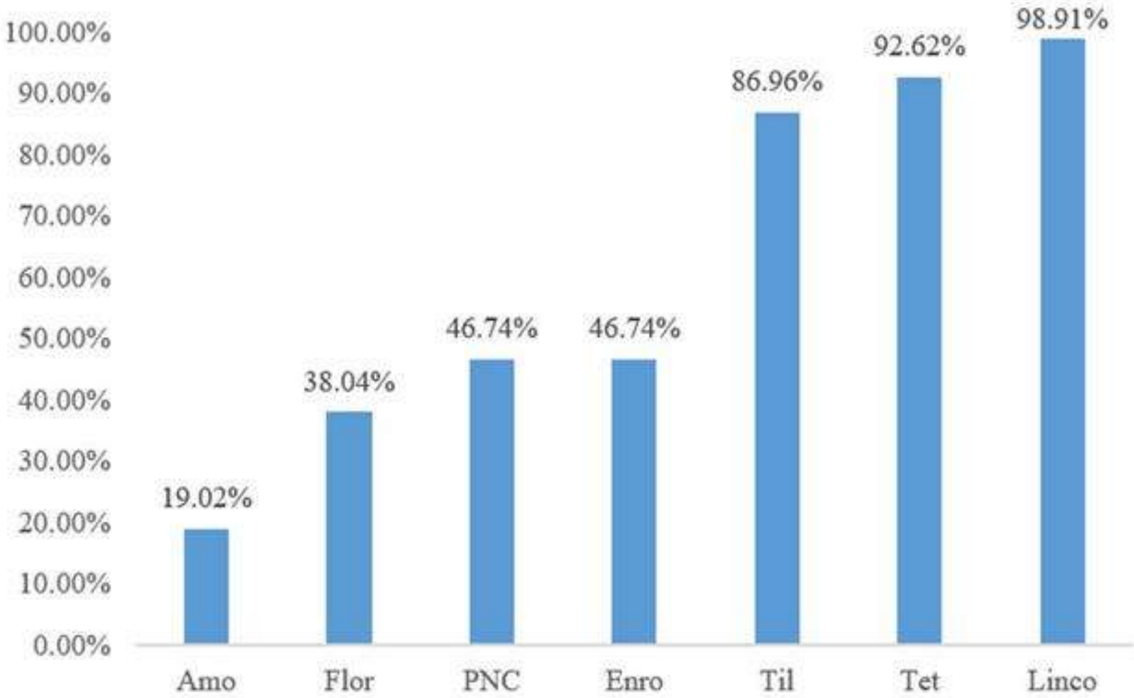


Fig. 2 Resistance rate to 7 antibiotics of *S. suis*

O-N-001

COMPARATIVE EVALUATION OF SYNTHETIC CHOLINE AND HERBAL BIOCHOLINE IN PIGLETS

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[1]Indian Herbs

Introduction

Choline is essential nutrient for swine because it is needed for phospholipid synthesis, cell membrane integrity, lipid transfer and for methylation of homocysteine to methionine (Russett et al., 1979). Choline deficiency in piglets causes poor coordination of movements and fatty liver . Currently, there are few studies on adequate level of choline in piglet diets. Supplementing choline have beneficial effect on fat metabolism, uweight gain & piglet survival (Alexandre et al., 2015). Supplementing choline as choline chloride presents a low availability, trimethylamine formation, high hygroscopicity causing operational problems in feed production due to oxidative loss of water soluble vitamins (Bryant, 1976). Natural choline supplement Biocholine® contains choline as phosphatidylcholine that assists in hepatic metabolism, is trimethylamine free, non-hygroscopic(Alexandre de Mello Kessle, 2015). This trial testifies hypothesis that piglets perform better when supplemented with natural choline source.

Material and Methods

A trial was undertaken at commercial farm at Sanluc, KUleuven, Belgium. 80 weaned piglets with avg. body weight 7.5 kg were randomly allocated into 2 groups of 40 piglet each and reared on standard diet for 6 weeks. Treatment 1 pilgets were fed with standard basal ration supplemented with choline chloride 60%@1kg/ton of ration. Treatment 2 piglets were fed with standard basal ration supplemented with BioCholine by completely replacing synthetic choline at one-fourth inclusion rate of 250g/ton of feed for 6 weeks.

Results

Average daily weight gain and feed intake were significantly better in BioCholine supplemented group. ADG (g) in BioCholine supplemented group T2 was 372.92 ±10.52 than ADG (g) in synthetic choline chloride group T1, 353.42±10.42. Average daily feed intake (ADFI) in group T2 was 448.36 ±11.46 than 523.21±11.46 in group T1. FCR in group T2 was lower 1.472 than FCR in group T1, 1.481.The present trial confirms hypothesis that piglets perform better during post-weaning period when supplemented with choline source. From the results set, it is proven that 250 mg kg-1 of Biocholine® to weaned piglets increased ADG and optimized FCR. For total growth period of 6 weeks, 1 unit of Biocholine® was found to be equivalent to 2.5 units of pure choline supplied by Choline Chloride.

Conclusions and discussion

Addition of Choline to diet of weaned piglets is helpful in better fat mobilization, metabolism and energy production (Mersmann, 1974). The growth & performance results in present are in corraboration with the findings of Bryant, 1976, since addition of choline source has performance parameters better than unsupplemented control group (Cast et al., 1976). It can be concluded on basis of trial findings that Natural choline BioCholine can completely replace synthetic choline chloride at a lower inclusion rate. Inclusion of natural choline BioCholine@250mg kg-1 has positive impact on growth and performance of weaned piglets.

References
Comparative study on zootechnical performance and blood parameters of pigs in the creche phase receiving two honey sources in the diet. Report, Prof. Alexandre de Mello Kessler Prof. Andréa Machado Leal Ribeiro; Execution: Marcos Speroni Ceron Porto Alegre, RS – Brasil 2015
RUSSETT, J. C.; KRIDER, J. L.; CLINE, T. R.; Underwood, L. B., 1979. Choline Requirement of Young Swine. Journal of Animal Science, v.48, p. 1366-1373.
Bryant, K. L. 1976. Effect of supplemental choline on sow and pig performance. M.S. Thesis. Univ. of Florida, Gainesville.
Cast, W. R., B. D. Moser, E. R. Peo, Jr. and P. J. Cunningham. 1977. Fat, choline, and thyroprotein additions to the diet of lactating swine. J. Anita. Sci. 45 (Suppl. 1):80.
Mersmann, H. J. 1974. Metabolic patterns in the neonatal swine. J. Anim. Sci. 38:1022.
Snyder, R. A. 1979. Source of energy for sow and effect on baby pig survival. M.S. Thesis. Univ. of Georgia, Athens.

O-N-002

CORRELATION OF SOUND-BASED MONITORING SYSTEM (SOUNDTALKS™) AND RESPIRATORY HEALTH CONDITION IN SMART PIG FARM IN THAILAND

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Introduction
Precision Livestock Farming (PLF) is a trend for the future of farming. As a result, many producers have installed various types of sensors in the farm to support better decisions regarding animal welfare, management, and responsible use of antibiotics. SoundTalks™ is a sound-based PLF tool which allows producers to detect, earlier and objectively, respiratory clinical signs 24/7 (24 hours a day, 7 days a week) for them to early intervene. The monitors collect the sound data from the animals and transforms it into a metric (0 - 100) that represents the animals' Respiratory Health Status (ReHS). When the ReHS value falls below a certain threshold, the system emits early warnings (yellow/red LED alerts instead of green)[1]. This early signal allows the farm staff to investigate the early cause of the respiratory onset by checking environmental conditions (i.e., temperature, humidity, air flow) and animals individually as well as to initiate diagnostic sampling. Despite all these advantages, such a technology has never been tested under farming conditions in Thailand. Therefore, the objectives of this study were twofold: to describe the respiratory health status based on SoundTalks™ from a sound-monitored production site and to correlate this information with production performance and medication usage.

Materials and Methods
The study was performed in a wean-to-finish farm in Thailand (4 buildings, 1500-1700 pigs/building) from June 2021 – Nov 2022. A total of 32 SoundTalks™ monitors were installed (8/ building). Variables related to health status (i.e., ReHS value, mortality by cause, number of alarms), environment (i.e., T, %RH, wind speed) and treatments (i.e., antibiotic doses/pig) were collected and consolidated for the analysis. Correlation between daily alert status, environment and production performance were analyzed.

Results
A total of 12 batches from 3 production cycles were analyzed for the study. Data collected from one of the batches is represented in Fig 1. An average ReHS of 79 (Max=98; Min=33) or 14% of days in yellow and red alarms were observed during the study.

Results demonstrated a significant correlation between average % green monitors/batch (lack of alarms) and respiratory mortality ($R^2 = 0.583$; p=0.010, Fig 2) as well as antibiotic usage ($R^2 = 0.475$; p=0.019).

Conclusions and Discussion
SoundTalks™ technology allows producers to objectively monitor respiratory health condition in real-time and to provide interventions based on LED status. This totally differs from conventional methods using producers subjective, and experience- dependent evaluation. Results of this study demonstrated the accuracy of the metric allowing producers to improve production performance; decrease antibiotic use (as previously demonstrated by Rathkjen et al.)[2] and to support the job operators to work more precisely under farming conditions in Thailand.

References
1. Alonso, C. et al., 2022. Using a sound-based monitoring of respiratory health status to improve grow-finish production performance. IPVS, 2022.
2. PH Rathkjen et al. 2022. Sensor based monitoring using SoundTalks supports timely and accurate interventions in nursery facilities based on early and accurate diagnostic detection of respiratory pathogens. IPVS, 2022.

Graphs or Images 1

Figure 1. Data collected by SoundTalks™: Respiratory health status (ReHS) graph with the 3 zones (green, yellow, and red) visualized in the dashboard for the producer to intervene.

Graphs or Images 2

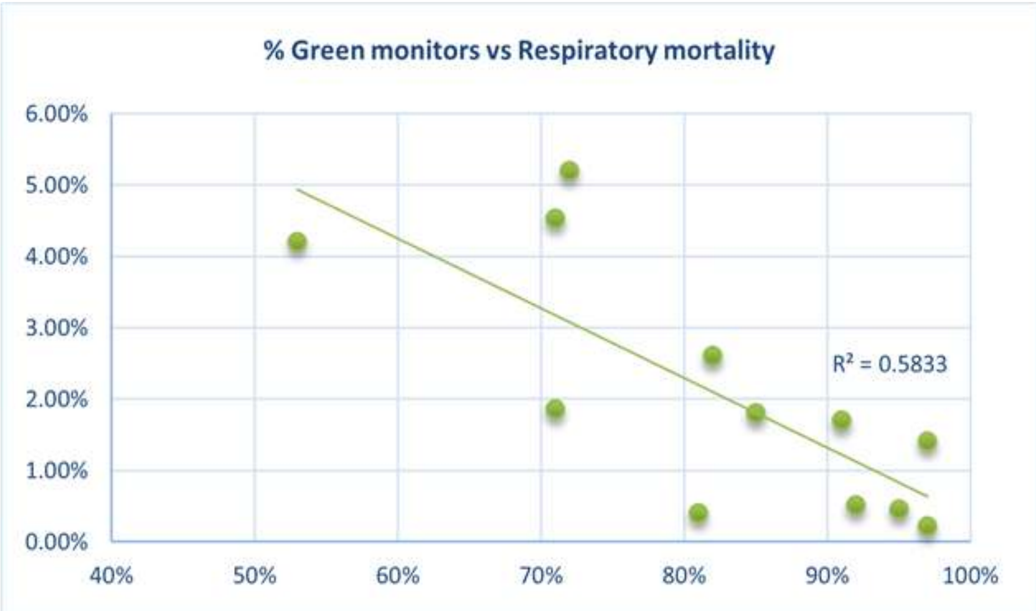

Figure 2. Correlation between % Green monitors vs Respiratory mortality (P = 0.010). X axis = % green monitors, Y axis = % Respiratory mortality, each dot represents each batch

O-N-003

EFFECT OF CATOSAL® ON PRODUCTION PERFORMANCE OF POSTPARTUM SOWS IN THAILAND

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Introduction
The swine industry aims to improve production performance by increasing the number of pigs weaned and minimizing pre-weaning mortality through genetic improvements and various farm management techniques. One of the critical factors is the postpartum recovery time of sows which Catosal® could play a vital role as a tonic and metabolic stimulant. The recovery period significantly impacts the production performance in the farrowing unit. The objective of this study was to evaluate the effect of Catosal® administration on the production performance of lactating sows.

Materials and Methods
The study was carried out on two swine farms in central Thailand with multiparous and primiparous sow herds. A total of 300 sows were included in the study. Two hundred multiparous sows were divided equally into the control (C1) and treatment group (T1). Likewise, one hundred primiparous sows were divided equally into the control (C2) and treatment group (T2). The treatment groups received Catosal® within 24 hours postpartum as a single intramuscular injection of 20 mL per sow, while the control group received the usual farm management. The sow performance, backfat thickness (at 109 days of gestation and weaning day), piglets born alive, birth weight, weaning weight, pre-weaning mortality, and medication cost were recorded and analyzed using the R program.

Results and Discussion
In the multiparous herd, the treatment groups had a lower backfat loss during lactation and significantly higher piglet weaned per litter due to lower pre-weaning mortality than the control group. The treatment group also had lower medication costs. The lower weaning weight observed in the treatment group may be due to higher litter size born alive negatively affecting the birth weight. In the primiparous herd, the weaning weight and average daily litter weight gain of the treatment group were higher than the control group. (Table1)

Conclusion
The Catosal® administration in sow after parturition helps reduce pre-weaning mortality and backfat loss during the lactation period.

Acknowledgment
This work was supported by Elanco Co. Ltd.

Reference
1. Lanfranchi E et al., 2008, Proc IPVS, P 479
2. A Nuntaprasert et al., 2006, Proc IPVS

Table 1

Table 1 Effect of Catosal® on production performance

Data	Multiparous sows			Primiparous sows		
	T1 n=98	**C1** n=98		**T2** n=50	**C2** n=50	
	Mean ± SD		*P*	Mean ± SD		*P*
Sow backfat thickness (mm)						
Gestation (d 109)	19.60±1.19	19.50±1.24	0.1839	22.60±1.69	23.12±1.69	0.1098
Weaning	19.00±1.06	18.88±0.97	0.5076	19.10±1.09	19.80±1.18	0.0063
Gestation to Weaning change (%)	-2.81%	-2.98%	0.7132	-15.41%	-14.36%	0.4415
Piglet performance						
Piglets born alive / litter (n)	12.23±2.72	11.93±2.56	0.4180	12.54±3.49	12.56±3.6	0.8680
Piglets birth weight (kg)	1.53±0.24	1.62±0.29	0.0351	1.49±0.24	1.35±0.27	0.0110
Piglets weaned / litter (n)	11.04±1.82	10.53±1.79	0.0207	11.52±1.01	11.66±1.06	0.4461
Piglet weaning weight (kg)	6.19±1.32	6.49±1.16	0.0373	8.38±1.16	7.22±1.19	<0.01
ADLWG (kg/d)	1.93	1.99		2.53	2.25	
Pre-weaning mortality (%)	9.76	11.72	-	8.13	15.87	-
Lactation length (d)	25.4	25	-	31	30	-
Medication cost/litter (baht)	487.41	1440.67	-	238.22	123.92	-

O-N-004

EFFICACY OF A MODIFIED CLINOPTILOLITE MYCOTOXIN BINDER AGAINST ZEARALENONE AND OCHRATOXIN A CONCURRENT INFECTION IN WEANED PIGS

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Introduction
Mycotoxins are secondary metabolites of certain fungal species that can be found in grains worldwide. Zearalenone (ZEN) is produced by Fusarium species, in grains, and acts by binding to estrogen receptors. It is rapidly absorbed after consumption of contaminated feed and extensive liver phase I and II biotransformation occurs. Phase I metabolism results in α- and β- Zearalenol (α, β- ZEL), α- and β- Zearalenol and zearalenone. Prepubertal gilts are the most susceptible age group to ZEN intoxication (Dänicke and Winkler, 2015). Ochratoxin A (OTA) is produced by Aspergillus and Penicillium species. It is a potent inhibitor of protein synthesis, considered as major nephrotoxic agent inducing porcine nephropathy (interstitial fibrosis and tubular alterations) due to binding of OTA in specific renal organic ion transporters. (Battacone et al., 2010). The aim of this study was to document the efficacy of an in-feed zeolite-based mycotoxin binding substance (Minazel® Plus, Patent Co, Misicevo, Serbia - TP) under concurrent feed contamination with ZEN and OTA, during nursery in pigs.

Materials and Methods
A total of 60 weaned pigs at the age of 28 days were evaluated. The total study period lasted 42 days. Animals were allocated to four treatment groups as follows: Group A: received feed without mycotoxins or the test product; Group B: received contaminated feed with 1 mg ZEN/kg and 0.5 mg OTA/kg without the TP (LC MS/MS actual contamination levels of 0.992 mg ZEN/kg feed and 0.531 mg OTA/kg) ; Group C: received group B feed with 1.5 kg TP /t feed; Group D: received group B feed with 3 kg TP /t feed. Serum biochemical parameters and hematological parameters were evaluated. Vulva length X width measurements (as sign of hyperestrogenism) were performed. Performance data, such as body weight (BW)/week, feed conversion ratio (FCR) and average daily gain (ADWG). Residues of ZEN, α-ZEL, β-ZEL and OTA in kidney and liver samples collected at the end of the study period were measured.

Results
Group D showed numerical improvement of ADWG and FCR mean values in comparison to Group B, reaching 0.47 kg greater mean body weight than group B at the end of the study (Fig. 1). The ADWG values of group D at the second part of the study were almost similar to the control group (571.43 Vs. 569.84). Significant reduction of alanine aminotransferase (ALT) mean values, which is usually used as indicator of hepatic pathology, was observed in both groups C and D in comparison to group B, though within normal limits. A significant reduction was observed in the mean values of bZEL in kidneys samples in group D (P = 0.005). Moreover, a tendency for significant reduction of the total bZEL mean values in the total samples' evaluation was present (P = 0.070). Reduction of residues reached maximum of 28.01% for total ZEN and metabolites in kidneys in group D when compared with group B.

Conclusions and Discussion
Though not always statistically significant, the dosage levels used in group D showed an overall greater beneficial effect than group C. Indications of a positive effect of the TP in the dosage regime of group D was obvious in mycotoxins residues reduction when compared with group B.

Acknowledgement and References

- Battacone G, Nudda A, Pulina G. Effects of ochratoxin a on livestock production. Toxins (Basel). 2010 Jul;2(7):1796-824. doi: 10.3390/toxins2071796.
- Danicke, S.; Winkler, J. Diagnosis of zearalenone (ZEN) exposure of farm animals and transfer of its residues into edible tissues (carry over). Food Chem. Toxicol. 2015, 84, 225–249, doi:10.1016/j.fct.2015.08.009.

Graphs or Images 1

Efficacy of a modified clinoptilolite mycotoxin binder against zearalenone and ochratoxin A concurrent infection in weaned pigs

Figures

Figure 1

ADWG mean values among treatment groups for the total study period

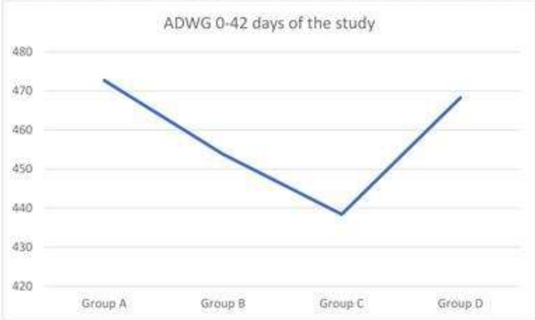


Figure 2

Mean ZEN and metabolites residues values in tissues samples among treatment groups

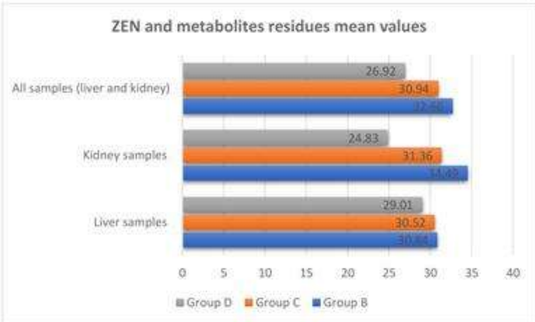
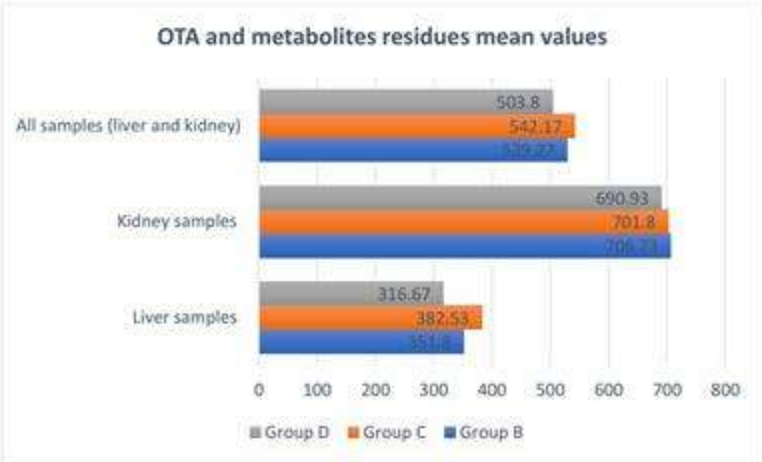


Figure 3

Mean OTA and metabolites residues values in tissues samples among treatment groups

Graphs or Images 2



O-N-005

EFFICACY OF HUMIC ACIDS SUPPLEMENT ON LACTATING PIGLETS FEED IN THAILAND

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Introduction
Humic acids (HA) are three dimentional macrocolloidal molecules resulting from decomposition of organic substance. Their functional groups are carboxylic (-COOH) and phenolic hydroxy (-OH) groups. In nature, HA and their salts (sodium or potassium) are in water, soil, and lignite (brown coal). HA and their sodium salt (so-called Sodium Humate-HNa) can be administrated orally in all food animals for the control of diarrhoea, dyspepsia, and acute intoxication from their protective action on intestinal mucosa. Furthermore, HA and HNa have anti-inflammatory, antitoxic, and antimicrobial properties when supplement to animals. Moreover, HA and HNa contain the nutritional value including protein, fiber, carbohydrate and trace minerals in chelated form (1, 2, 3, 4). This study aimed to evaluate the efficacy of HNa supplement in piglet feed during lactation in a Thai pig farm having high incidence and loss from diarrhoea although there was antimicrobial treatment frequently.

Materials and Methods
Studying in lactating unit, 198 sucking piglets from 17 liters were divided into two groups. 91 piglets in control group (C) were fed with normal liquid feed as the conventional practice in farm while another 107 piglets (HNa) were supplement by HNa 2kg/ton dry feed (equi. To HA 1.2kg/ton dry feed) in the conventional liquid feed from 5-20 wk old (16 days). During supplement feeding, clinical diarrhoea signs of individual piglets were recorded from the first sign until recovery. All pigs were weighted at birth and the weaning day. Statistical analysis by t-test and chi-square at $P<0.05$, were calculated as well as risk by ODD ratio.

Results
Results of study are illustrated in table 1-2. Diarrhoeal are 28.8% and 18.2% and loss&cull piglets are 19.5% and 8.5% from Control and HNa supplement groups, respectively. Recovery days from diarrhoea and mean diarrhoeal days from HNa supplement group are less than Control group, $P<0.001$ (table 1). ODD ratio is 3.306 (95%CI:1.844-5.920), $P<0.001$ (table 2).

Conclusions and Discussion
According to this study, HNa feed supplement for lactating piglets from 5-20 days old provides the significant less incidence of diarrhoea and faster recovery. The ODD ratio is represented the significant risk of diarrhoea in non-supplement group (control) at 3.306 times more than the HNa supplement group.
In conclusions, HNa 2kg/ton feed (HA 1.2 kg/ton feed) supplemented during sucking period of piglets can be an alternative to antimicrobial to reduce risk and incidence of piglet diarrhoea.

References
1. EMEA/MRL/554/99-FINAL, February 1999
2. Makhanon et.al., 2019, The 3rd ATA 2019 Proc., IN2, p.121

3. Makhanon et.al., 2020, Thai J Vet Med, 50 (Suppli.):245-246
4. Visscher et.al., 2019, Front. Vet. Sci., 6:29

Table 1

Table 1. Recovery days from diarrhoea and mean diarrhoeal days

Group	N wean (pigs)	Recovery days		Mean diarrhoeal days
		2 days	3 days	
C	91	13 (14.4%)	44 (48.4%)	1.74±1.39
HNa	107	36 (33.6%)	0	0.67±0.95
P-value		<0.001*		<0.001**

*Chi-Square test; **t-test

Table 2

Table 2. Risk of diarrhoea

Risk	N. of pigs (%)	
	Diarrhoea	Normal
No HNa (C)	57(28.8%)	34 (17.2%)
HNa 2kg/T (16d)	36 (18.2%)	71 (35.9%)
ODD	3.306 (95% CI:1.844-5.920)	
Fisher's Exact test	*P*<0.001	

O-N-007

MAINTAINING THE PERFORMANCE AND HEALTH OF NURSERY PIGS UNDER INCREASING REGULATORY PRESSURE ON WHAT WERE STANDARD PROCEDURES

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[1]Jefo Nutrition Inc., [2]Rivalea

The literature is littered with papers reporting the potential of various additives as replacements for growth promoting antibiotics and high levels of ZnO. The former are largely banned from use in pork production and the latter is being increasingly restricted. The problem with most of the published studies is that they are of small scale, generally poorly designed and few if any have been validated under commercial situations where performance and health can be adversely affected especially in the first 7-10 days after weaning. This paper deals largely with what has happened commercially and the results of industry-based experiences to reduce the level of ZnO in first stage nursery diets and what has proven to be reliable alternatives.

Commercial studies were conducted to investigate the use of a microencapsulated ZnO and an exogenous multi-component protease as replacements to high level supplementation of ZnO. Two large integration pork production companies in Australia conducted studies in newly weaned piglets where high doses of ZnO (2,500-3,000 ppm) were replaced with a microencapsulated ZnO product at 400 ppm (Jefo Zinco PLUS S). The results showed no difference in growth performance and incidence and severity of post-weaning diarrhoea between the two groups. Similarly, studies conducted in two commercial farms in Canada and Colombia revealed that the supplementation of a microencapsulated ZnO (400 ppm) can be a good alternative to high level use of free ZnO (3000 ppm) in weaned piglets. Microencapsulated ZnO worked equally in reducing the incidence of diarrhoea but promoted better performance in terms of average daily gain (5% improvement) than piglets fed with high levels of free ZnO. The lower inclusion rate also resulted in decreased Zn excretion in the manure (75% reduction vs free ZnO). The microencapsulated ZnO used in these studies is made with a matrix of triglycerides designed to facilitate the targeted release of ZnO in the small intestine where it exerts its gut-health promoting effects. Correspondingly, feed mills in Asia conducted research with Dankook University using nursery diets reduced in CP (-3% unit CP) without high level of ZnO (-2,400 ppm) but supplemented with high doses (500-700 ppm) of a multi-component protease (Jefo Protease). In this 5-week study, piglets fed low CP diets with protease had better overall performance (474 g vs 442 g ADG; 1.37 vs 1.42 FCR) than piglets fed standard diets with pharmacological ZnO. Accordingly, nitrogen digestibility (79.0 vs 75.3%) was improved in piglets fed protease. A multi-component protease increases digestibility of essential and non EAAs, thus, providing nutritional benefits while lowering nitrogen excretion in farms. The same protease has also been shown to consistently improve gut morphology and integrity by reducing the levels of undigested proteins in the hindgut which serve as substrate for pathogens, leading to lower incidence of diarrhoea in piglets.

Overall, managing piglet nutrition and health under increasing regulatory pressure from what were standard procedures will require a multi-faceted approach based on combinations of optimal management and nutritional strategies. Dietary solutions such as the use of microencapsulated ZnO, low CP diets, and supplementation of protease offer opportunities to reduce post-weaning diarrhoea, support optimal growth, and promote environmental sustainability against the backdrop of restricted use of antibiotics and ZnO.

O-N-008

MEASURING SERUM D-ROMS AS OXIDANTS AND BAP AS ANTIOXIDATIVE CAPACITY IN SOWS IN THE PERIPARTURIENT PERIOD.

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Introduction
Reactive oxygen species (ROS) are energy production metabolites and play a central role in the immune system (1). However, excessive production of ROS and free radicals may result in oxidative stress, which negatively impacts reproductive performance and growth speed and may lead to diseases (2,3). Reactive oxygen metabolite-derived compounds (d-ROMs) and biological antioxidant potential (BAP) are measurable markers of oxidative stress. Since these factors have not yet been estimated in sows, we examined the values at parturition and weaning and whether the values varied by sow parity and farms.

Materials and Methods
The study was conducted on two commercial pig breeding farms (farm A with 1,100 sows and farm B with 1.000 sows) in Kyusyu, Japan. A total of 40 sows, 20 from each farm, were grouped by parity: young group (parity 1–2: n=10) and elderly group (parity 4–6: n=10). All sows were fed the same diet with adequate minerals and vitamins. Blood samples were collected during parturition and weaning. The d-ROMs and BAP were measured, and the OSI was calculated from their ratios. The reproductive data collected included total litter size and the number of live births, stillborn births, and suckling terms that took place from November 2022 to June 2023.

Results
From parturition to weaning, BAP significantly decreased, particularly in young sows, whereas OSI significantly increased ($p < 0.05$ for both). d-ROMs had a negative relationship with the suckling period ($p < 0.05$). At the time of weaning, young sows had significantly higher d-ROMs and OSI than elderly sows ($p < 0.05$).
Farm A had a significantly higher number of live births than farm B. The d-ROMs were higher in Farm A than in Farm B at weaning ($p < 0.05$), whereas BAP was not significantly different. The OSI was significantly higher in Farm A than in Farm B at both parturition and weaning ($p < 0.05$ for both).

Conclusions and Discussion
In this study, d-ROMs tended to decrease with longer suckling periods, which may suggest that oxidants generated at parturition are metabolized during this period (4). The significant decrease in the BAP after parturition was similar to that observed in a previous study (5). It was reported that reproductive performance decreased (2), whereas sows on the farm with a higher number of live births expressed stronger oxidative stress in our results. This might be because sows on this farm had a long period of parturition, which is related to enhanced oxidative stress (5).
In terms of parity, young sows have higher antioxidant capacity than elderly sows. Further research is required to determine the cause of these results.

In conclusion, the estimation of d-ROMs and BAP indicated that oxidative stress in sows was elevated at weaning rather than at parturition, and reproductive performance and parity were related to the generation of oxidants and reduction of antioxidative capacity. d-ROMs and BAP seem to be useful indicators of oxidative stress in sows.

Acknowledgment and References

[1] Genestra M. Cell Signal. 2007 Sep;19(9):1807-19.
[2] Wang H, Hu C, Cheng C, Cui J, Ji Y, Hao X, Li Q, Ren W, Deng B, Yin Y, Deng J, Tan C. Theriogenology. 2019 Sep 15;136:131-137.
[3] Smith F, Clark JE, Overman BL, Tozel CC, Huang JH, Rivier JE, Blikslager AT, Moeser AJ. Am J Physiol Gastrointest Liver Physiol. 2010 Mar;298(3):G352-63.
[4] Szczubiał M. Pol J Vet Sci. 2020 Jun;23(1):185-190.
[5] Szczubiał M, Dąbrowski R, Bochniarz M, Komar M. Theriogenology. 2013 Oct 15;8

O-N-009

PROBIOTIC EFFECT ON SOW AND WEANLING PERFORMANCE CHALLENGED WITH E. COLI K88

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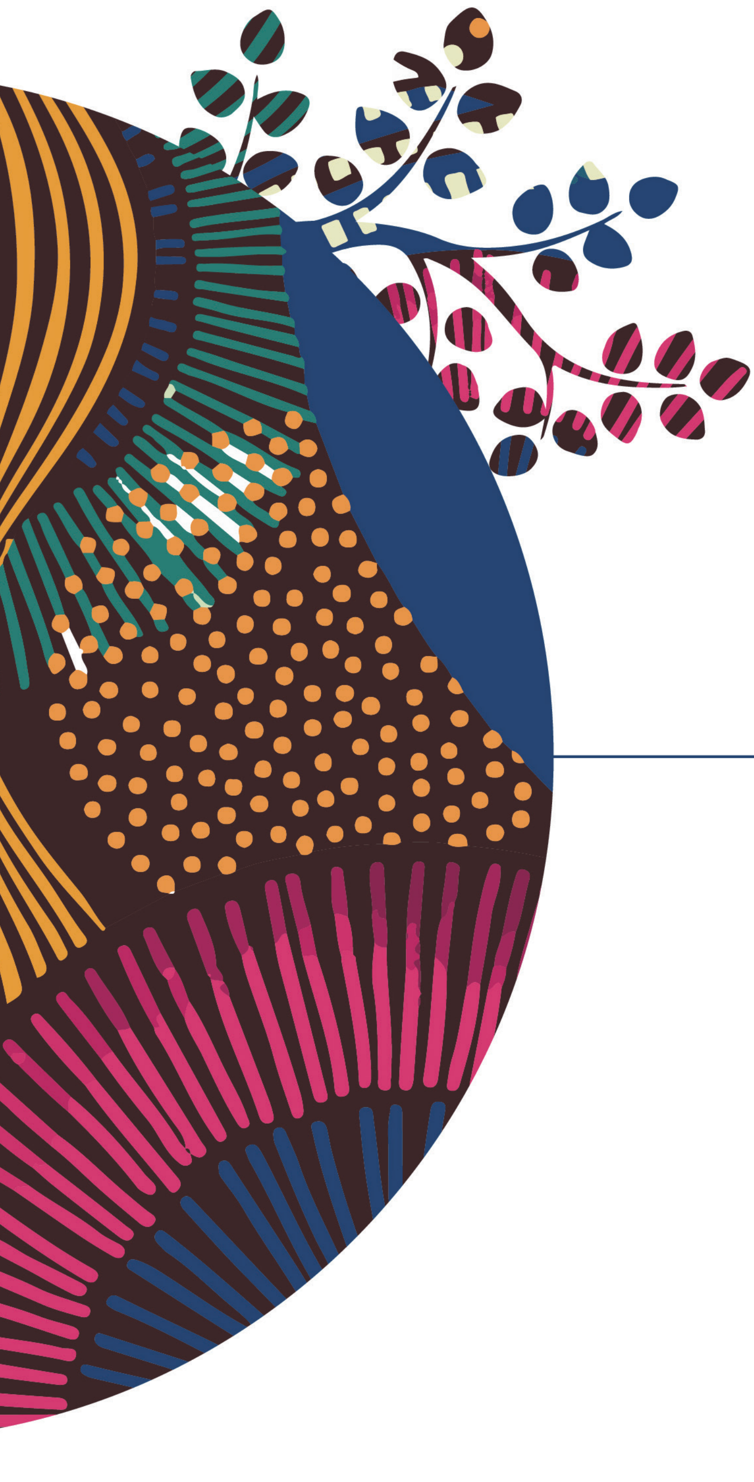
Introduction
Restrictions on antibiotic use in swine production in many countries have resulted in the assessment and adoption of management changes including the use of antibiotic alternative feed additives. This study assessed piglet and post-weaning growth performance, diet digestibility, blood cytokine response, and fecal microbiota composition in response to a multi-Bacillus strain probiotic (Bacillis subtilis PB6, B. subtilis FXA and B. licheniformis G3).

Materials and Methods
Two experiments were conducted in which the probiotic was included at 500 grams/ton in sow feed from 100 days of gestation through to weaning at 21 days and in weanling feed to 9 weeks of age. Ten Landrace x Yorkshire sows served as controls and 10 received the probiotic. At weaning in experiment 1, 8 replicate pens of 5 pigs/pen were allocated to 1 of 4 treatments (160 pigs in total): (1) control sow and weanling diets, (2), control sow diets then probiotic treated weanling diet, (3) probiotic treated sow diets then control weanling diet, and (4) probiotic treated sow and weanling diets. In experiment 2, an additional 40 weaned pigs from the same 4 treatments as used in experiment 1 (i.e., 10 pigs/treatment) were individually penned and challenged with E. coli K88 at 14 days post-weaning.

Results
The probiotic significantly increased piglet weaning weight at 21 days ($p<0.05$) and significantly increased the digestibility of Dry Matter, Nitrogen, and Gross Energy in the sow's lactation diet ($p<0.05$). There was also a significant increase in Dry Matter and Nitrogen digestibility in the sixth week after weaning ($p<0.05$) and significant increases in average daily weight gain to 9 weeks of age ($p<0.05$). The increase in nutrient digestibility may have contributed to the milk nutrient supply to the suckling piglets. In a similar study, Zhang et al. (2022) reported that B. subtilis PB6 supplementation during gestation and lactation resulted in an increase in colostrum fat content and a non-significant numerical increase in milk fat content. The results of experiment 2, the probiotic resulted in significantly heavier pigs at 9 weeks of age ($p<0.05$), a significantly reduced increase in serum Interleukin-6 ($p<0.05$), and changes in fecal microbiota composition. The multi-Bacillus strain probiotic improved growth performance in the absence and presence of E.coli K88, improved diet digestibility, attenuated blood cytokine response to the E. coli K88 challenge, and modified the taxonomic distribution of fecal microbiota. Taxonomic distribution changes observed significantly reduced prevalence of Clostridia and Brachyspira in fecal microbiota. Surfactins produced by B. subtilis and B. licheniformis have been shown to exhibit activity against B. hyodysenteriae and C. perfringens (Horng et al., 2019).

References

Australian Pork. 2021. Antimicrobial stewardship in Australian Livestock Industries, 2nd. edition. ISBN: 978-1-922529-33-6
Hanson, J., Harms, P., Culbertson, M., Hahn, J., Moore, S., Yersin, A.G. 2009. Utilization of a direct-fed microbial (Bacillus subtilis PB6) to improve performance of nursing piglets: comparison of sow, piglet, and combination regimens. Amer. Assoc. Swine Vet. p.301-303
Zhang, Q., Li, J., Cao, M., Li, Y., Zhuo, Y., Fang, Z., Che, L., Xu, S., Feng, B., Lin, Y., Jiang, X., Zhao, X., Wu, D. 2020. Dietary supplementation of Bacillus subtilis PB6 improves sow reproductive performance and reduces piglet birth intervals. Animal
Horng, Y-B., Yu, Y-H., Dybus, A., Hsiao, F. S-H., Cheng, Y-H. 2019. Antibacterial activity of Bacillus species-derived surfactin on Brachyspira hyodysenteriae and Clostridium perfringens. AMB Express 9:188



Poster Abstracts

P-T-001

A FIELD STUDY OF THE APPLICATION OF ZEOMIC-T (SILVER ION) TO CONTROL AFRICAN SWINE FEVER VIRUS INFECTION

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Introduction
African swine fever (ASF) is a harmful disease for pig industry. The disease is caused by African swine fever virus (ASFV), a sole member of the Family of Asfarviridae. ASF outbreak is started in China in 2018 and then spread to several countries in Asia (1). In Thailand, the Department of Livestock Development was announced the detected ASFV in a pig carcass sample in a slaughterhouse in Jan, 2022. Silver has antimicrobial property against bacteria, virus and fungi with low toxicity, and has been commonly used in human. Silver ion is studied for some viruses but not ASFV (2). Thus, Zeomic-T® (silver ion) may be suitable for control ASFV infection.

Materials & Methods
Ten 5-week-old pigs from a pig farm with the history of ASF outbreak were selected in this study. All pigs were adlib fed normal feed with Zeomic-T® for 3 months. Whole blood was collected from all pigs into EDTA treated tube at before mixing Zeomic-T® (day 0) and 35, and 90 days after taking Zeomic-T®. EDTA-blood samples were performed DNA isolation using Geneaid™ DNA isolation kit (Geneaid Biotech Ltd., Taiwan) and followed the product instruction. For ASFV detection, the extracted DNA was carried out WOAH recommended real-time PCR (3). The cycle threshold (Ct) from QuantStudio3 was used to classify into 5 groups and calculate the average Ct value (Table 1). Ct value >40 was negative reaction.

Results
The results of this study showed in Table 1. By interpretation of Ct value, at the starting of the study, 9 out of 10 were infected with ASFV. After feeding Zeomic-T® for 35 days, there was a pig became negative. At the end of the study, there was not detected ASFV in all pigs.

Conclusions & Discussion
ASFV is a strong virus and tolerate to wide range environmental conditions. There is no prospective medication and vaccine. This is a field study in a pig farm with ASF outbreak history. Silver ion has potent antiviral action (2). All pigs became negative and could survive until the end of the study after taking Zeomic-T® more than 35 days. The study has evaluated the antiviral activity of Zeomic-T® (silver ion). That is Zeomic-T® could be beneficial virucide against ASFV.
In conclusion, it is confirmed that Zeomic-T® could provide antiviral activity to control the infection and spread of ASFV in pigs.

Acknowledgements
I would like to thank Shield Innovation Co., Ltd. for supporting Zeomic-T® in this study.

References
1. Mighell, E. et al. (2021). Transbound. Emerg. Dis., 68, 2722–2732
2. Soliman, M.Y.M. et al. (2020). Water Res. X, 9, 100077
3. King, D.P. et al. (2003). J. Virol. Methods, 107, 53–61

Table 1

Table 1 Results of ASFV detection by TaqMan® real-time PCR

Zeomic-T® (days)	**0**	**35**	**90**
Ct <20	2	-	-
Ct 20 – 35	6	8	-
Ct >35 – 38	1	-	-
Ct >38 – 40	-	-	-
Ct >40 (negative)	1	2	10
Average Ct	**27.282**	**30.212**	**>40**

P-T-002

BROAD ANTIGENIC COVERAGE OF HIGH POTENCY O1 CAMPOS VACCINE AGAINST TYPE O FOOT-AND-MOUTH DISEASE VIRUSES CIRCULATING WORLDWIDE

Dr. Juver Membrebe[1], Facundo Romero[2], Cecilia Caldevilla[2], Dung Nguyen[1], Sabrina Cardillo[2], Jorge Filippi[2], Romina Scian[2], Cristian Malnero[2], Marisa Martinez[2]
[1]*Biogenesis Bago Asia,* [2]*Biogenesis Bago S.A*

Introduction
Foot-and-mouth disease (FMD) virus serotype O is the most prevalent worldwide. FMD outbreaks of type O viruses, belonging to Middle East South Asia (ME-SA), Southeast Asia (SEA), and CATHAY topotypes highly affect Asian and Southeast Asian countries. Meanwhile, East Africa (EA) topotype cause outbreaks in North Africa and East Africa. The O/ME-SA-2018 was reported as an emerging lineage in South Asia and Middle East. FMD is a transboundary disease, transpool movement of viruses among regions became a continuous threat of incoming new viruses. A potent vaccine with broad antigenic coverage has a key role in the control of FMD worldwide. The present report provides evidence that O1 Campos Biogenesis Bago high potency vaccine induces satisfactory cross-protection against different field isolates circulating globally.

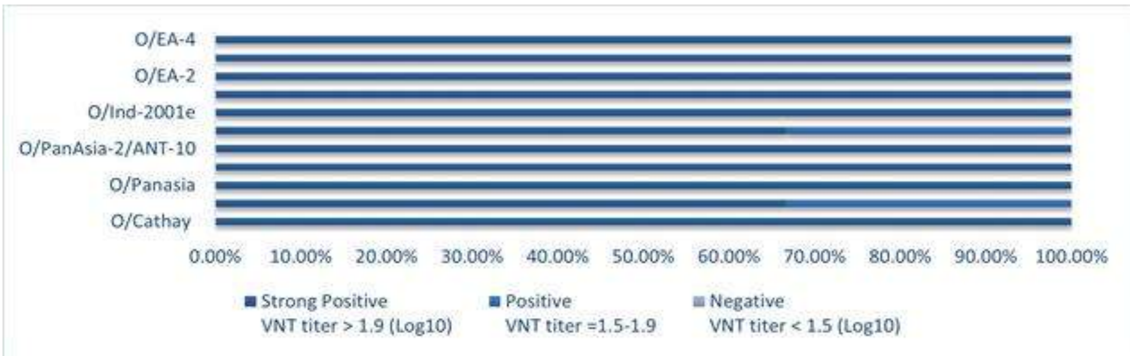
Materials and Methods
Monovalent water-in-oil single emulsion (>6PD50) vaccines containing highly purified O1 Campos antigen were used, manufactured by Biogenesis Bago (BB) in compliance with Good Manufacturing Practices (GMP). All animals assayed came from FMD free zones and tested negative for FMD antibodies prior to enrollment; were vaccinated at day 0 and bleed for sera collection at 28 days post vaccination (dpv). Serum samples were tested for in vitro vaccine matching determined by the bi-dimensional virus neutralization test (VNT), in compliance with World Organization for Animal Health (WOAH, former OIE) recommendation, and performed at The Pirbright Institute, World Reference Laboratory for FMD (WRLFMD, Pirbright, UK) and at SENASA (National Service for Agrifood Health and Quality), the WOAH reference laboratory for Foot-and-Mouth Disease in Argentina In this study a total of 95 O serotype FMD viruses (O/SEA/Mya-98, O/ME-SA/PanAsia, O/ME-SA/PanAsia-2, O/ME-SA/Ind-2001, O/ME-SA/SA-2018, O/CATHAY, and O/EA) isolated from 2013 to 2022 from 1 to 6 regional pools were characterized by VNT. In the present study, VN antibody titer of the post-vaccinal sera greater or equal to 1.5 (Log10) was considered as an indicator of minimum heterologous cross-protection, as previously proposed [1-3]. For a better characterization, heterologous VNT (expressed as Log10) were classified into three vaccine matching categories: < 1.5 (negative), 1.5 - 1.9 (positive), and > 1.9 (strong positive)

Results
Virus neutralization titers > 1.5 (Log10) were observed against all type O viruses tested, with strong positive results in 96% of the isolates belonging to O/Cathay, O/PanAsia, O/PanAsia-2, O/Ind-2001e, O/ME-SA/SA/2018 and O/EA lineages, as well as for most O/Mya-98 and O/Ind-2001d isolates. Results of this investigation demonstrated that Biogenesis Bagó FMD vaccines conferred high cross-reactivity, predicting protection against a wide spectrum of type O FMDV circulating worldwide.

Conclusion and Discussion
Highly immunogenic and widely cross-protective vaccine strains are key factors for the success of vaccination programs. The results of this study demonstrate the broad antigenic coverage of Biogenesis Bago world-class vaccines formulated with O1 Campos vaccine strain against circulating viruses worldwide. Strong heterologous VNT titers support the use of these vaccines not only for systematic vaccination in these endemic settings but also to control emergencies in free regions.

References
1. Hammond, J.M. et al. Targeted FMD Vaccines for Eastern Africa: The AgResults Foot and Mouth Disease Vaccine Challenge Project. Viruses 2021, 13, 1830. https://doi.org/10.3390/v13091830.
2. Ludi AB, et al. Selection and use of reference panels: a case study highlighting current gaps in the materials available for foot and mouth disease. Rev Sci Tech. 2021 Jun;40(1):239-251. doi: 10.20506/rst.40.1.3221. PMID: 34140727.
3. Karabassova AS, et al. Toward the calibration of serological assays using sera collected from cattle and sheep following a single dose of foot-and-mouth disease vaccine. Vet World. 2022 Feb;15(2):524-530. doi: 10.14202/vetworld.2022.524-530. Epub 2022 Feb 28. PMID: 35400944; PMCID: PMC8980403.

Graphs or Images 1



P-T-003

BROAD ANTIGENIC COVERAGE OF HIGH-POTENCY VACCINES CONTAINING A24 CRUZEIRO AND A2001 ARGENTINA FOOT-AND-MOUTH DISEASE VACCINE STRAINS AGAINST TYPE A FOOT-AND-MOUTH DISEASE VIRUSES CIRCULATING WORLDWIDE

Dr. Juver Membrebe[1], Facundo Romero[2], Cecilia Caldevilla[2], Dung Nguyen[1], Sabrina Cardillo[2], Jorge Filippi[2], Romina Scian[2], Cristian Malnero[2], Marisa Martinez[2]
[1]Biogenesis Bago Asia, [2]Biogenesis Bago S.A

Introduction:
Foot-and-mouth disease viruses (FMDV) from serotype A exhibit high antigenic diversity, and several variants caused outbreaks of Foot and Mouth Disease (FMD) in endemic regions despite regular vaccination. Type A viruses, from topotype ASIA, lineage Sea-97 (A/ASIA/Sea-97) are restricted to Southeast Asia and East Asia (pool 1). A/ASIA/Iran-05 lineage is predominant in West Eurasia & Middle East (pool 3) while A/ASIA/G-VII, previously limited to South Asia (pool 2), emerged in 2015 in pool 3. A/AFRICA topotype, lineages G-I and G-IV cause outbreaks in North Africa, Eastern Africa (pool 4) and West /Central Africa (Pool 5). FMD is a transboundary disease, transpool movement of viruses among regions became a continuous threat of incoming new viruses. The use of a potent vaccine with broad antigenic coverage has a key role in the control of FMD worldwide. The aim of the present study is to assess the cross-reactivity induced by Biogenesis Bago high potency vaccines against type A field isolates circulating globally.

Materials and Methods:
Trivalent water-in-oil single emulsion (PD50>6) vaccines containing highly purified antigens A24 Cruzeiro/A2001 Argentina/O1 Campos were used, manufactured by Biogénesis Bagó (BB) in compliance with Good Manufacturing Practices (GMP). All cattle (5) and pigs (5) enrolled came from FMD free zone and were free of FMDV antibodies before vaccination. Sera were collected at 30 days post vaccination (dpv), 60 dpv and 30 days post revaccination (dprv) in cattle and at 28 dpv and 28 dprv in pigs. Virus neutralization test (VNT) was performed at the Pirbright Institute, World Reference Laboratory for FMD (WRLFMD, Pirbright, UK). Sera were tested against the following Type A field viruses: A/ASIA/Sea-97, A/ASIA/G-VII, A/ASIA/Iran05, A/AFRICA/G-I and A/AFRICA/G-IV. The antibody titer against heterologous A viruses was established for each serum and the mean group antibody titer was calculated. For the present study, VNT titer of the pool sera greater or equal to 1.5 (Log10) was considered as an indicator of minimum heterologous cross-protection, as previously proposed1-3.

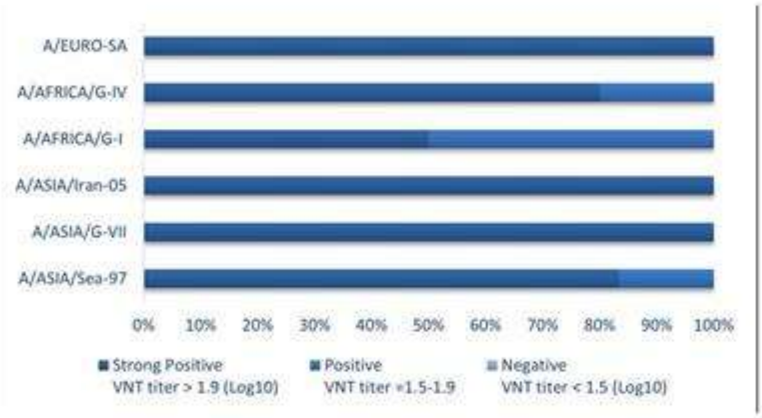
Results:
After a single dose, vaccines containing A24 Cruzeiro and A2001 induced a protective broad immunological coverage, showing titers above 1.50 (Log10) in cattle and pigs. These titers increased at 60 dpv.

Conclusion and Discussion:
Highly immunogenic and widely cross-protective vaccine strains are key factors for the success of vaccination programs. The results of this study demonstrate the broad antigenic coverage of Biogénesis Bagó world-class vaccines formulated with A24 Cruzeiro and A2001

Argentina vaccine strains against circulating viruses worldwide. Strong heterologous VNT titers support the use of these vaccines not only for systematic vaccination in these endemic settings but also to control emergencies in free regions.

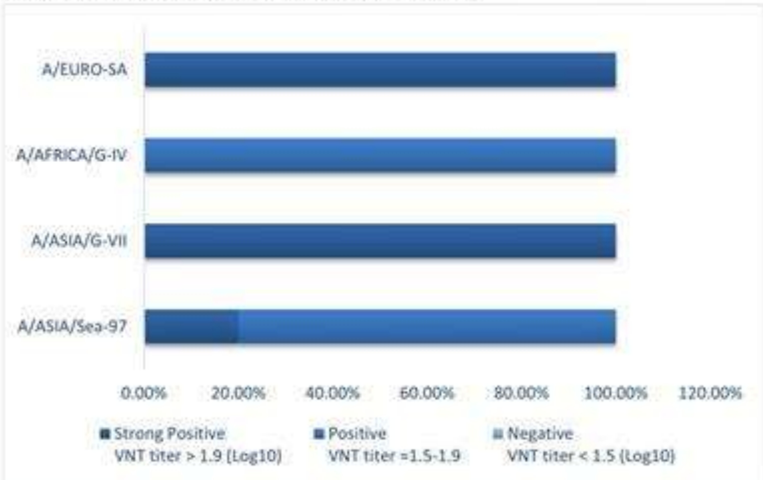
Graphs or Images 1

Figure 1. Vaccine matching studies for A field isolates belonging to different topotypes/lineages. VNT was performed at Pirbright Institute (WRL for FMD) employing a trivalent A24 Cruzeiro/A2001 Argentina/O1 Campos **bovine sera (30 dpv)** from Biogenesis Bago vaccine. Data are shown as the proportion (%) of isolates in different vaccine matching categories (>1.9; 1.5-1.9; <1.5 log10) according to the VNT titer obtained. VNT titers = > 1.5 log10 was considered as an indicator of minimum heterologous cross-protection, as previously proposed.



Graphs or Images 2

Figure 2. Vaccine matching studies for A field isolates belonging to different topotypes/lineages. VNT was performed at Pirbright Institute (WRL for FMD) employing a trivalent A24 Cruzeiro/A2001 Argentina/O1 Campos **pig sera (28 dpv)** from Biogenesis Bago vaccine. Data are shown as the proportion (%) of isolates in different vaccine matching categories (>1.9; 1.5-1.9; <1.5 log10) according to the VNT titer obtained. VNT titers = > 1.5 log10 was considered as an indicator of minimum heterologous cross-protection, as previously proposed.



P-T-004

CHANGES IN NEUTRALIZING ANTIBODY TITERS AFTER ADDITIONAL VACCINATION OF GILTS WITH CLASSICAL SWINE FEVER VACCINE

Ms. Kana Fukushima[1], Mr. Yoshihiro Mizukami[1], Mr. Mitsugi Ito[1]
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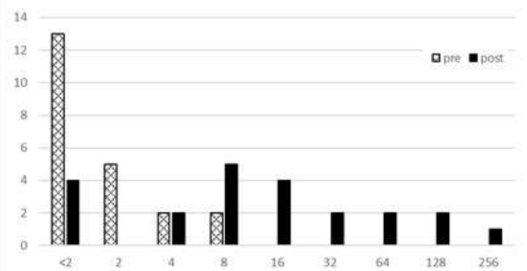
Introduction :
In September 2018, an outbreak of classical swine fever (CSF) was confirmed at a swine farm in Japan for the first time in 26 years. Infection of wild boars has also been confirmed, and swine are being vaccinated regionally to prevent outbreaks on swine farms. Stabilization of antibody titers in sows is a very important factor in considering the appropriate age at which to vaccinate piglets on farms.
In Japan, the vaccination schedule for sows is set at a maximum of four times: (1) during piglethood, (2) six months after the first vaccination, (3) one year after the second vaccination, and (4) one more year after the third vaccination.
The purpose of this additional vaccination is to raise the extremely low antibody titer of some sows.Therefore, we conducted a study to confirm changes in neutralizing antibody titers before and after the second vaccination, approximately six months after the first vaccination.

Materials and Methods :
Gilts introduced from the outside of farm were tested (n = 126) at a swine farm with a sow size of 400 in Aichi Prefecture. Since introduction was at 4-5 months of age, the gilts at the time of introduction had only been vaccinated as piglets. Neutralizing antibody titers were tested (pre) and gilts with low neutralizing antibody titers (<2x to 8x, n = 22) were tested again (post) after additional vaccination.

Results :
The results of the pre-test showed an extremely high number of gilts with low titers of less than 2x . Comparing the neutralizing antibody titers of the post-tested gilts with those of the pre-test, many gilts had increased neutralizing antibody titers. (Graph)

Conclusions and Discussion :
These results suggest that additional CSF vaccination of gilts six months after vaccination as piglets is important to reduce the number of sows with extremely low neutralizing antibody titers on the farm.

Graphs or Images 1



Comparing the neutralizing antibody titers of the post-tested gilts with those of the pre-test

P-T-005

COMPARISON THE SPECIFICITY OF 3 ELISA KITS USED FOR FOOT-AND-MOUTH DISEASE VIRUS NONSTRUCTURAL PROTEIN SEROLOGY IN PIGS

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Introduction
Foot and mouth disease is one of the most contagious diseases among the cloven-hoofed animals. It causes severe economic losses in the meat exportation countries. In Taiwan, vaccination program has been introduced after massive culling of infected pigs and FMD incidents has been brought to control soon after the vaccination. The established surveillance method for sampling farms for reactivity to NSP, in conjunction with the tracing back of reactors to detect swine with clinical signs and to conduct follow-up serological testing has been successfully applied in Taiwan to show the situation of FMD virus circulation in the fields (Chen et al., 2011). The application of NSP kits is able to differentiate the infected animals from the vaccinated one, which have been recommended from WOAH as one of the methods for national FMD surveillance. In our previous studies the commercialized NSP ELISA kits are reliable to apply as a herd test to demonstrate the absence of FMDV circulation by showing that vaccinated pig herds are free from antibody to FMD NSPs. These tests have been applied in Taiwan for pig farm surveillance by testing appropriate numbers of serum samples from the finishers. This study is to demonstrate the estimated specificity of these kits in the pigs without use FMD vaccination in Taiwan from 2020 to 2022.

Materials and methods

Samples collected
Sera were collected from all auction markets annually from 2020 to 2022. A total of 109,416 serum samples were collected from pigs in the auction markets in 3 years.

The NSP ELISA kits used in the serosurveys
Three commercially available ELISAs for detection of antibodies directed against FMDV non-structural proteins (NSP ELISAs) were used. The different combination of two ELISA kits for annual serosurvey was used as the screening test and confirmatory test, respectively (Table 1).

Diagnostic specificity
From July 2019, Taiwan has stopped the vaccination against foot-and-mouth disease, the FMD cases reported to WOAH from Taiwan has dropped to zero and the pig sera were assumed to be negative for FMD NSP reactors from which the diagnostic specificity for different test kits was calculated according to their application as screening test kits.

Results

Table 1 summarizes the test results for a total of 109,416 serum samples collected from auction markets from 2020 through 2022. FMD NSP positive reactors were recorded using an initial NSP ELISA screen test followed by a different NSP ELISA confirmatory test. In the samples from auction markets the NSP primary screening test positive ranged from 2.99 (95% CI, 2.84-3.15), 0.21 (95% CI, 0.17-0.26) to 0.13 (95% CI, 0.09-0.19) in 2020, 2021 and 2022, respectively.

Conclusions and Discussion

Looking at the data for NSP screening tests in Table 1 it is evident that the years with the Sentinel tests as the screening test showed a substantially higher percentage of reactors than with the PrioCHECK or ID Vet tests. The combination of two NSP ELISA kits tested in series identified suspect farms based on confirmed FMD NSP antibody detection and this facilitated detailed on-farm investigation using clinical examination of all age groups of pigs and serological investigation by NSP ELISA tests to determine the farm status as infected or not infected.

Reference

Chen, S.P., Lee, M.C., Sun, Y.F., Yang, P.C., 2011. Application of non-structural protein ELISA kits in nationwide FMD surveillance in pigs to demonstrate virus circulation in Taiwan. Vet Microbiol 152, 266-269.

Chen T.H., Fan L., Lin Y. L, Dekker A., 2011, Differentiation of FMD-infected pigs from vaccinated pigs using antibody-detection sandwich ELISA. J. Vet. Med. Sci. 73(8): 977-984.

Table 1

Table 1, 2020~2022 FMD NS Positive reactions in Auction markets

Year	Tested Sample	Primary screening positive %	Positive 95% CI	Confirm positive %	Positive 95% CI	Screening test kit	Confirmatory test kit
2020	45,301	2.99	2.84-3.15	0.01	0.01-0.03	Sentinel	PrioCHECK
2021	43,889	0.21	0.17-0.26	0.03	0.02-0.05	PrioCHECK	ID.vet
2022	20,226	0.13	0.09-0.19	0.03	0.01-0.06	ID.vet	Sentinel

P-T-006

EFFICACY OF A HIGH-POTENCY POLYVALENT FOOT-AND-MOUTH DISEASE VIRUS VACCINE IN PIGS AGAINST HETEROLOGOUS CHALLENGE WITH SOUTH KOREAN FIELD ISOLATES

Dr. Juver Membrebe[1], Facundo Romero[2], Cecilia Caldevilla[2], Dung Nguyen[1], Sabrina Cardillo[2], Jorge Filippi[2], Romina Scian[2], Cristian Malnero[2], Marisa Martinez[2]
[1]Biogenesis Bago Asia, [2]Biogenesis Bago S.A

Introduction:
in vivo vaccine matching offers crucial information for the proper selection of adequate vaccine strains against field outbreaks and for the successful implementation of Foot-and-Mouth Disease (FMD) vaccination policy to control the disease. South Korea experienced several outbreaks since 2010, when a new lineage of serotype O FMD Virus (Mya-98) spread to the country, resulting in devastating economic consequences. In spite of a compulsory vaccination campaign, clinical disease has been reported sporadically in 2014. Since September 2016, a vaccine containing O1 Campos FMDV (Biogenesis Bago) showing satisfactory in vitro and in vivo cross immunity results1-2, has been introduced in the market and is being administered in vaccination programs in pigs. Here we extend those studies and report satisfactory efficacy results against O/Ind-2001e and A/ASIA/Sea-97, lineages responsible for the last reported outbreaks, in pigs after vaccination with one dose of a vaccine containing O1 Campos, A 24 Cruzeiro, and A 2001 Argentina.

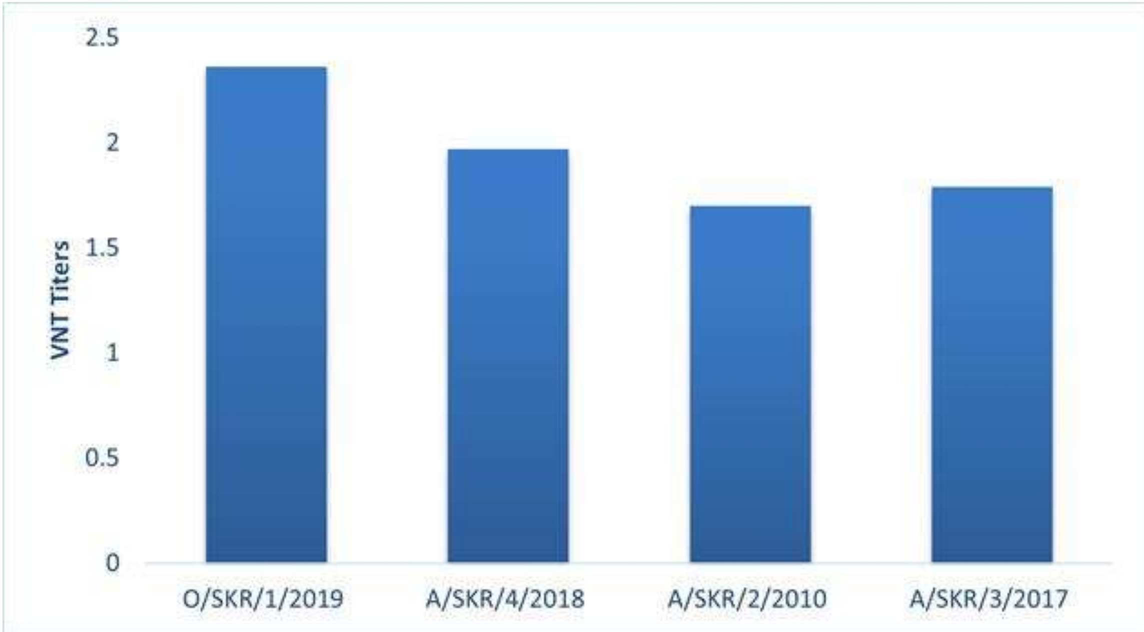
Materials and methods:
Trivalent water-in-oil single emulsion (>6PD50) vaccines containing highly purified antigens A24 Cruzeiro/A2001 Argentina/O1 Campos were used, manufactured by Biogénesis Bagó (BB) in compliance with Good Manufacturing Practices (GMP); and approved by SENASA (National Service for Agrifood Health and Quality- WOAH Reference Lab) for safety, purity, and potency in swine. Four independent in vivo challenge tests were performed, in compliance with the Argentinian Animal Ethics Code, in the BSL4 animal facility of SENASA, Argentina. Challenge viruses were O/ME-SA/Ind2001-e (Anseong O/SKR/1/2019) and three viruses of A/ASIA/SEA-97 lineage (Gimpo A/SKR/4/18; Pocheon A/SKR/2/2010 and Yeoncheon A/SKR/3/2017); all had two previous passages in pigs for adaptation. Twenty pigs were enrolled in each trial. Eighteen pigs were allocated in three groups and received intramuscular vaccination in the neck region with 2 ml (full dose), 0.5 ml (1/4 dose), and 0.125 ml (1/16 dose), respectively; while two remained unvaccinated and were included as controls. At 28 days post vaccination, all 20 pigs were challenged with 100.000 ID50. The PD50 result for each FMDv strain was calculated following the Spearman-Karber method.

Results:
All animals vaccinated with a highly potent (>6PD50) FMD vaccine were protected upon challenge against O/SKR/1/2019 (8PD50), A/SKR/4/18 (9PD50), A/SKR/2/2010 (12,70PD50), and A/SKR/3/2017 (16PD50) isolates, in accordance with in vitro results against O/ME-SA/Ind2001-e and A/ASIA/SEA-97 lineages, as reported 3.

Conclusion and Discussion: The results of this in vivo study were in agreement with previously reported in vitro results against isolates belonging to type O lineage O/ME-SA/Ind2001-e and type A lineage A/ASIA/SEA-97(Figure 1). The successful results of these in vivo tests, as well as the current outcome of field performance seen in South Korea, confirm that Biogénesis Bagó world-class vaccines can be used successfully applied not only for systematic vaccination in endemic settings but also for emergency attendance and outbreaks control.

References:
1- Galdo Novo, S., V. Malirat, E. D. Maradei, A. M. Espinoza, E. Smitsaart, A. R. Pedemonte, N. Mattion and I. E. Bergmann (2017). “Antigenic and immunogenic spectrum of foot-and-mouth disease vaccine strain O1 Campos against representative viruses of topotypes that circulated in Asia over the past decade.” Vaccine 35(18): 2303-2307.
2- Galdo Novo, S., V. Malirat, E. D. Maradei, A. R. Pedemonte, A. M. Espinoza, E. Smitsaart, K. N. Lee, J. H. Park and I. E. Bergmann (2018). “Efficacy of a high-quality O1/Campos foot-and-mouth disease vaccine upon challenge with a heterologous Korean O Mya98 lineage virus in pigs.” Vaccine 36(12): 1570-1576.
3- High cross-reactivity of O1 Campos FMD vaccine strain against circulating Foot and mouth disease viral lineages in pool 1 and pool 2 (Abstract, pending approval)

Graphs or Images 1



P-T-007

GOOD AGRICULTURE PRACTICE ASSESSMENT OF THE RESTART SMALL HOLDER PIG FARMS AFTER AFRICAN SWINE FEVER OUTBREAK.

Dr. Panuwat Yamsakul[1], Terdsak Yano[2], Pakpoom Tadee[2], Prapas Pachanee[2]
[1]*Faculty Of Veterinary Medicine, Chiang Mai University,* [2]Swine Clinic, Department of Food animal clinic, Faculty of Veterinary Medicine, Chiang Mai University, Thailand

Introduction
After Africa swine fever outbreak in Asia was found in 2019. It started from China, Vietnam, Cambodia, Laos, and finally in Thailand respectively. In Thai, it was reported in 2021. There were decreasing of pig population in each country which found the outbreak. Presently, farmers want to return to raising pigs again, especially small holder farms. Good Agriculture Practice assessment is necessary thing before restart for checking and preparing.
Materials and methods
There were 5 small holder pig farms which had a history of ASF outbreak. These farms were assessed to follow the GAP guideline of DLD of Thailand. The GAP guideline of DLD compose of 11 core-sections and more than 200 sub-sections. Assessors are the expert person from DLD and university which compose of 5 persons. They had conclusion by consensus from assessment form. All data were analyzed by descriptive analysis from excel program v.2016.

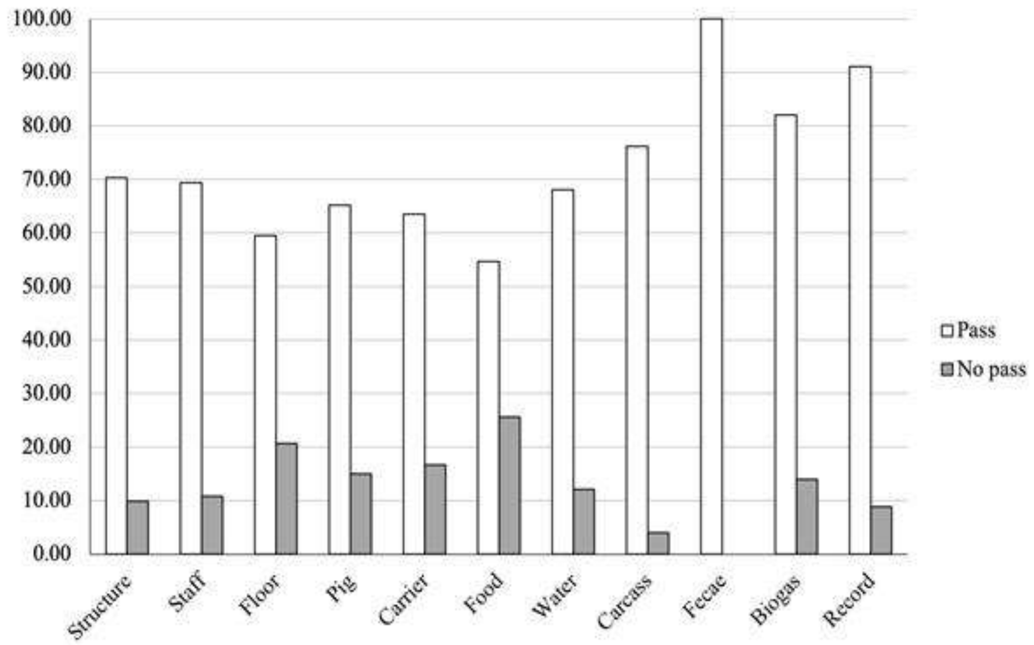
Results
Average of each expert person assessment were shown in Figure 1. The top 3 of assessment form, which those passed the GAP assessment, included; 1. fecal management 2. Record system and 3. West management (Biogas). It means that the farmers can make those follow the GAP guideline as well. Unfortunately, the top 3 of assessment form, which those did not pass the GAP assessment, included; A. Food management, B. Floor surface and C. Control carrier which those are the high risk of ASF outbreak into the pig farm.

Discussion and conclusion
Therefore, at least these risk factors should be considered if farmers want to come back to raise pigs again.

Figure 1 Average of GAP assessment which were assessed by the expert persons

Acknowledgement and References
Thank you U2T project to support budget.
Bora, M., et al., 2020. Assessment of Risk Factors of African Swine Fever in India: Perspectives on Future Outbreaks and Control Strategies. Pathogens 2020, 9(12), 1044; https://doi.org/10.3390/pathogens9121044

Graphs or Images 1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P-T-008

HIGH CROSS-PROTECTION OF O1 CAMPOS FOOT-AND-MOUTH DISEASE VACCINE STRAIN AGAINST CIRCULATING VIRAL LINEAGES IN POOL 1 AND POOL 2

Dr. Juver Membrebe[1], Facundo Romero[2], Cecilia Caldevilla[2], Dung Nguyen[1], Marisa Martinez[2], Sabrina Cardillo[2], Jorge Filippi[2], Romina Scian[2], Cristian Malnero[2]
[1]Biogenesis Bago Asia, [2]Biogenesis Bago S.A

Introduction:
Foot-and-mouth disease (FMD) remains endemic in pool 1 (Southeast Asia-Central and East Asia) and pool 2 (South Asia) causing significant economic losses. Most of the FMD outbreaks in the region are caused by serotype O. Several topotypes and lineages have been circulating, such as O/ME-SA/Ind-2001, O/ME-SA/PanAsia, O/SEA/Mya-98, and O/CATHAY. The O/ME-SA/Ind-2001e viruses have now become established; being during 2021-22 the only lineage detected. The challenge for vaccination programs to be successful is to select appropriate cross-reactive vaccine strains. The aim of this study was to assess the cross-reactivity of O1 Campos (Biogenesis Bago) bovine and swine post-vaccinal sera with the field viruses circulating in pools 1 and 2. The present report provides evidence that O1 Campos Biogenesis Bago high-potency vaccine induces satisfactory cross-protection against relevant Asian strains.

Materials and Methods:
Monovalent water-in-oil single emulsion (>6PD50) vaccines containing highly purified O1 Campos antigen were used, manufactured by Biogénesis Bagó (BB) in compliance with Good Manufacturing Practices (GMP). All animals essayed came from FMD -free zones and tested negative for FMD antibodies prior to enrollment. Cattle were vaccinated and bleed for sera collection at 28 days post vaccination (dpv). Five serum samples were tested for in vitro vaccine matching determined by the bi-dimensional virus neutralization test (VNT), in compliance with World Organization for Animal Health (WOAH, former OIE) recommendation, and performed at The Pirbright Institute, World Reference Laboratory for FMD (WRLFMD, Pirbright, UK). Pooled sera collected from pigs at 21 dpv were assayed in SENASA (National Service for Agrifood Health and Quality), the WOAH reference laboratory for Foot-and-Mouth Disease in Argentina. Type O field viruses (50 isolates collected over a period of 10, from 2013 to 2022) belonging to O/ME-SA/Ind2001, O/ME-SA/PanAsia, O/SEA/Mya98, and O/CATHAY were tested in all cases. In the present study, VN titer of the pool sera greater or equal to 1.5 (Log10) was considered as an indicator of minimum heterologous cross-protection, as previously proposed1-3.

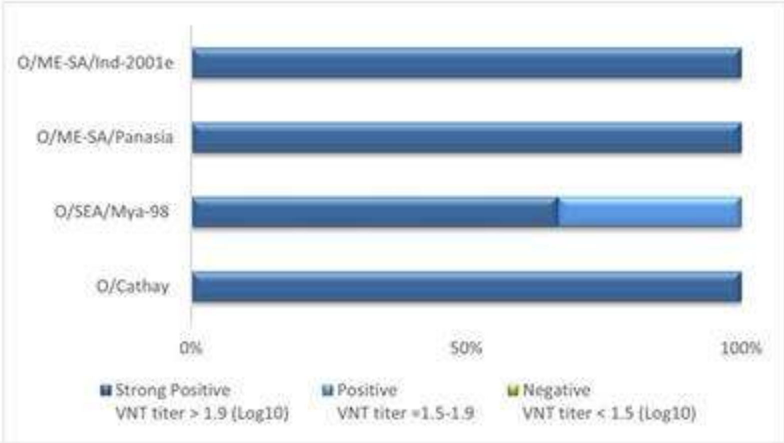
Results:
Virus neutralization titers obtained with both bovine and swine sera against all type O viruses tested were above the established protective threshold, indicating a close relationship between the field viruses and the O1 Campos vaccine strain. Furthermore, strong positive results (titer greater than 1.9 Log10), were obtained in 94% (N=47) of the isolates tested.

Conclusion and Discussion:
Highly immunogenic and widely cross-protective vaccine strains are key factors for the success of vaccination programs. The results of this study demonstrate the wide immunogenic spectrum of Biogenesis Bago world-class vaccines formulated with O1 Campos

vaccine strain against circulating viruses in Pools 1 and 2. Strong heterologous VNT titers support the use of these vaccines for systematic vaccination in these endemic settings, and the inclusion of such vaccines to control emergencies in free regions.

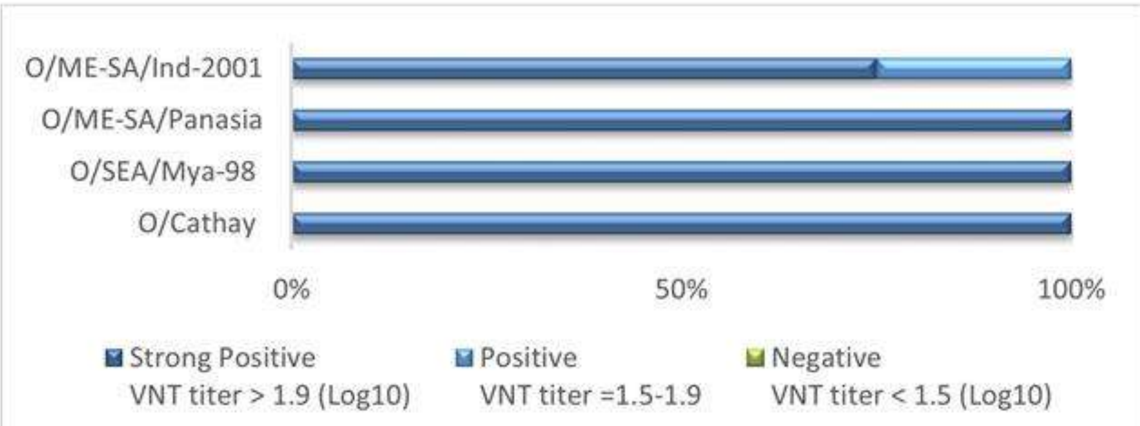
Graphs or Images 1

Figure 1. Vaccine matching studies for O field isolates collected during 2017-2022 belonging to different topotypes/lineages (n=34). VNT was performed at Pirbright Institute (WRL for FMD) employing O1 Campos **bovine** pooled sera (28 dpv) from Biogenesis Bago vaccine. Data are shown as the proportion (%) of isolates in different vaccine matching categories (>1.9; 1.5-1.9; <1.5 log10) according to the VNT titer obtained. VNT titers = > 1.5 log10 was considered as an indicator of minimum heterologous cross-protection, as previously proposed



Graphs or Images 2

Figure 2. Vaccine matching studies for O field isolates collected during 2013-2019 belonging to different topotypes/lineages (n=16). VNT was performed at SENASA, Argentina employing a O1 Campos **pig** pooled sera (21 dpv) from Biogenesis Bago vaccine. Data are shown as the proportion (%) of isolates in different vaccine matching categories (>1.9; 1.5-1.9; <1.5 log10) according to the VNT titer obtained. VNT titers = > 1.5 log10 was considered as an indicator of minimum heterologous cross-protection, as previously proposed.



P-T-009

PHYLOGEOGRAPHIC INVESTIGATION OF LINEAGE 1A PRRSV 2 TRANSMISSION IN TAIWAN FROM 2018 TO 2022

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- Introduction

In recent years, the study of porcine reproductive and respiratory syndrome virus (PRRSV) showed the significant increase in the detections of lineage 1A, and that may have been caused by the international spread of NADC34-like strains. In 2018, the lineage 1 strain was first detected in PRRS outbreak herd in Taiwan [1]. In early 2021, severe clinical outbreaks related to lineage 1A PRRSV strains occurred on many pig farms in Taiwan, including "abortion storms" in the sow herd with high mortality in piglets. The lineage 1A PRRSV strain has spread among pig farms in Taiwan since its introduction in 2018. The aim of this study was to understand molecular epidemiology, evolution model and phylogeographic distribution of lineage 1A PRRSV in Taiwan

- Materials and Methods

ORF5 nucleotide sequences of lineage 1A PRRSV (n=46) from field isolates were obtained from 2018 to 2022. Markov chain Monte Carlo (MCMC) sampling using BEAST v1.10.4 was employed to infer the time-scaled phylogenies of ORF5 genes. GTR+Γ+I substitution and uncorrelated relaxed clock were used prior to setting coalescent population constants in the MCMC simulations. Five hundred million MCMC cycles were performed and set the parameter to all 50,000 states to infer the posterior evolutionary parameters. Finally, the phylogeographic reconstructions were visualized on the EvoLaps website [2].

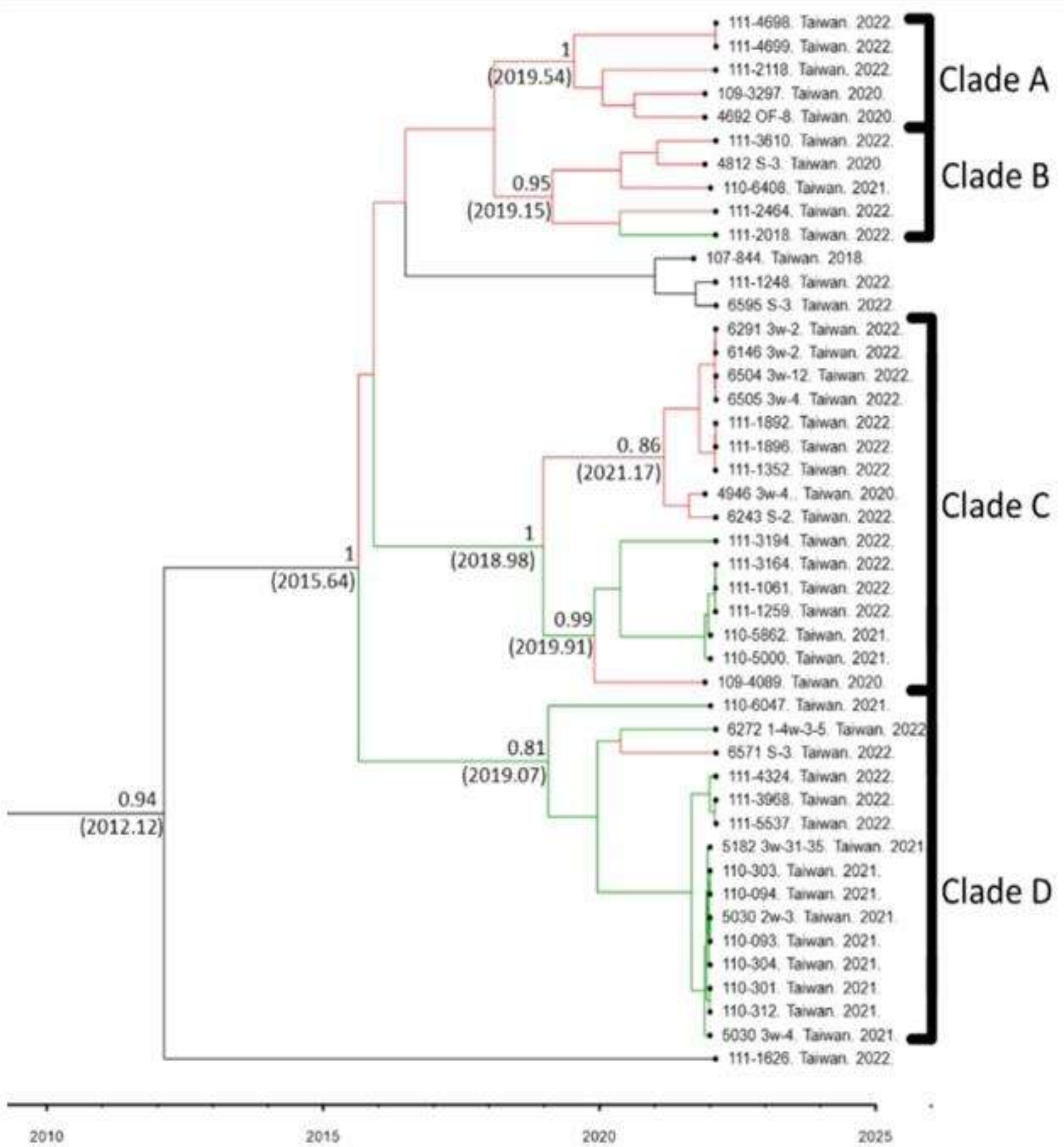
- Results

Our results showed that Taiwanese lineage 1A strains from 2018 to 2022 were divided into four clades (A, B, C and D). Four clades exhibited statistically significant posterior probabilities (>0.8), including 41 viral strains on 4 monophyletic branches. The most recent common ancestor (MRCA) for four clades was traced to June 2015. The phylodynamic coalescent tree indicated the involvement of multiple virus strains in the outbreak. Molecular sequence data were visualized using EvoLaps website. The preliminary introduction of the virus was inferred in northern and western regions of Taiwan. Subsequently, the western strains spread to the north and south, among which the southern group evolved into clade A-D. Later, clade B, C and D circulated both in western and south region until today.

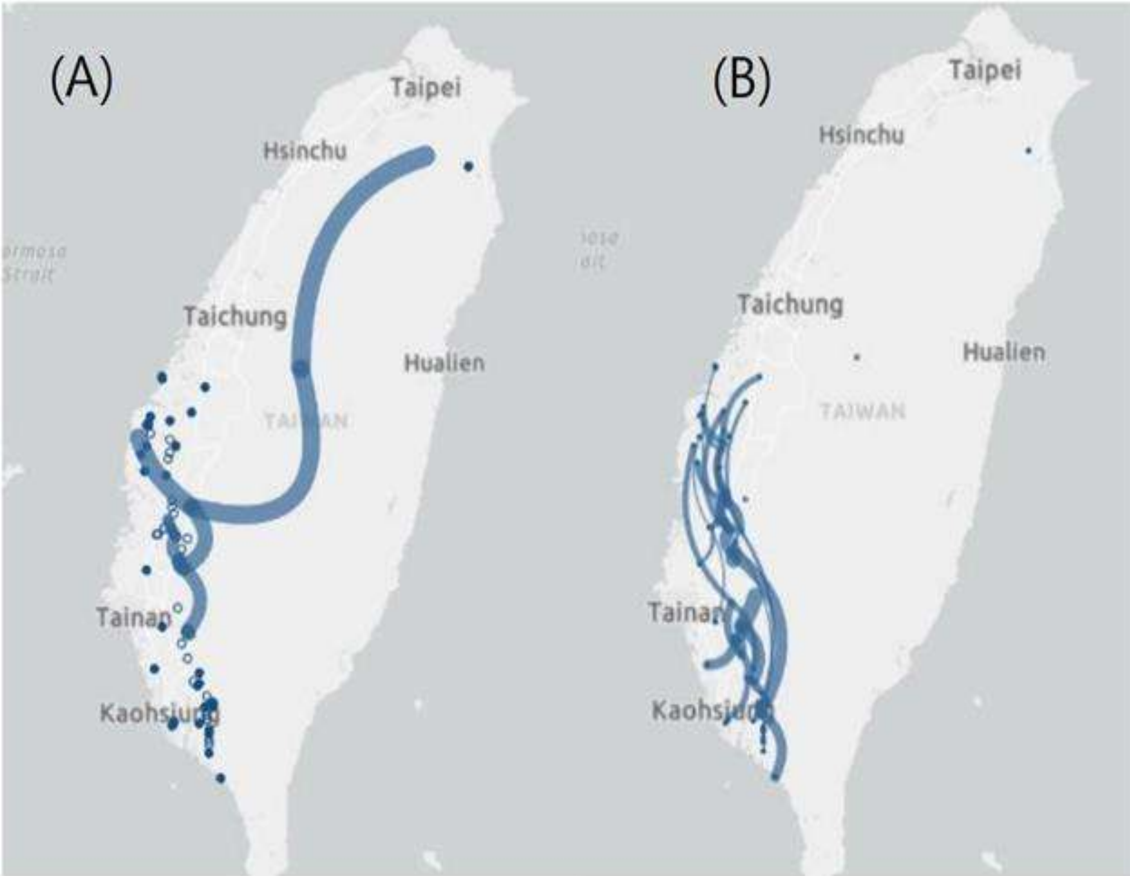
- Conclusions and Discussion

In the present study, we provide up-to date knowledge with regard to the phylogeographic pattern of circulating lineage 1A PRRSV strains in Taiwan in the outbreak after 2018. Our results can provide helpful information for disease control strategies.

Graphs or Images 1



Graphs or Images 2



P-T-010

POSITIVE EFFECTS OF ALGAL BETA GLUCAN ON VIRAL REPLICATION OF PRRSV AND ASFV IN PULMONARY ALVEOLAR MACROPHAGES (PAM).

Dr. Alex de Leon[1], Dr. Rafael Hermes[1], Valentine Van Hamme[2], Ricardo Neto[2], Susanne Kirwan[2], Siti Fatimah[1], Hans Lee[1], Tinh Nguyen[1], Vannie Wan[1], Hà Thanh[3], Dang Vu Hoang[3]
[1]Kemin Asia, [2]Kemin Europa , [3]Department of Biochemistry and Immunology, National Institute of Veterinary Research

PRRS and ASF cause a huge economic impact on pig producers worldwide (OIE) and there is a need for additional tools to help control them (Cubillos et al., 2013; Kapoor et al., 2017). Two studies were done to determine the effect of Algal Beta Glucan in the replication of PRRSv and ASFv in PAM cells. Both studies used an in vitro macrophage model, as the virus is macrophage trophic. In the PRRS study, PAM cells were incubated for 1 hour with Algal Beta Glucan at a rate of 0.1, 1, and 10 µm/mL and then challenged with PRRSV strain MN184. The supernatant was collected at different time points (0, 12, 24, and 36 hours after infection) for the determination of viral growth by qRT-PCR. The results showed that the highest concentration of Algal Beta Glucan provided the most reduction in 24 hours and the response was dose dependent.

The overall results show suppression of viral growth as low as 0.1 µm/mL dilution of algae beta-glucan. In the ASF study, PAM cells were incubated at different times (15, 30, 60, and 120 mins) and concentrations of Algal Beta Glucan of 30 and 50 µm/mL before being challenged with 10^2-3 HAD50/ml ASFv Ha Nam strain. PAM cells incubated with β-glucan for 2 hours reduced significantly ASFv invasion up to 12 hours post-infection compared with the control group.

Moreover, the titer of ASFv confirmed by HAD assay showed that the titer of ASFv was significantly reduced at 12 hours post-infection when compared to the positive control. IFN-α and IL-6 production induced by Algal Beta Glucan was also assessed in this ASF study using sandwich ELISA. Production of IFNα and IL-6 were significantly increased in both treated groups (30 µg/ml or 50 µg/ ml) at 12 hours and remained until 24 hours when compared with the control groups ($p < 0.05$) This indicated that the high dose of β-glucan enhanced significantly the IFN-α and IL6 production in PAMs in response to ASFv infection. The results showed that Algal Beta Glucan is potentially a useful tool for a better PRRS and ASF-protected pig herd through a hypothetically direct effect on viral replication.

References:

OIE. 2012. African swine fever. In: Manual of diagnostic tests and vaccines for terrestrial animals.
Cubillos, C., Gomez-Sebastian, S., Moreno, N., Nun ez, M.C., Mulumba-Mfumu, L.K., Quembo, C.J., Heath, L., Etter, E.M., Jori, F., Escribano, J.M. and Blanco, E. 2013. African swine fever virus serodiagnosis: a general review with a focus on the analyses of African serum samples. Virus Res. 173, 159–167.
Kapoor, R., Sharma, B. and Kanwar, S.S. 2017. Antiviral phytochemicals: an overview. biochem physiol: open access 06.

P-T-011

SEROLOGIC INVESTIGATION OF FOOT-AND-MOUTH DISEASE IN PIGS IN KINMEN FROM 2020 TO 2022

Mr. Ming-Chang Lee[1], Ms. Ze-Hui Huang[1], Ms. Yun-Hsuan Chiang[1], Dr. Shih-Ping Chen[1]
[1]*Agricultural Technology Research Institute*

Introduction
Foot-and-mouth disease (FMD) is caused by infection with FMD virus and is one of the most contagious infectious diseases of cloven-hoofed animals. Vaccination has been successfully used for controlling FMD in Taiwan, which has been recognized as an FMDV-free zone without use vaccine but Kinmen not included. To achieve this it was necessary to demonstrate freedom from virus circulation in the field and that required effective means of differentiating vaccinated from convalescent animals (Chen et al., 2007). This is a major requirement for countries with compulsory FMD vaccination programs. Recently, methods such as NSP ELISA kits have become available that provide a reliable procedure to detect infected animals in vaccinated populations. Several commercialized FMDV NSP ELISA kits have recently been validated and shown to be useful in monitoring NSP antibody responses in pigs (Chen et al., 2011).

Materials and methods

Sera collecting：

For serologic investigation, a total of 4,081 sera collected from 136 pig farms from 2020 to 2022 were used. (Table 1)

Measurement of antibody to NSPs:
Serum antibodies to FMDV NSPs were measured by PrioCHECK FMDV NS Antibody ELISA kit (Prionics AG, Zurich, Switzerland), ID Screen® FMD NSP Competition ELISA kit (ID.vet, Grabels, France) and the Sentinel® FMDV NSP Ab ELISA kit (Excelsior Bio-Systems Inc., Taipei City, Taiwan) are all competitive ELISA tests to detect serum antibodies against FMDV non-structural 3ABC recombinant protein.

Results
1. Three of 2,023 sera (0.29%) collected from 62 pig farms were shown NSP positive in 2021. All the sera collected from 2020 and 2022 were shown NSP negative. (Table 2)
2. FMD virus has never been detected from the OP fluid by virus isolation and polymerase chain reactions. Meanwhile, there are no clinical observations found in pigs.
3. Although no clinical case was confirmed through further investigation, all NSP positive pigs were then culled and slaughtered to minimize the risk of potential FMDV transmission.

Conclusions and Discussion
1.The comparative findings indicated FMD viruses not circulated in pigs even though the few of positive serological evidences found in pigs in Kinmen.
2. The FMD surveillance program is supervised by the LADIAs, AHRI, ATRI and BAPHIQ. Once the positive of NSP of FMD was detected in the serum samples collected. It will be trace back to the original farm by veterinarians of the LADIA to examine the clinical situation of the animals and another 15 serum samples will be collected for NSP test. The detailed follow-up investigations involving clinical, serological and virologic testing. By doing this, we can verify the infection status more clearly and stop the virus spreading as possible as it can.

3. For the biological security concern, movement of man, animal, equipment and trucks must be controlled. Selecting the appropriate disinfectant for each disease is also essential.

References
1. Chen, S.P., Ellis, T.M., Lee, M.C., Cheng, I.C., Yang, P.C., Lin, Y.L., Jong, M.H., Robertson, I.D., Edwards, J.R., 2007. Comparison of sensitivity and specificity in three commercial foot-and-mouth disease virus non-structural protein ELISA kits with swine sera in Taiwan. Vet. Microbiol. 119, 164–172.
2. Chen T.H., Fan L., Lin Y. L, Dekker A., 2011, Differentiation of FMD-infected pigs from vaccinated pigs using antibody-detection sandwich ELISA. J. Vet. Med. Sci. 73(8): 977-984.

Table 1

Table 1. The number and source of the sera used in the study.

Year / Source	2020		2021		2022	
	Farm no.	Sera no.	Farm no.	Sera no.	Farm no.	Sera no.
Field	18	293	18	368	18	364
Slaughter house	0	0	44	1,655	38	1,401

* no. = number.

Table 2

Table 2. The NSP Ab detecting results of this investigation.

Year	2020	2021	2022
Farm no.	18	62	56
Sera no.	293	2,023	1,765
NSP (+) no.	0	3	0

* no. = number.

P-T-013

DEVELOPMENT OF HIGH-EFFICIENCY CELL-ADAPTED VACCINE CANDIDATE AGAINST AFRICAN SWINE FEVER VIRUS

In-pil Mo[1], Prof. Jong-Soo Lee[2], Sung-sik Yoo[3], Hyeok-il Kwon[3], Seung-Chul Lee[3], Min-ho Kim[3], Joo yong Lee[3], In-joong Yoon[3], Yeonji Kim[4], Yongkwan Kim[4], Weonhwa Jheong[4]
[1]Avinext, [2]College of Veterinary Medicine, Chungnam National University, [3]ChoongAng Vaccine Laboratories Co., Ltd, [4]National Institute of Wildlife Disease Control and Prevention

African swine fever (ASF) is a highly contagious hemorrhagic disease of domestic pigs and wild boars caused by the African swine fever virus (ASFV). Recently, ASFV has extended its geographical area from Central Europe to East Asia, including Korea, causing tremendous economic losses to the local swine industry. Although experimental vaccines derived from naturally occurring, genetically engineered or cell culture-adapted ASFV have been tested, there is no still commercial vaccine available for ASFV.

In this study, we developed a cell-adapted live attenuated ASFV vaccine candidate and the vaccine efficacy was tested in domestic pigs. We isolated ASFV from domestically infected wild boars and the virus was continuously passaged into the CA_CAS-01_A cell line. Replication of ASFV in CA_CAS-01_A cells increased with serial passages, and a specific single clone of ASFV (ASFV-11893) from several clones were isolated after 18 passages. NGS analysis was performed on passaged ASF virus and we found specific deletions and mutations in several genes. In order to define the safety and efficacy of this attenuated vaccine candidate (ASFV-11893), ASFV-11893 were administrated intramuscularly to domestic pigs.

As a result, ASFV-11893 was completely attenuated in pig as pigs inoculated with 2 doses of 105 HAD50 remained healthy without signs of the disease. Importantly, all immunized pigs conferred 100% protection against lethal parental ASFV challenge, which correlated with high ASFV-specific antibody titers. Overall, these results suggest that ASFV-11893 could be a safe and potential attenuated candidate against the current ASFV outbreak.

P-T-014

THE SUPPLEMENTATION EFFECT OF SPORULATED IMMUNOGEN PROBIOTICS (SIP) THAT CARRIED SYNTHETIC ASF IMMUNOGEN EXPRESSION ON THE LEVEL OF SECRETORY-IMMUNOGLOBULIN A IN SALIVA/ORAL FLUID OF REPLACEMENT GILTS

Dr. Yaowaluck Maprang Roshorm[2], Dr. Kanokporn Polyiam[2], Dr. Namphueng Butkhot[2], Dr. Jaturong Ekawatpanich[3], Dr. Sujin Sukchai[3], Prof. Kampon Kaeoket[1]

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Introduction

African swine fever is a devastating viral disease, caused a high lethality of nearly 100% in domestic pigs and wild boars (1). Currently, ASF outbreaks continuously spread across the world including Asia. At the moment, various vaccines were launched and tested for efficiency and safety in pigs with variation of results such as the deletion of the I177L conserves gene attenuated vaccine, seven genes deleted attenuated vaccine (HLJ/18-7GD) and the SY18-1226RGD as a conserved gene (2, 3). In some countries, unlicensed ASF vaccines were withheld because of their turning virulence and leading to genetic mutation combined with field strains and vaccines (1). Bacillus subtilis is widely used as a probiotic in pig farm worldwide as it regulates intestinal flora, improves feed efficiency, enhances mucosal immunity, has short fermentation cycle and able to form heat tolerance spores. With the DNA recombination technology, B. subtilis can be used as a potential immunogen expression vector for the prevention of various diseases caused by viruses (4). Thus, we validated the Sporulated Immunogen Probiotic (SIP) by using B. subtilis'spores as an ASF virus immunogen expression vector, with an aim to provide mucosal immunity against ASF virus (S-IgA) in saliva/oral fluid of pigs.

Materials and Methods

The immunogen genes (CD2V, P72 etc.) was synthetically made and integrated into the genome of Bacillus subtilis using double-crossover homologous recombination. Recombinant clones were selected based on auxotrophic marker and grown in special medium. PCR amplification confirmed a successful integration of the immunogen gene in the B. subtilis chromosome at the desired locus and then candidate clones was culture in sporulating medium. When detected by Western blot, the immunogen could be detected in the crude proteins extracted from the B. subtilis spore coat layers, so called Sporulated Immunogen Probiotics (SIP). SIP carried ASF virus immunogen expression in this study was mixed with other species of Lactobacillus acidophilus, Lactobacillus plantarum and Pediococcus pentasaceus. Each species contains 1.0 x 1010 cfu/kg. We used 90 replacement gilts. They were allocated into 3 groups: group A (control, n=30) was fed with commercial probiotics 1 kg/ton for a period a month, Group B was fed with SIP product 1 kg/ton for a period of a month and Group C was fed with SIP product 1 kg/ton for two months. Saliva samples from 10 gilts in each group were collected by rope-assisted swab method before and 2, 4 weeks after feeding, and were further analysis for S-IgA by using indirect ELISA.

Result and Discussion

The level of S-IgA antibody against all synthetic peptides in pig saliva is showed in Figure 1. At 4 weeks after feeding with SIP, there was a higher level of S-IgA in saliva/oral fluid in groups 2 and 3, compare with control. This indicated that the SIP carried ASF virus immunogen expression is able to stimulate pig mucosal immunity particularly in the small intestine (mucosal inductive site) and homing to other mucosal tissue (mucosal effector site) which may lead to the prevention of ASF virus infection by oronasal route (5). However, further study is needed in order to investigate whether S-IgA in the saliva/oral fluid of gilts has the ability to neutralize the ASF virus. In conclusion, feeding gilts with SIP carried ASF virus immunogen expression product lead to an increased level of S-IgA that specific to synthetic peptides in the saliva/oral fluid.

References

1. Li Z. et al. (2022). Life. 12(8):1–41.
2. Borca MV. et al. (2020). J Virol. 94(7):1–15.
3. Chen W. Sci China Life Sci. 63(5):623–34.
4. Penghao LV. et al. (2020). J. Vet. Med. Sci. 82(11): 1693–1699.
5. Brandtzaeg P. et al. (2008). Mucosal Immunol. 1(1): 31-37.

Graphs or Images 1

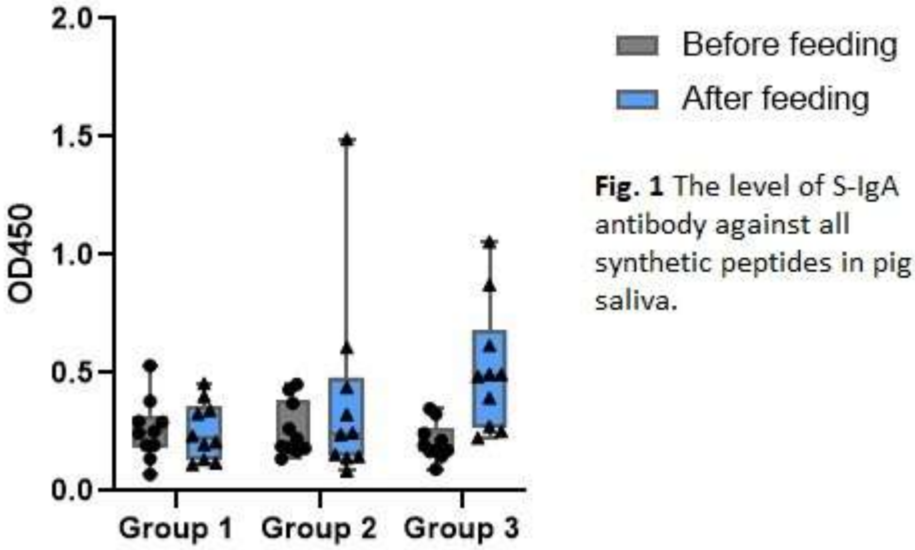

Fig. 1 The level of S-IgA antibody against all synthetic peptides in pig saliva.

P-T-015

VETERINARY AYURVEDA : A HOLISTIC APPROACH TO ONE HEALTH MISSION

Dr. Shivi Maini[1]
[1]Indian Herbs

One Health Mission
For most of the 20th century, society viewed veterinary medicine primarily as an animal health medical discipline. In recent years, highly publicized public health threats have helped many realize the fundamental linkages between veterinary medicine and human health. According to the World Health Organization (WHO), one or more new infectious diseases have emerged each year since the 1970s (WHO 2007). The majority of these have been zoonoses, diseases caused by pathogens that can be transmitted between animals and humans, with more than three-quarters originating from wildlife. Of the 1400 diseases now recognized in humans, 64% are caused by pathogens transmissible across species. These trends have led to support for a more integrated and holistic approach to human, animal and environmental health. Known as One Health, this approach has received growing attention over the past decade among policy makers, practitioners and funding agencies seeking more effective prevention, control and treatment responses in an increasingly populous and globalized world.
Holistic Approach in Veterinary Medicine
Holistic Veterinary Medicine is a term that the American Veterinary Medical Association defines as “a comprehensive approach to health care employing natural and conventional diagnostic and therapeutic modalities. In practice, it incorporates but is not limited to the principles of acupuncture and acutherapy, botanical medicine, chiropractic, homeopathy massage therapy, nutraceuticals and physical therapy as well as conventional medicine, surgery and dentistry”. The vision of this approach is to take the best of both holistic, natural approaches to animal health and combine them with the best of conventional veterinary medicine. No one form of medicine has all the answers, cures to all problems. It just seems prudent to take the best from all fields and integrate them into a new comprehensive form of animal health care. Where do we start with integrative animal health care then?
Holistic approach is to offer the livestock a balanced approach to health care, offering the best of both conventional or natural and complementary medicine. Ayurvedic preparations incorporate ingredients derived from plant origin. Emergence of the resistant pathogenic strains against antibiotics & deadly chemicals coupled with ever growing concerns of toxicity & environmental contamination has lead to scientific & technological advancement in last few decades, reviving the interest of modern scientist & health care practitioners in herbals. Herbal medicine is today a valid & proven science, with great potential for integration with allopathic medicine to the benefit of human & animal health.The medicinal or antimicrobial properties of plant-derived substances have been well known for centuries. This property is mainly attributed to the essential oils and plants viz carom, thymol, curcumin, anthraquinones & many more. Oregano and thymol are among those which have received a great deal of interest. These plants contain the monoterpenes, carvacrol and thymol. Many other essential oils and bioactives. Eucalyptus oil, Ocimum sanctum and Cedrus deodara oils have demonstrated high efficacy in vitro against several pathogens found in the intestinal and respiratory tract. This suggests that phytogenic feed additives are suitable replacements for in-feed antibiotics to improve livestock health and growth performance, for control of gut & respiratory infections and for prevention of zoonotic diseases. Traditional system of medicines has stood the test of tie for over 4000 years and should not be considered as an alternate to the modern medicine; rather they complement and enhance the production of livestock. Researchers have found that ethnoveterinary treatments are primary recourse for

livestock owner when their animal is ill. If ethnoveterinary practices blend with complementary medicine this will open a wide scope towards one health mission.

P-T-016

WHOLE GENOME ANALYSIS OF AFRICAN SWINE FEVER VIRUS IN THE 2022 OUTBREAKS IN DOMESTIC PIGS IN SOUTH KOREA

Mr. Tae-Young Suh[1], Mr. Ji-Hoon Park[1], Ms. Hae-Eun Kang[1], Mr. Jun-Gu Choi[1]
[1]*Animal And Plant Quarantine Agency*

- Introduction

African swine fever (ASF) is a highly contagious and lethal disease affecting both domestic pigs and wild boars. It is caused by the African swine fever virus (ASFV), a large enveloped virus with a double-stranded DNA genome of approximately 190 kilobase pairs. In recent years, the rapid spread of ASF across the world has caused significant economic losses in the pig industry. In Korea, the disease has continued to occur sporadically in pig farms and spread among wild boars since the first outbreak in 2019. To effectively control the spread of ASF, it is crucial to identify the genetic mutations and epidemiological characteristics of the African swine fever virus. Next-generation sequencing (NGS) technology provides a powerful tool for analyzing the large genome of ASFV.

- Materials and Methods

Whole blood samples used for this study were collected from ASF outbreak pig farms. The viral DNA was extracted and fragmented to approximately 200 bp for NGS library preparation. To capture the target regions, the NGS libraries and probes were hybridized using the Celemics target capture kit (Celemics, Korea). The captured DNA libraries were subsequently sequenced on the Illumina NextSeq 500 system as paired-end reads. The DNA reads generated from NGS were paired, and low-quality reads were trimmed using the BBDuk trimmer in Geneious Prime Version 2023.0.4 (BIOMATTERS INC., U.S.A.). The trimmed reads were then mapped against the Georgia strain (ASFV Georgia 2007/1, GenBank: FR682468.2) using the Geneious mapper.

- Results

In this study, seven ASFVs isolated from 7 domestic pig farms in Gyeonggi and Gangwon province in Korea in 2022 were analyzed with NGS. All viruses belonged to genotype Ⅱ and intergenic region type Ⅱ and showed 99.9 genetic identity with both the Georgian strain(ASFV Georgia 2007/1) and the first Korean isolate: ASFV/Korea/pig/Paju1/2019(Paju1/2019). Compared to ASFV Georgia 2007/1, all eight Korean isolates, including Paju1/2019 strain shared most of the nucleotide changes in coding and non-coding regions and showed close phylogenetic relatedness with each other.

- Conclusions and Discussion

Our analysis indicates that the genomes of the 2022 domestic ASF outbreaks in Korea are closely related to that of the Paju1/2019 strain. Further investigation is needed to elucidate the biological consequences caused by such nucleotide changes. The results of this study will provide valuable information on the molecular epidemiology and genetic diversity of ASFV outbreaks in Korea.

- Acknowledgement and References

1. Cho KH, Kim DY, Jang MK, Hong SK, Ryu JH, Kang HE, Park JY. Genetic Characterization of African Swine Fever Virus from Pig Farms in South Korea during Outbreaks in 2019-2021. Viruses. 2022 Nov 24;14(12):2621. doi: 10.3390/v14122621. PMID: 36560625; PMCID: PMC9784228.

2. Kim G, Park JE, Kim SJ, Kim Y, Kim W, Kim YK, Jheong W. Complete genome analysis of the African swine fever virus isolated from a wild boar responsible for the first viral outbreak in Korea, 2019. Front Vet Sci. 2023 Jan 11;9:1080397. doi: 10.3389/fvets.2022.1080397. PMID: 36713858; PMCID: PMC9875005.
3. Forth JH, Tignon M, Cay AB, Forth LF, Höper D, Blome S, Beer M. Comparative Analysis of Whole-Genome Sequence of African Swine Fever Virus Belgium 2018/1. Emerg Infect Dis. 2019 Jun;25(6):1249-1252. doi: 10.3201/eid2506.190286. Epub 2019 Jun 17. PMID: 30907724; PMCID: PMC6537744.

P-V-001

A CASE STUDY OF STABILIZING A PIG FARM INFECTED BY NADC30-LIKE PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS THROUGH HERD CLOSURE AND MEDICATION PROGRAMS

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Introduction:
Since the first isolate of NADC30-like PRRS virus was reported in 2013, this Lineage 1 PRRSV strain has been circulating in the field and presenting major health challenges in the Chinese swine industry. Unfortunately, the current commercial vaccines cannot provide sufficient protection against NADC30-like strain, and it was found to be very difficult to eliminate the virus from infected farms [1-2]. The study objective was to evaluate the effects of herd closure, medication programs and monitored using processing fluids and production data as strategic management protocols to stabilize a NADC30-like PRRSV-infected farm.

Materials and methods:
In early 2021, a 3000-sow farrow-to-finish farm in Fujian Province experienced a severe PRRS outbreak. The strain circulating in the farm was later identified as NADC30-like PRRSV by gene sequencing. A commercial PRRS-modified live vaccine (CH-1R strain) was used but turned out to be a failure. The overall survival rate of different batches of commercial pigs ranged from 70% to 85%.

Starting from October 2021, a few interventions were systematically performed as follows: [i] Herd closure for 12 months (no introductions to the defined population after proper cleaning and disinfection procedures). [ii] Stop using PRRSV MLV. [iii] For the sow herd medication, 1.5 kg RuiTiMei� (Tilmicosin Premix, Zoetis) and 2kg RuiDuoKang� (Doxycycline Hyclate Soluble Powder) were added into each ton of feed for 21 days continuously medication, once every 3-month. [iv] For the newborn piglets, 0.2 ml of Excede� (Ceftiofur crystalline free acid, Zoetis) per pig was intramuscularly injected at three days of age while 0.2ml of Draxxin� (Tulathromycin, Zoetis) per pig were injected at 21 days of age. [v] For the wean-to-finish pigs, 0.5ml of Draxxin� per pig was injected at 70 days of age. Lastly from 100 days of age, 1.5 kg RuiTiMei� and 2 kg RuiDuoKang� were added into each ton of feed for 14 days continuously medication. [vi] From January 2022, McREBEL (Management Changes to Reduce Exposure to Bacteria to Eliminate Losses from PRRS) procedures were implemented, and all the weaned pigs were transferred to another clean site.

From July 2021 to September 2022, 34 consecutive batches of processing fluids were collected from all litters for PRRSV loads assay by qPCR. Meanwhile, the survival rate of each batch of commercial pigs was recorded.

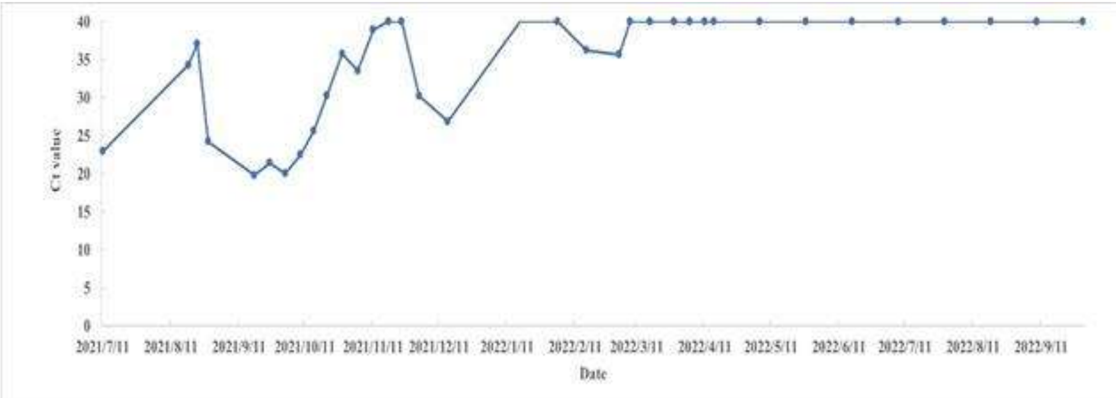
Results:
Based on the qPCR results for PRRSV, Ct values of processing fluids samples were relatively low in September and early October 2021. After the intervention measures were taken (October 2021), the Ct values gradually increased, indicating the viral load decreased. Ct

values reached negative status in March 2022 and lasted for six months (Fig. 1). Production data showed that before the intervention, the survival rate of each batch ranged from 75% to 88.5%, with an average survival rate of 84.1%. After the interventions, the survival rate of each batch ranged from 87.7% to 96.9%, with an average survival rate of 93.1% (Fig. 2). The absence of clinical respiratory signs in subsequent batches supported the NADC30-like PRRSV negative status. These intervention measures improved the survival rate of production pigs by 9% on average.

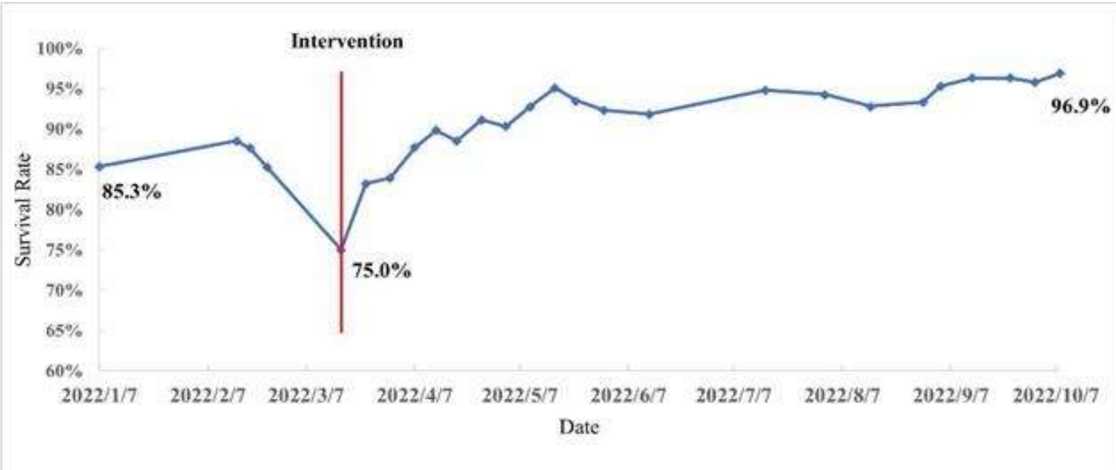
Conclusions and discussion:
Based on this exercise, using a period of 12 months for herd closure, medication programs and other strategic management protocols (McREBEL, multi-site production and aggressive monitoring by processing fluids and production data) greatly helped to stabilize the subsequent production herds in this farm.

References:
[1] Trevisan G et al. Porcine Health Management (2019) 5:18
[2] Trevisan G. et al, J Swine Health Prod. (2020) 28 (5): 244-253

Graphs or Images 1



Graphs or Images 2



P-V-002

A CSF VACCINE BASED ON E2 RECOMBINANT GLYCOPROTEIN AND ADJUVANTED WITH OIL-IN-WATER EMULSION INDUCES A FULL PROTECTION IN A PIG FIELD TRIAL

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[1]*Seppic*

Introduction
Classical swine fever (CSF) is a highly contagious viral disease which causes important economic losses in the pig industry. Even if many countries have succeeded in eliminating CSF, it remains endemic in South and Central America, Eastern Europe and Asia. Systematic prophylactic vaccination is the most effective strategy to control CSF in the endemic zones. In this aim, live attenuated vaccines have been widely used to control the disease but these conventional vaccines fail to identify infected from vaccinated animals (Differentiating Infected from Vaccinated Animals). Thus, a new generation of CSF DIVA vaccines are needed.

Materials and Methods
In this study, a recombinant vaccine, based on the highly immunogenic structural glycoprotein E2 of CSF virus, is assessed in a pig trial.
Two groups of 7 pigs are intramuscularly injected in the neck with 2 ml of the E2 recombinant CSF vaccine at day 0 (D0) and D21. The vaccines are either adjuvanted with a carbomer or with an oil-in-water emulsion (Montanide™ ISA 28R VG ; 15/85 w/w). A third control group is left unvaccinated. After each vaccination, the body temperature and the local reactions at the injection site are monitored. The body weight gain is also controlled at D21 and D35. Moreover, blood samples are taken at different dates in order to assess the E2 specific antibodies by ELISA. At D35, a lethal dose of Shi-Myn strain of the CSF virus is intramuscularly injected into the posterior femoral muscle. After challenge, the vaccine protection is evaluated by calculating the survival rate and by PCR detection of CSF virus in the blood samples and in the spleens and lymph nodes.

Results
In terms of safety, the 2 adjuvanted vaccines were very well tolerated: no abnormality was noticed in the injection site, the body temperatures did not exceed one celsius degree after injections and the body weight gains were normal. Regarding the efficacy, vaccine groups showed a similar antibody profile with a positive threshold reached after the boost at D21. After challenge, only the vaccine adjuvanted with the Montanide™ ISA 28R VG induced a survival rate of 100% while that based on carbomer failed to fully protect the pigs (57%). The monitoring of the viremia also underlined an early and total clearance of the virus with the emulsion based vaccine compared to the carbomer vaccine.

Conclusions and Discussion
Taken together, these results showed that E2 recombinant CSF vaccine associated with an oily adjuvant as Montanide™ ISA 28R VG is a good vaccine candidate and adapted to protect pigs from the disease while maintaining a good safety profile.

Acknowledgement and References

Table 1

	Nb of survival pigs D21 after challenge	% of protection
ISA 28 R	**6 / 6***	**100 %**
Carbomer	4 / 7	57 %
Infected ctrl	0 / 5	0 %

Table 2

Rate of positive piglets in virus detection after challenge (D35)

		D39	D43	D47	D51	D56
Blood Sample	ISA 28R	66%	50%	0	0	0
	Carbomer	100%	100%	100%	83%	0
Lymph Node	ISA 28R					0
	Carbomer					85%
Spleen	ISA 28R					0
	Carbomer					29%

P-V-003

A NEW FORMULA OF CLASSICAL SWINE FEVER E2 SUBUNIT VACCINE AGAINST CLASSICAL SWINE FEVER VIRUS ALD STRAIN

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Introduction
Classical swine fever (CSF) caused by CSF virus (CSFV), is one of the most devastating and highly contagious pig diseases that affect the swine industry worldwide with a past outbreak in Taiwan (1,2). Continued vaccination research with live attenuated CSF vaccine and E2 glycoprotein subunit vaccines offer an efficacious measure of disease control and eradication (3). Elimination of live vaccines and cost-effectiveness analysis of adjuvanted subunit vaccines ready for use are important topics to consider for the possibility to declare Taiwan free from CSF (4,5).

Materials and Methods
In this study, a novel recombinant E2 protein vaccine was developed and its efficiency on specific-pathogen-free (SPF) pigs was compared with a conventional E2 vaccine. The new formula of this vaccine consists of the E2 recombinant antigen expressed by a eukaryotic expression system, species-specific CpG adjuvant and a dual phase adjuvant. Humoral response detection by ELISA. The serum CSFV-anti-E2 antibodies were quantified by ELISA, (IDEXX CSFV Ab test) based on the kit's instructions. Antibody levels were represented as blocking percentage ≥ 40% was considered positive and suitable for CSFV infection prevention.

Results
The E2 subunit vaccine composition can confer effective immune protection against the strong virulent ALD strain of CSFV via a single vaccination without boost vaccination (Figure 1).

Conclusions and Discussion
In this study, all groups, except for the negative control group and positive control group, produced CSFV E2-specific antibodies (higher than the 40% cut off) after 4 weeks of vaccination. Strangely, the amount of E2-specific antibody produced by the double-dose group was slightly lower than the single-dose groups, but altogether they were higher than the positive control group. In conclusion, this study presents evidence that a single dose of a novel E2 subunit vaccine can effectively stimulate immunity against heterologous CSFV (CSFV ALD strain). The present invention relates to an E2 subunit vaccine composition against CSFV. These results support a new perspective to reduce the concentration and dosage of E2 recombinant proteins required to induce humoral immune responses in the piglet population. The present vaccine not only is safer but also has equal or even better immune efficiency than conventional ones. In addition, immunity responses of piglets vaccinated with this E2 subunit vaccine are differentiated from those inoculated with conventional vaccines and can be detected using differential diagnostic kits. A new formula of CSF E2 subunit vaccine is ready for use to control measures to CSF outbreak any time.

Acknowledgment
We kindly thank all members in the RCAB, VMII207, and FS105 of NPUST, Taiwan, for providing computing, storage resources, and assisting the diagnostic works. We also thank

ATRI (Agricultural Technology Research Institute, Taiwan) and ADIB (Animal Drugs Inspection Branch, Animal Health Research Institute, Taiwan) for providing animal house and assistance.

References

1. Pan, C.H. et al. Phylogenetic analysis of classical swine fever virus in Taiwan. Arch. Virol. 2005, 150(6), 1101-1119.
2. Zhou, B. Classical swine fever in China-an update minireview. Front. Vet. Sci. 2019, 6, 187.
3. Bouma, A. et al. Efficacy and stability of a subunit vaccine based on glycoprotein E2 of classical swine fever virus. Vet. Microbiol. 1999, 66(2), 101-314.
4. Chen, J.Y. et al. Evaluation of classical swine fever E2 (CSF-E2) subunit vaccine efficacy in the prevention of virus transmission and impact of maternal derived antibody interference in field farm applications. Porc. Health Manag. 2021, 7(1), 9.
5. Madera, R. et al. Pigs immunized with a novel E2 subunit vaccine are protected from sub-genotype heterologous classical swine fever virus challenge. BMC Vet. Res. 2016, 12, 197.

Table 1

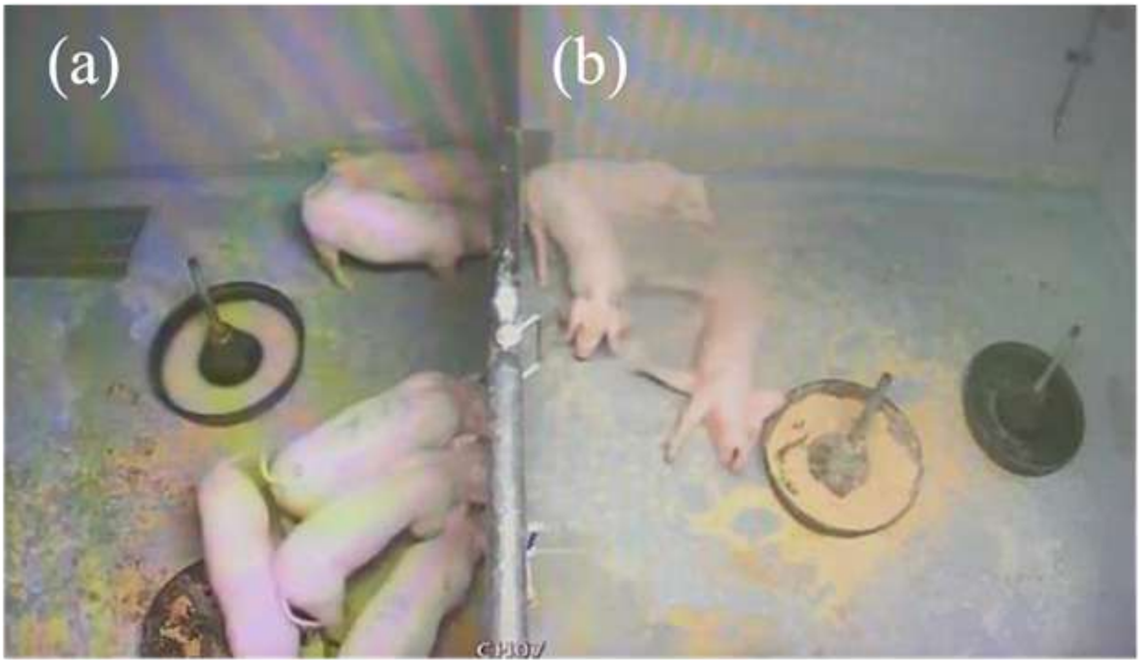

Figure 1. Clinical signs：5 days post-challenge (a) Six vaccinated pigs (single dose & two doses) normal. (b) Three no-treatment pigs (N) showed depression, anorexia, and shivering.

P-V-004

A NEW VACCINE CONTAINING TWO ANTIGENS, PCV2A AND PCV2B, SHOWED IMPROVED GROWTH PERFORMANCE UNDER PCV2 SUBCLINICAL INFECTION IN JAPAN

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Introduction
Porcine circovirus virus-associated disease (PCVAD) caused by porcine circovirus type 2 (PCV2) infection is still economically important diseases despite current PCV2 vaccines can prevent and control this disease. In recent years, it is becoming evident that PCV2 subclinical infection (PCV2 SI) causes important economic losses comparable to PCVAD [1]. Recently, a new vaccine containing two antigens, PCV2a and PCV2b, has shown to provide broad cross-protection against major circulating PCV2 genotypes[2]. The objective of this study was to evaluate the efficacy of the vaccine under PCV2 SI in Japan.

Materials and Methods
This field study was conducted in a commercial Farrow to Finish 700 sow farm. PCV2a monovalent vaccine (vaccine A) had been routinely administered to piglets at 21 days old. No clinical symptoms had been observed and PCV2 virus had not been detected at any stage by PCV2 real-time PCR, but seroconversion to PCV2 was observed by ELISA testing in 60-day-old pigs, and mortality increased in the period from 60 - 120 days of age. Therefore, the farm veterinarian decided to switch from Vaccine A for the new PCV2 vaccine, with the aim to evaluate the relation between mortality and PCV2 SI, and the efficacy of the vaccine controlling PCV2 SI. Blood samples were collected at 30, 60, 90, 120 and 150 days of age (5 pigs per time point). All samples were tested with a PCV2 ELISA test and with a PCV2 real-time PCR. Mortality was calculated and compared with the data obtained in the previous period when using Vaccine A.

Results
The total of 18,438 piglets, equivalent to a year of weaned pigs, were included the study. The new PCV2 vaccine significantly reduced mortality in the period of 60 - 120 days of age compared to previous Vaccine A, despite neither clinical symptoms were seen nor PCV2 virus was detected at any age by PCV2 real-time PCR ($p<0.05$, Table 1). There was no difference in ADG (Table 1). Seroconversion to PCV2 was detected by ELISA test at 60 days of age in both periods, when using the Vaccine A and during the study with the new vaccine (Graph 1).

Conclusions and Discussion
Use of the new PCV2 vaccine resulted in better performance in a PCV2 SI compared to the period using Vaccine A. To reduce the negative economic impact caused by PCV2, it is important to estimate the timing of PCV2 infection by not only real-time PCR but also ELISA test, and select an appropriate PCV2 vaccine for farms with or without clinical symptoms caused by PCV2.

Acknowledgement and References
1) Alarcon, P., et al., Prev. Vet Med. 2013. 110(2):88-102.
2) Bandrick, M., et al.,Vet Immunol Immunopathol. 2020. 223:110034.

Table 1

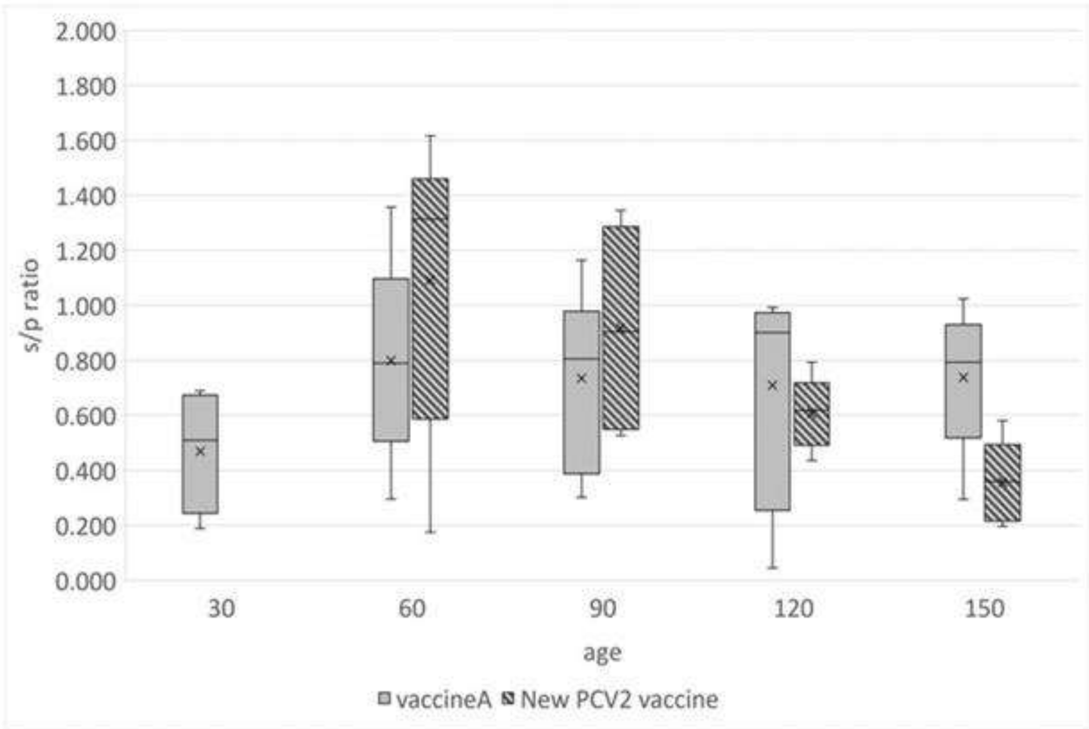
Table 1: Comparison of Mortality between Vaccine A and the new PCV2 vaccine

PCV2 Vaccine	Vaccine A	New PCV2 vaccine
Number of piglets	9,367	9,071
Post weaning Mortality	1.0%	0.8%
Mortality from 60 to 120 daysof age	0.6%[a]	0.3%[b]
ADG	730 g/ day	738 g/day

a, b: $p<0.05$ by Chi-squared test

Graphs or Images 1

Graph 1: s/p ratio of PCV2 ELISA from 30 to 150 days of age



P-V-005

A NOVEL BIVALENT SUBUNIT VACCINE IN A SINGLE DOSE AGAINST CLASSICAL SWINE FEVER VIRUS ALD STRAIN AND PORCINE CIRCOVIRUS TYPE 2

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Introduction
Porcine circovirus type 2 (PCV2) infection, a high-risk factor, impairs pig immune response development during its weaning to growth stage, which is also a crucial period to piglets for receiving the vaccine against classical swine fever virus (CSFV) (1). Huang et al. (2011) demonstrated that PCV2 infection may attenuate the CSFV vaccine strain (LPC vaccine) efficacy (2). It's important to prevent piglets against both virus infections. However, no commercialized bivalent vaccine of PCV2 and CSFV is currently available.

Materials and Methods
In this study, we developed a novel bivalent subunit vaccine and evaluated its efficiency as using a single dose against CSFV ALD strain and PCV2 on SPF pigs. This bivalent subunit vaccine consists of soluble CSFV-E2 and PCV2-ORF 2 antigens, species-specific CpG adjuvant and a dual phase adjuvant. A safety evaluation of the bivalent subunit vaccine was performed at Agricultural Technology Research Institute (ATRI) and a total of 20 SPF pigs were separated into vaccinated and non-vaccinated piglets (n=10 in each group). Then, both of the vaccinated and non-vaccinated piglets were further divided into 2 sub-groups for two efficacy trials by challenged with either CSVF or PCV2 (n=5 in each sub-group), which were performed by Animal Health Research Institute, Council of Agriculture (AHRI) and National Pingtung University of Science and Technology (NPUST), respectively (Figure 1).

Results
Results show that piglets vaccinated with this bivalent subunit vaccine in a single dose significantly increased the antibody titers to against both CSFV ALD strain and PCV2 and these high titers can last at least two months after vaccination.

Conclusions and Discussion
In this study, a novel CSFV/PCV2 bivalent subunit vaccine was developed and results confirmed that the bivalent subunit vaccine is safe when given at the ages of 4 or 5 weeks in pigs and its high efficacy in a single dose to significantly increase antibody titers and efficient protection against CSFV and PCV2. Thus, it will be benefit to pig farmers to control both diseases in infected areas.

Acknowledgment
We kindly thank all members in the RCAB of NPUST, Taiwan, for providing computing, storage resources, and assisting the diagnostic works. We also thank ATRI (Agricultural Technology Research Institute, Taiwan) and AHRI (Animal Health Research Institute, Council of Agriculture, Taiwan) for providing animal house and assistance.

References
1. Chen, J. et al. The impact of porcine circovirus associated diseases on live attenuated classical swine fever vaccine in field farm applications. Vaccine. 2019, 37 (43), 6535-6542. https://doi.org/10.1016/j.vaccine.2019.08.039.

2. Huang, Y.L. et al. Porcine circovirus type 2 (PCV2) infection decreases the efficacy of an attenuated classical swine fever virus (CSFV) vaccine. Vet Res. 2011, 42, 10.1186/1297-9716-42-115.

Graphs or Images 1

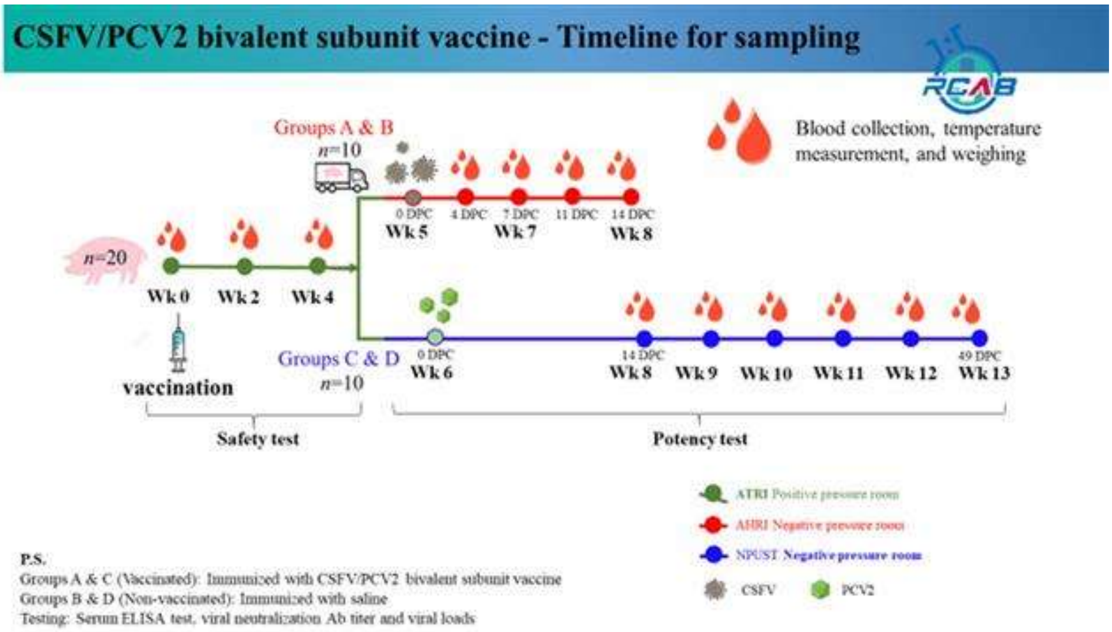

Figure 1. Schema of the experimental design in this study.

P-V-007

AFRICAN SWINE FEVER VIRUS B175L INHIBITS TYPE I INTERFERON PATHWAY TARGETING STING AND 2’3’-CGAMP

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Introduction:
The type I interferon pathway (IFN-I) pathway operates a pivotal role in the first line of host immune defense against pathogens. This study found B175L as one of the IFN-I antagonists in the African Swine Fever Virus (ASFV).

Materials and Methods:
Our STING-mediated IFN-β luciferase activity found B175L as one of IFN-I antagonists. First, we assessed the GFP-tagged DNA virus phenotypes in PK15 and PAM stable cells by fluorescence microscopy and cytokine ELISA kits. Second, we estimated the phosphorylation of IFN-I molecules using immunoblotting. Third, the STING and B175L binding was confirmed by mass-spectrometry, followed by different overexpression and endogenous immunoprecipitation assays in HEK293T, PK15, and PAM stable cells. The confocal imaging was used to visualize the co-localization of STING and B175L in HeLa and PAM stable cells. Specifically, the SDD-AGE assay was used to test the STING polymerization, and the in vitro binding assays were performed with 2’,3’-cGAMP-Biotin or Cy5 conjugates and purified STING/B175L proteins.

Results:
We found that the conserved zf-FCS motif of pB175L bound with both cGAMP and STING obstructed the cGAMP/STING interaction. The pB175L/STING binding occurred at the cyclic dinucleotide binding domain (CBD) of STING (185-270aa), and we found STING R238 and Y240 residues were crucial for this interaction.

Conclusions:
Our results demonstrate the tight control of STING signaling by ASFV B175L, which could be one of the broad operative strategies used by ASFV to escape from the host immunity during infection.

Acknowledgement:
This work was supported by National Research Foundation (Grant no. 2021R1A6A1A03045495), Ministry for Food, Agriculture, Forestry and Fisheries (Grant no. 119081-5) and Ministry of Environment (Grant no. NIWDC-2021-SP-02), Republic of Korea.

References:
1. Ahn. et al. 2019. Exp & Mol Med. 51: 1-10.
2. Cui S. et al. 2022. Virus Research: 198872

P-V-008

African Swine Fever virus EP364R and C129R degrade 2'3'cGAMP and Negatively Regulate the cGAS STING Pathway

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Introduction:
For efficient virus infection, ASFV has developed complex strategies to evade the key components of antiviral innate immune responses. In this study, we discovered the host immune invasion mechanism of ASFV nuclease homologs EP364R and C129R proteins.

Materials and Methods:
The effect of ASFV genes on DNA virus replication was evaluated by infecting GFP tagged ADV, HSV and VACV viruses into ASFV EP364R and C129R plasmid transfected PK-15 cells, PAMs and MA104 cells. To determine the target of ASFV genes, IFN-β and NF-κB luciferase assays were conducted. cGAMP degradation was evaluated by cGAMP ELISA and HPLC in vitro using purified ASFV proteins. IBMX, a pan phosphodiesterase inhibitor was used to inhibit ASFV gene-induced cGAMP degradation to check the ASFV genes-cGAMP interaction. STING aggregation assay was performed to check the STING activation by cGAMP.

Results:
EP364R and C129R proteins increased DNA virus replication and reduced IFN-β and IL-6 production resulting inhibited type I IFN-induced ISG gene transcription. Luciferase assays found that both ASFV genes target mammalian second messenger 2’3’cGAMP. ASFV genes inhibited the type I IFN production degrading 2’3’cGAMP exerting their phosphodiesterase activity. HPLC data showed cGAMP degraded by-products were neither AMP nor GMP. Sequence analysis of EP364R gene found a cGAMP binding motif similar in STING that competes with STING for cGAMP interaction. In vitro mutagenesis of ASFV-EP364R indicated that the Y76 and N78 amino acids interact with 2’3’cGAMP and amino acid mutations restored subsequent antiviral responses.

Conclusions:
Results highlight a critical role of EP364R and C129R concerning inhibition of IFN responses and could be provided for the development of ASFV live attenuated vaccine.

Acknowledgement:
This work was supported by the National Research Foundation (Grant no. 2021R1A6A1A03045495) and the Ministry of Environment (Grant no. NIWDC-2021-SP-02).

References:
1. Li L. et al. 2014. Nat. Chem. Biol 10: 1043-1048.
2. Hernáez B. et al. 2020. Sci. Adv. 6: 38.

P-V-009

AFRICAN SWINE FEVER VIRUS PROTEIN L11L EVADES HOST TYPE-I INTERFERON SIGNALING BY TARGETING IRF3

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African swine fever (ASF) is a highly contagious disease caused by the African swine fever virus (ASFV), which renders 100% mortality in domestic and wild pigs. ASFV holds diverse immune evasion mechanisms to increase its pathogenesis by producing different kinds of viral proteins with specific functions. L11L is one of those ASFV proteins produced at late time points by ASFV, and the role of L11L is unknown. In this study, we investigated the molecular mechanism of L11L in evading the type-I interferon pathway in order to escape the host immune response. L11L downregulated the IFN-β production by interacting with the interferon regulatory factor 3 (IRF3). Further examination revealed that L11L binds with the autoinhibitory domain (AIE) of IRF3, which is responsible for the phosphorylation of IRF3 by the tank binding kinase I (TBK1).
Moreover, we found that L11L loses the binding with IRF3 5D mutant, containing five amino acid (aa) mutations in the AIE domain of IRF3 and these amino acids are essential for this L11L-IRF3 interaction. Consequently, L11L inhibited IRF3 phosphorylation and subsequently impaired IRF3 dimerization. Altogether these findings conclude that L11L escapes host immune response by targeting IRF3 and subsequently antagonizing type-I IFN production.
[National Research Foundation (2021R1A6A1A03045495), Ministry for Food, Agriculture, Forestry and Fisheries (119081-5) and Ministry of Environment (NIWDC-2021-SP-02)], Republic of Korea]

P-V-010

AFRICAN SWINE FEVER VIRUS UK GENE TARGETS IRF3 TO DOWNREGULATE THE CGAS MEDIATED IFN PATHWAY

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The highly contagious African swine fever virus (ASFV) is currently causing a pandemic affecting wild boar and domestic pigs, causing catastrophe for the swine industry. ASFV has evolved multiple strategies to eliminate the host's innate immune responses for efficient infection. However, the evasion mechanism of ASFV remains elusive. Upon ASFV infection, the cytosolic DNA sensor cyclic GMP-AMP (2'3'cGAMP) synthase (cGAS) induced anti-viral signals transferred to interferon regulatory factor 3 (IRF3) and inhibitor of nuclear factor kappa-B (NF-κB) kinases (IKKs) via stimulator of interferon genes (STING) to produce interferons (IFNs) and pro-inflammatory cytokines, respectively. This study demonstrated a novel immune evasion mechanism of the ASFV DP96R (UK gene) that blocks IRF3 & IKK subunit alpha (IKKα) dependent anti-viral responses. Mechanistically, ASFV DP96R interacted with the IRF3 nuclear localization signal (NLS), inhibiting IRF3 nuclear translocation. Ectopic expression of DP96R reduced cGAS-STING signaling and anti-viral gene transcription, enhancing DNA and RNA virus replication and blocking the IFN-β and interleukin-6 (IL-6) production in porcine alveolar macrophages (PAMs). Most notably, the ASFV DP96R is associated with the major karyopherin alpha (KPNA) binding residues of IRF3 to inhibit KPNA-IRF3 interaction. Besides, DP96R specifically interacted with and inhibited the phosphorylation of IKKα, hampering the activation downstream of NF-κB. In particular, we discovered that ASFV DP96R central region is crucial in suppressing IFNs and pro-inflammatory cytokines production. Although the DP96R gene is widely used to develop live attenuated vaccines, no precise mechanism of innate immune invasion has yet been discovered. Our results highlight the crucial role of the highly conserved ASFV DP96R in invading the innate immune system. [National Research Foundation (2021R1A6A1A03045495), Ministry for Food, Agriculture, Forestry and Fisheries (119081-5) and Ministry of Environment (NIWDC-2021-SP-02)], Republic of Korea]

P-V-011
AN EFFICACY OF PRRSV-2 MLV (PRIMEPAC™ PRRS) AGAINST AN EXPERIMENTAL CHALLENGE WITH A TYPE 2 PRRSV FIELD ISOLATE

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[1]*Immunopathology Lab, College of Veterinary Medicine, Konkuk University,* [2]*Farm&Pharm veterinary hospital*

Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) causes severe economic losses worldwide by inducing various problems including reproductive failure and respiratory distress [1]. Vaccination is considered one of an effective measure to control this disease worldwide. In this study, we evaluate the efficacy of a type 2 PRRS modified live vaccine (PrimePac™ PRRS) against challenging of a type 2 PRRSV isolated in Republic of Korea in experimental conditions.

Materials and Methods: Thirty-four 3-week-old PRRSV naïve pigs were divided in 5 groups and individually identified by ear tag. Intra-dermal (ID) vaccinated challenged group (group 1, n = 8), intra-muscular (IM) vaccinated/challenged group (group 2, n = 9), ID vaccinated/unchallenged group (group 4, n = 7), and IM vaccinated/unchallenged group (group 5, n = 7) were immunized with 0.2ml (ID) and 1ml (IM) of PrimePac™ PRRS vaccine at 3 weeks old. Placebo group (group 3, n = 3) was injected with 1ml of PBS at the same time. At 28 days post vaccination (dpv), group 1,2 and 3 were intranasally challenged with 5ml of inoculum containing 105.01 TCID50/ml of type 2 PRRSV isolated from commercial pig farm and characterized by viral genomic sequencing. Blood samples were collected at 0, 3, 6, 13, 20, 27, 31, 33, 35, and 42 dpv. PRRSV-specific serum antibody response was determined by commercial ELISA kit (IDEXX PRRS X3 Ab test, USA) and viremic statue was analyzed by real-time PCR.

Results: All the groups were virus free and -seronegative to the PRRSV at 0 dpv. Seroconversion was detected in all vaccinated groups (group 1, 2, 4 and 5) from 13 dpv. All vaccinated groups showed higher PRRSV-specific antibody titer than placebo group at 31, 33 and 35 dpv (Fig. 1). After vaccination, most of pigs in vaccinated groups started showing viremia until 6 dpv. The challenged virus in sera of both vaccinated challenged group decreased from 31 dpv more than that of placebo group and no virus was detected at 42 dpv in group 1 (Fig. 2).
The clinical score and fever in both vaccinated-challenged group also were lower than placebo group after challenge.

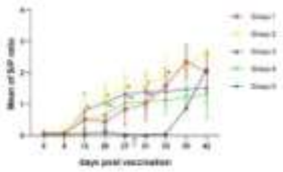
Conclusions and Discussion: In this study, we evaluated the efficacy of PRRSV-2 MLV (PrimePac™ PRRS). Based on our results, this MLV showed PRRSV specific humoral immune response, decreased viremia, less febrile reaction and better clinical scores after field isolate challenge that indicate the vaccine is efficacious against type 2 PRRSV isolated in pig farms suffering from PRRSV in the Republic of Korea.

Acknowledgement and References
[1] Kwon, Taeyong, et al. Virology 530 (2019): 89-98.

Figure legends
Figure 1. Mean PRRSV ELISA S/P ratio of each group. Red arrow indicates the point of challenge. An asterisk (*) and double asterisks (**) indicate the significant difference (p <

0.05; Kruskal–Wallis Dunn's Multiple comparison) in group 1 vs 3 and group 5 vs 3, respectively.
Figure 2. Mean PRRSV titers (log10TCID50/ml) in sera of each group. Red arrow indicates the point of challenge.

Graphs or Images 1



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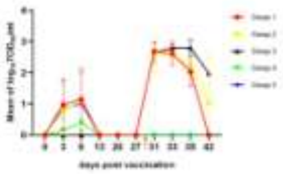

P-V-012

ANALYSIS OF E2 GENE SEQUENCES OF ATYPICAL PORCINE PESTIVIRUS IN TAIWAN

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Introduction:
Atypical porcine pestivirus (APPV) is a positive single-stranded RNA virus, belonging to species Pestivirus K of genus Pestivirus, family Flaviviridae. APPV was first characterized in the United States in 2015 and then reported as the causative agent of congenital tremor type A-II in newborn piglets in many countries, threatening the global pig industry [1, 2]. In Taiwan, APPV-positive cases have been reported in recent years; nonetheless, the investigation of the genomic sequence has not been conducted yet. In order to clarify the characteristics of APPV local strains in Taiwan, the aims of this study were to perform the phylogenetic analysis of the E2 gene and compare the sequences of both nucleotides and amino acids with APPV strains from other countries.

Materials and Methods:
Neurological (cerebrum, cerebellum, and lumber spinal cord), submandibular lymph nodes, tonsil, and serum specimens were collected from piglets with congenital tremor, sent to Animal Diseases Diagnostic Center, National Pingtung University of Science and Technology from January 2020 to April 2022. APPV E2 gene cloning and sequencing were performed for positive specimens detected by RT-qPCR [3]. All E2 sequences obtained from clinical specimens were aligned with 126 E2 sequences downloaded from NCBI GenBank (access date: 1 June 2022) for phylogenetic analysis, nucleotide and amino acid similarity analysis.

Results:
A total of 16 cases were detected as positive from 50 symptomatic cases. The E2 gene was successfully sequenced from only one case. This may be related to the high complexity and variability of the innate characteristic of APPV E2 gene. According to the phylogenetic analysis of E2 genes, APPV strains could be divided into three major clades. The virus strains in the second and third clades were from China and Japan respectively. By contrast, strains belonging to the first clade were widely distributed in several countries. The local strain, APPV1092520Taiwan2022, was classified into the first clade as the red dot shown in Figure 1 and presented a distinctive branch. In the nucleotide similarity analysis, the average nucleotide identity (ANI) of local strain and other references showed the range 80.2-88.7%, with the lowest to strain AHSG201801 from China and the highest to strain wbOR13370 from South Korea. In addition, the amino acid similarity accounted for 88.8-95.9%, with the lowest to the same Chinese strain AHSG201801 and the highest to strain HN2018 which was also from China.

Conclusions:
This study provided the initial phylogenetic analysis result of APPV E2 gene in Taiwan and showed the unique classified branch comparing to other virus strains within clade 1. Since the primary neutralizing epitope (PNE) or decoy epitope (DCE) of APPV E2 sequences has not been mentioned and elaborated so far, further research is needed to reveal the mutations of nucleotides and amino acids and their effects on viral pathogenesis.

Acknowledgement:
We gratefully acknowledge the technicians and veterinarians in ADDC of NPUST for their contributions to this study.

References:
[1] Gatto I.R.H. et al., 2019. Front. Vet. Sci. 6: 35
[2] Hause B.M. et al., 2015. J. Gen. Virol. 96(10): 2994-2998.
[3] de Groof A. et al., 2016. Viruses. 8(10): 271

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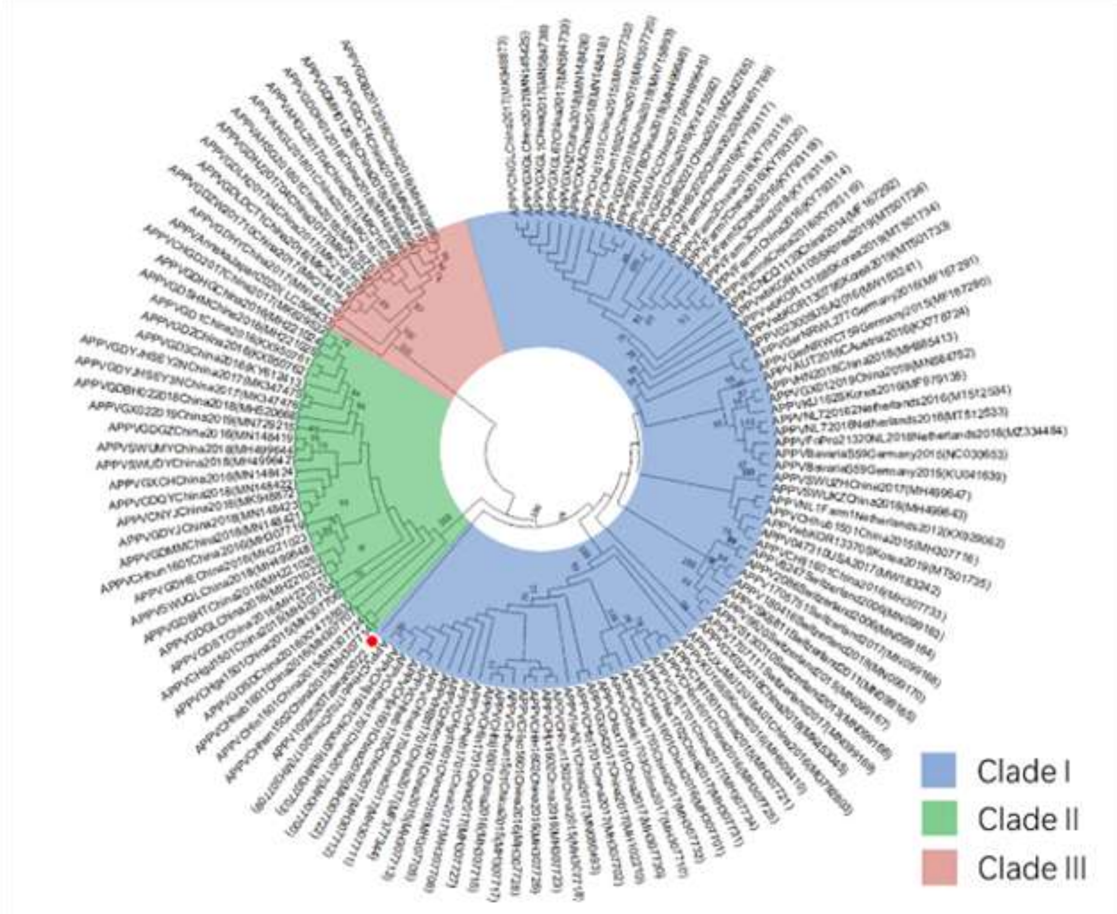

Figure 1. Phylogenetic analysis of 127 APPV E2 genes by neighbor-joining method, 1000 bootstraps. The red dot is the local strain, APPV1092520Taiwan2022.

P-V-014

ASSESSMENT OF THE EFFECTIVENESS OF PCV2D AND PCV2B INACTIVATED VACCINES IN PIGS CHALLENGED WITH PCV2D

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Introduction
Porcine circovirus type 2 (PCV2) is an important swine pathogen causing porcine circovirus-associated diseases (PCVAD) and finding ways to effectively control it is crucial for the well-being of pigs and the economic viability of the swine industry. Most of the current commercial PCV2 vaccines are based on PCV2a and the experimental and field evaluations of the commercially available PCV2a-based vaccine have shown its effectiveness in reducing viremia caused by both PCV2a and PCV2b, as well as the associated lesions and diseases. However, as the global PCV2 genotype shift from PCV2b into PCV2d, concerns regarding vaccine failure have been raised. The objective of this study was to evaluate the efficacy of inactivated and adjuvanted PCV2b- and PCV2d-based vaccines in the specific pathogen-free (SPF) pig model against PCV2d field isolate challenge.

Materials and Methods
Thirteen three-week-old SPF pigs that are free of PCV2 were randomly divided into three groups, a non-vaccinated group of three pigs, a group of five pigs vaccinated with the commercial PCV2b-based inactivated vaccine (SuiShot® Circo-ONE) and a group of five pigs vaccinated with the inactivated and adjuvanted PCV2d-based vaccine. At 21 days post vaccination, all pigs were simultaneously challenged with a PCV2d field isolate (PCV2d/CBNU0324) via both the intranasal and intramuscular routes of inoculation. The experiment was concluded 21 days post virus challenge.

Results
The study found that PCV2d/CBNU0324 infection alone can trigger PCVAD. Both PCV2b- and PCV2d-based vaccinations demonstrated remarkable efficacy in protecting against PCV2d infection compared to the non-vaccinated challenged group, as indicated by a reduction in viral loads in nasal swabs, serum samples, and lymphoid tissues and improved growth performance. Furthermore, both types of PCV2 vaccines were found to effectively protect PCV2-associated pathological lesions based on histopathology and immunohistochemistry.

Conclusions
In conclusion, the results of this study demonstrate that the inactivated and adjuvanted PCV2b- and PCV2d-based vaccines are effective in protecting against PCV2d infection in specific pathogen-free pigs. The findings also suggest that a PCV2b commercial vaccine induced effective cross-protection against a heterologous PCV2d challenge as equivalent to the homologous PCV2d vaccination.

Discussion
Globally, vaccination has been identified as one of the most crucial and effective strategies to control PCV2-related diseases. As observed with the use of PCV2 vaccines in 2–3-week-old antibody-positive pigs, vaccination has had a major positive impact on decreasing PCVAD in the field. The study was conducted to evaluate the protective efficacy of heterologous

(PCV2b-based) and homologous (PCV2d-based) PCV2 vaccines against PCV2d challenge. Vaccinated groups (PCV2b-Vac and PCV2d-Vac), in contrast, showed significantly higher protective efficacy against PCV2d challenge than the Non-Vac group.

Acknowledgement
This study was funded by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through the “Technology Development Program for Bioindustry (315029-3)” and the “BioGreen21 Agri-Tech Innovation Program (Project No. PJ015969)” of the Rural Development Administration, Republic of Korea.

P-V-015

CASE REPORT: NOVEL PRRS VACCINE BY CPD IN NSP1 PROTECTED PIGS AND SHOWED STABLE GROWTH PERFORMANCE IN KOREAN PIG FARMS.

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[1]*Biopoa*, [2]*Eulji University*

Field porcine reproductive and respiratory syndrome viruses (PRRSV) isolated from PRRS-affected swine farms were attenuated by codon pair deoptimization in NSP1. This chimeric PRRSV live vaccine which is developed by reverse genetic engineering in Korea protected pigs and showed enough serological result and growth performance without severe clinical signs, which were observed in pig infected with the wild type viruses. Especially this vaccine showed significantly lower replication ability and higher secretion of IFN-γ. Pigs vaccinated with novel vaccine show stable growth performance against wild type PRRS virus. Conclusively, the vaccine developed by CPD, reverse genetic engineering in this case demonstrated proven effectiveness as novel vaccine to provide new control option to swine veterinarian against current PRRSV status in Korea.

P-V-016

CASE REPORT: RESOLUTION OF CSF & PRRS OUTBREAK BY WHOLE HERD INGELVAC PRRS MLV AND PESTIFFA VACCINATION

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[1]AGRITECH ENTERPRISE SDN BHD, [2]BOEHRINGER INGELHEIM (MALAYSIA), [3]FACULTY OF VETERINARY MEDICINE UPM

Introduction: Classical swine fever (CSF) is an economically important swine disease with high prevalence in Southeast Asia countries (1). Studies have proven that PRRS infection could suppress immunity response induced from CSF vaccination, leading to vaccination failure (2). This report describes a case of CSF and PRRS coinfection in which resulted in a farm loss in high porker mortality due to secondary respiratory bacterial diseases. This field case also summarises the improvement of production followed with changes in CSF breeder vaccination program in combination of whole herd PRRS control using PRRS MLV vaccine.

Material and Methods: A 450-sow farrow-to-finish pig farm was reported with high weaner mortality (17.99%) and sudden mortality spike of starter-grower (1.14%) in November 2020. All gilts/sows were vaccinated against CSF, FMD, AD and parvovirus. Porkers were vaccinated against CSF, Mycoplasma hyopneumoniae and PCV2 during weaning time around 28-30 days post farrowing. Both weaners and porkers showed clinical signs of depression, anorexia, conjunctivitis, puffy eyes, redden extremities and respiratory distress. Most porkers died from coinfections of CSFv, Glaeserella parasuis and Actinobacillus pleuropneomoniae with significant pathological lesions such as haemorrhagic tonsils, splenic infarction, fibrinous pleuritis and fibrinous peritonitis. CSFv was confirmed by PCR detection in both sera and lymph node of weaners. Serum Neutralisation Test (SNT) performed 6 to 12-weeks-old weaners (n=30) sera revealed non-protection (SNT<8). At the same time, blood samples collected from sows (n=15) and 3-day-old piglets (n=10) showed vertical infection from sows to piglets and heterologous challenge of both type I and type II PRRSv. In this case, PRRS mass vaccination using Ingelvac PRRS MLV were immediately carried out in breeder herd followed with immunisation in piglets one week before weaning. Concurrently, CSF vaccination program in sows was changed from post-farrowing (previously attenuated GPE negative strain live vaccine) to pre-farrowing using attenuated CL-strain (Pestiffa) due to restriction of previous CSF vaccine in pregnant sows. Farm also implemented several measures including sufficient colostrum intake for the first 6 hours after birth, no cross-fostering policy and culling of old sows (>parity 10). The statistical analysis was generated using Minitab software version 21. Blood samples were collected once again from sows (n=15) and processing fluid from 1-week-old piglets (n=150) from farrowing house 6 months after implementation of PRRS & CSF control and subjected to PRRS RT-PCR and CSF Elisa.

Results and discussion: Weaner mortality reduced from 17.99% to 8.95% within 8 months (Figure 1), while porker mortality also reduced to 0.39% (Figure 2). Calculated return on investment (ROI) was 12.9:1. After Ingelvac PRRS MLV vaccination, PRRSv was not detected from both sows and 1-weeks old piglets, suggesting the vertical transmission of PRRSv was stopped. PRRSV infection prior to CSF vaccination can significantly interfere with induction of anti-CSFV immunity. Previous studies also showed that PRRSV infection poorly induced innate immune responses followed by delayed viral-specific immunity, and impaired lung defence mechanisms (4) that led to bacterial problem in this case.

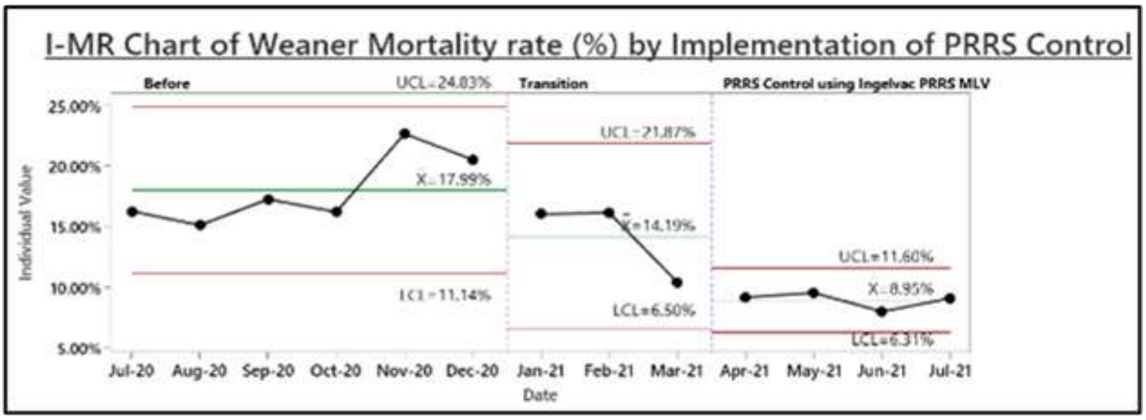
Figure 1: Weaner mortality.

Figure 2: Porker mortality.

Conclusion: The result that PRRSV infection significantly inhibited the CSF vaccine efficacy confers severe implications to the swine industry especially in CSFV-endemic countries with high prevalence of PRRSV. The finding from this case suggested that PRRS control should be prioritised during coinfection of PRRSV and CSFV. A holistic approach from vaccination optimisation to maximise the herd immunity and farm husbandry changes to minimise the pathogen load is crucial to control the outbreak.

References:
[1] Retrieved from WOAH website:
https://rr-asia.woah.org/en/projects/classical-swine-fever-csf/.
[2] S. Suradhat et al., 2006, Veterinary Microbiology 119 (2007). 1–9.
[3] S. Suradhat et al., 2005. Vaccine 24 (2006). 2634-2642.
[4] Beyer et al., 2000. J Vet Med 2000, B47:9-25.

Graphs or Images 1



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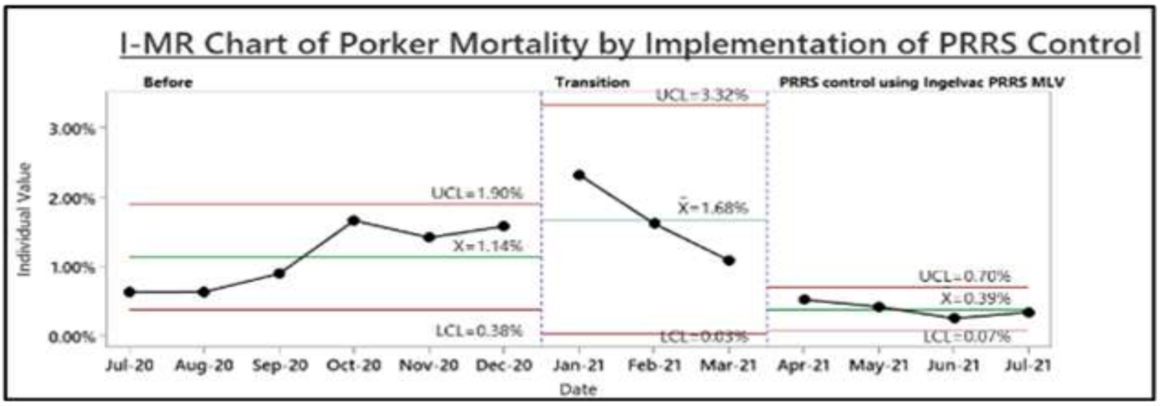

P-V-018

CHARACTERIZATION OF THE SUBCLINICAL INFECTION OF PORCINE DELTACORONAVIRUS IN GROWER PIGS

Mr. Lu Yen[1], Dr. Juan-Carlos Mora-Díaz[1], Mr. Rolf Rauh[2], Mr. William Nelson[2], Mr. Gino Castillo[1], Ms. Fangshu Ye[3], Dr. Jianqiang Zhang[1], Dr. David Baum[1], Dr. Jeffrey Zimmerman[1], Dr. Rahul Nelli[1], Dr. Luis Giménez-Lirola[1]

[1]Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, [2]Tetracore Inc., [3]Department of Statistics, College of Liberal Arts and Sciences, Iowa State Univeristy

Introduction

Porcine deltacoronavirus (PDCoV) is a transboundary and emerging pathogen that replicates in small-intestinal enterocytes, causing enteric disease in suckling pigs. Although less severe, the gross and histological changes in the gut of pigs infected with PDCoV are similar to those observed in the other two major enteropathogenic swine coronaviruses, transmissible gastroenteritis virus (TGEV) and porcine epidemic diarrhea virus (PEDV). There are no previous reports of PDCoV outbreaks in grower or adult pigs. Thus, the objective of this study was to characterize the susceptibility and dynamics of PDCoV infection in grower pigs under experimental conditions using a combination of syndromic and laboratory assessments.

Materials and Methods

Seven-week-old conventional pigs (n=24) that were pre-screened negative for different swine coronaviruses were randomly distributed into PDCoV (USA/IL/2014 strain, orally) (n=12) and control (culture medium, orally) (n=12) groups. Blood was collected from individual pigs at day post-inoculation (dpi) -7, 0, 3, 7, 10, 14, 17, 21, 28, 35, and 42 to evaluate viremia and humoral response by RT-qPCR (Tetracore Inc.) and indirect ELISA (based on S1 recombinant protein), respectively. Viral shedding (RT-qPCR) was evaluated every other day between dpi 0 to 42 using pen feces and oral fluids. In addition, the potential infectivity of the temporal fecal and oral fluid samples was assessed in monolayers of swine testicular (ST) cells via observation of potential virus-specific cytopathic effect and expression of nucleoprotein (N) via immunofluorescence assay.

Results

No clinical signs were observed throughout the study. No viable virus was detected in pen feces and oral fluids. PDCoV viremia was also not detected, but virus shedding was detected in feces (6-22 dpi) and oral fluids (2-30 dpi), with a peak of detection (% positive pigs and Ct values) at dpi 10. A moderate IgG response was first detected at dpi 10, being significant after dpi 14 compared to the control group, and coinciding with the progressive resolution of the infection.

Conclusions and Discussion

This study demonstrated that exposure of grower pigs to PDCoV results in subclinical infection, making active surveillance based on systematic sampling and laboratory testing critical for accurate detection of PDCoV infection and circulation in grower and adult animals.

P-V-019

CLINICAL PROTECTION AND REDUCED POST-CHALLENGE SHEDDING PROVIDED BY THREE DIFFERENT PSEUDORABIES MLV VACCINES AGAINST CHALLENGE INFECTION WITH VIRULENT NIA-3 STRAIN

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Background and objectives
Pseudorabies virus (PRV), originally named Aujeszky's disease virus, causes potentially devasting, reproductive failure, piglet and grower mortality, reduced growth and productivity, and compromised immunity against secondary infection and vaccine responses. Once infected the animal will be life-long infected with latent reactivation following stress and hormonal changes. The virus is shed in extreme levels for 3-4 weeks after infection and reactivation. The aim of this study was to determine the efficacy of three PRV-MLV marker vaccines, two based on the Ceva-China produced Bartha strain, and an international Bartha-K61 strain based PRV vaccine in a controlled virulent PRV challenge study.

Materials and methods
In a trial design according to the European Pharmacopeia forty-four eight-week-old pigs were randomly allocated to one of four groups, vaccinated (D0), and then boosted eleven weeks old (D21), either with A1-vaccine (O/W adjuvated Auphyl® plus; Ceva, China), A2-vaccine (non-adjuvated Auphyl® plus; Ceva, China), IA-vaccine (non-adjuvated international PRV-Bartha-K61 based), and non-vaccinated controls (ctrl) injected with phosphate-buffered saline (PBS). PBS is used as diluent in both non-adjuvated PRV-vaccines (A2 and IA). At 14 weeks of age (woa), three weeks after second vaccination (D42), all animals were challenged intranasally with 4 ml of the virulent NIA-3 PRV strain in a 7.7 log10 TCID50/ml solution. Seven days later, D49, all surviving animals are humanely euthanised and investigated.
All pigs were weighed at D42 and again at D49, nasal swabs were taken at days 2, 5 and 7 post-challenge and mortality was recorded. Average daily weight gain (ADG) post challenge per group was calculated as an objective measure for appetite and well-being. Swabs were washed into MDBK cell culture for determination of PRV titer. For each group, the average area-under-the-curve (AUC) was computed applying the Trapezoidal rule and used to provide an accurate estimate of post-challenge PRV shedding. These figures were calculated into residual rate of shedding for each of the vaccine group relative to the shedding from ctrl-group, and into the relative shedding to that of the best vaccine group (A1), set as 1, for easier comparison of relative efficacy. Data were analysed in R/Python. ADG and post-challenge shedding were analysed using the non-parametric Mann Whitney test. Results with p-values ≤0.05 are considered statistically significant.

Results
No pigs died in the vaccine groups and 10/11 pigs died in the ctrl group. ADG were 370, 350, 232, and -1812 g/day in A1, A2, IA, and ctrl group respectively (Fig 1). Hence A1: +5.7% vs A2, and 59% vs IA; A2: +51% vs IA (Fig 1).
Post-challenge (NIA-3-only) PRV AUC-shedding was 22.3log10, 23.6log10, 29.6log10, and 33.7log10 TCID50/ml in A1, A2, IA, and ctrl group respectively, providing shedding rates relative to the best of 20-, 19,952,623-, and 251,188,643,151-times A1a shedding in A2a, IAb,

and ctrlc groups respectively (Table 1). Statistically significantly different groups are indicated by letters: a, b, and c.

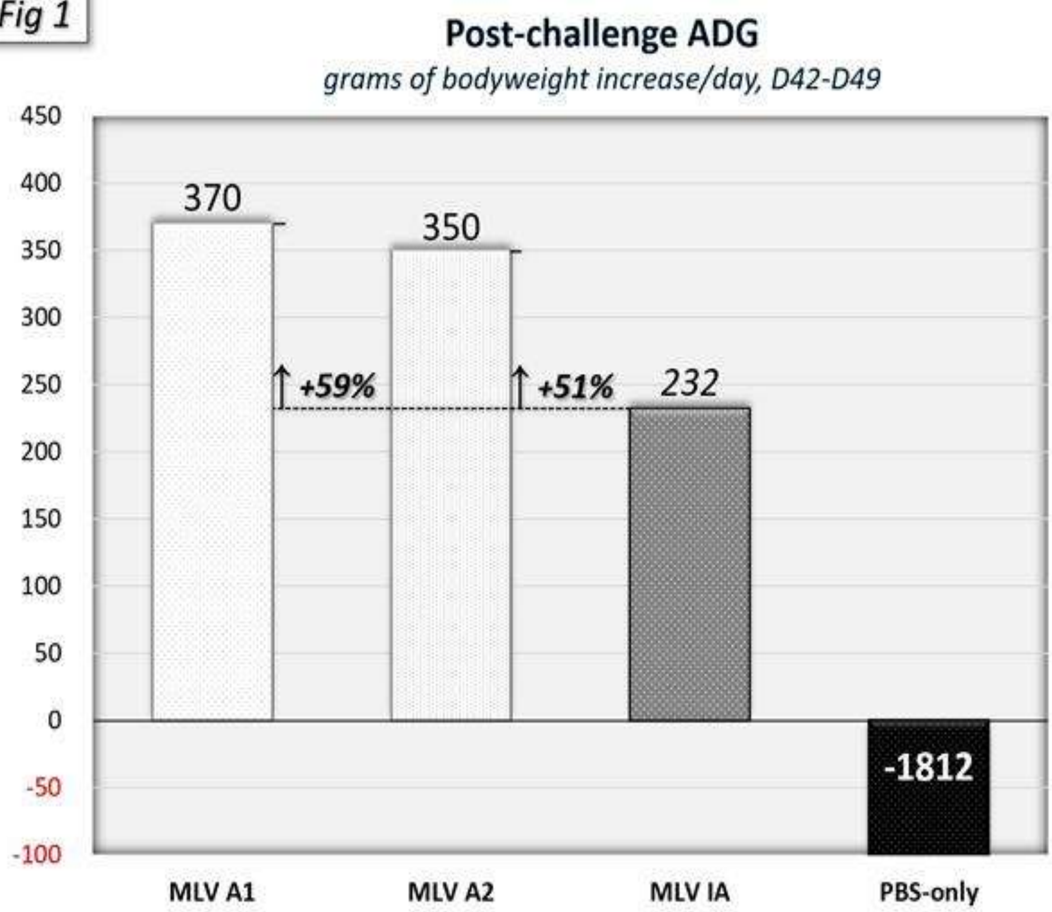
Conclusion
The A1 (O/W) vaccine demonstrated superior efficacy, even numerically compared to the A2 (PBS) vaccine, particularly in the viral control, but also on well-being and appetite. The superior PRV viral control, with a potential of eradication, of the A1 vaccine makes it a strong candidate for all breeding-stock PRV-vaccination. In more farm situations the A2 vaccine would likely be sufficient for pig(let) PRV-vaccination and protection.

Table 1

Table 1: **Post-challenge PRV AUC-shedding**	**A1 (O/W)[a]**	**A2 (PBS)[a]**	**IA (PBS)[b]**	**Ctrl (PBS-only)[c]**
Residual rate	0.000,000,000,4%	0.000,000,007,9%	0.007,9%	100%
Relative to best	**1**	*20* x A1	*≈20 million* x A1	> *¼ trillion* x A1

Graphs or Images 1

Fig 1



P-V-020

COMPARATIVE EFFICACY STUDY BETWEEN FRESHLY MIXED AND READY TO USE PCV2 AND MYCOPLASMA HYOPNEUMONIAE VACCINES IN A SWINE FARM IN JAPAN

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Introduction
Porcine Circovirus Type 2 (PCV2) and Mycoplasma hyopneumoniae (M. hyo) are the major causative pathogens of porcine respiratory disease complex (PRDC), which causes severe economic losses in the swine industry worldwide (1,2). PCV2 / M. hyo combination vaccines are widely used to protects pigs against those two separate infections. The objective of this study was to compare and evaluate the efficacy and economic impact of PCV2/Mhyo commercial vaccines (freshly mixed or ready to use) in a farrow to finish farm in Japan.

Materials and Methods
This study was conducted in a farrow to finish swine farm with 250 sows located in West Japan during Sept. 2021 to Apr. 2022. The farm was seropositive to PRRSv around 80 days of age. On the day of weaning, suckling piglets born from 11 sows were evenly divided into two groups by individual weight, sex, and parity. Group A (n=62) received a single 2 ml shot of Ingelvac® FLEXcombo Mix (freshly mixed vaccine, Boehringer Ingelheim Animal Health Japan Co., Ltd.), and Group B (n=73) received a single 2 ml shot of ready to use PCV2 / M. hyo vaccine at 21 days of age, respectively. Both vaccines were administered in accordance with the manufactures' instructions. Pigs were individually weighted at 21 (weaning), 75 (migrated to the finisher barn), and day to market. Blood samples were collected at 21, 75, and 125 days of age (5 pigs per group) and tested for PRRS ELISA / qPCR, PCV2 ELISA / qPCR, and M. hyo ELISA. Post-weaning mortality and average daily weight gain (ADWG) were statistically analyzed using the Chi-square test and Student's t-test, respectively.

Results
Results are shown in Table 1. Group A showed lower wean-to-finish mortality (4.84% vs 6.85%) and higher ADWG (666.4 g/day vs 664.2 g/day) compared to Group B. This difference resulted in a shorter period from weaning to market (164.9 vs 165.5). The percentage of lightweight (<89.2kg at 180 days old) pigs marketed was lower in group A (Group A: 0% and Group B: 7.35%). There was no statistically significant difference between the two groups, probably due to sample sizes in this study.
When evaluating the survival rate by day of age along the wean-to-finish period, it was observed an increase on mortality in Group B from 89 to 189 days of age (Figure 1). This late mortality has a negative effect on profitability as it impacts in a higher feed usage.

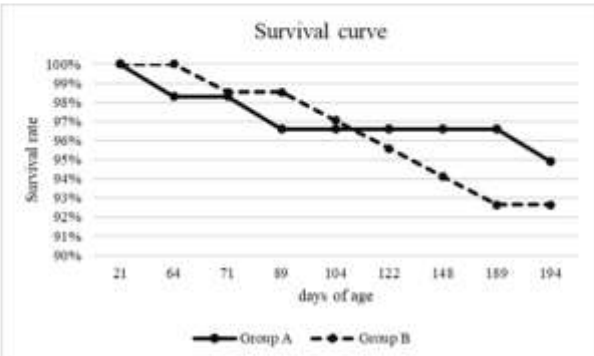
Discussion and Conclusion
Although there was no statistically significant difference in production parameters between the two groups, freshly mixed PCV2 / M. hyo vaccination showed lower post-weaning mortality, higher ADWG, shorter wean-to-market period and lower percentage of light-weight pigs marketed than the ready to use vaccine. The economic result was calculated based on the performance parameters, current Japanese feed/pork prices and vaccination costs. There was an estimated economic advantage of USD 4.01 per pig of Group A over Group B, at the end of the study.

References
1. Opriessnig T et al. 2011. Anim Health Res Rev 12, 133-148.
2. Lung O et al. 2017. Transbound Emerg Dis 64, 834-848.

Table 1

	Group A (Freshly mixed)	Group B (Ready to use)	p- value
Number of pigs	62	73	-
Average body weight (kg, 21 days of age)	5.95 ±1.14	6.03 ±1.40	0.71
Average body weight (kg, days to market)	114.98 ±2.87	114.88 ±3.56	0.89
Total Weight Gain (Kg)	109.0	108.7	-
Average study period (days, wean to market)	164.9	165.5	0.85
Post-weaning mortality (%)	4.84	6.85	0.62
ADWG (g/day)	666.4	664.2	0.92
Percentage of light-weight pigs marketed (%)	0	7.35	-

Graphs or Images 1



P-V-021

COMPARATIVE FIELD EFFICACY STUDY BETWEEN FLEXCOMBO® AND READY-TO-USE VACCINE IN TWO CHINESE GROWING FARMS

Dr. Carlo Magno Maala[1], Dr. Chunqing Sun[2], Dr. Qingxin Zhou[2], Dr. Changying Zhang[2], Dr. Lv Huang[2]

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Introduction

Porcine circovirus type 2(PCV2) and Mycoplasma hyopneumoniae(M.hyo), as the major pathogens of Porcine respiratory disease complex(PRDC), causing significant economic impact in Chinese swine industry. FLEXcombo® is a mixed PCV2 and M.hyo vaccine from Boehringer Ingelheim and was first registered ready-to-mix vaccine in Chinese market, to protect pigs against PCV2 and M.hyo. The aim of the two studies was to evaluate the efficacy of FLEXcombo® and a ready-to-use vaccine in two separate Chinese farms.

Materials and Methods

The two studies were double-blinded, randomized designed. Study 1 was conducted in a farrow-to-finish farm located in Anhui province, including 208 pigs in total. Study 2 was conducted in a two-site farm located in Hubei province, with 223 pigs in total. Both farms were confirmed M.hyo and PCV2 positive. The pigs were allocated into two groups randomly, blocking by initial weight and gender. One group was vaccinated with FLEXcombo®, another group was vaccinated with multinational brand ready-to-use vaccine (MNC RTU). Pigs of the two vaccine groups were mixed within pen to assure all groups were managed under same condition, and only vaccine was the variable. Both studies started after weaned at about 21-30 days of age. Tagging, weighting and vaccination day was as Day0. All pigs were weighed at the end of the trial to calculate Average Daily weight Gain (ADG -primary parameter). Detailed grouping information is listed in Table1.

Results

Although the two farm conditions were different, the pigs of two groups were randomized to reduce possible bias. Pigs in FLEXcombo® group showed significant better ADG in both studies. FLEXcombo® group shown also higher percentage of heavier pigs and lower lighter pigs, translated in better weight uniformity. The results of two studies are in Table 2, Table 3, Figure 1, and Figure 2.

Table 1

Study	Treatment Group	Number of trial pigs	Vaccination method and age	Study days
Study 1	FLEXcombo®	106	2ml neck injection at 33 days of age	127
	MNC-RTU	102	2ml neck injection at 33 days of age	127
Study 2	FLEXcombo®	110	2ml neck injection at 33 days of age	171
	MNC-RTU	113	2ml neck injection at 33 days of age	171

Table 1 - Trial design and grouping information

Table 2

Group	FLEXcombo®	MNC-RTU	Difference
# pigs	106	102	
Initial weight (D0, Kg)	11.12	11.13	
Average weight of 110 days of age (D78, Kg)	69.42	68.26	+1.16kg
Average weight at market (D127, Kg)	119.6	117.1	+2.5kg
ADG-Nursery (D0-D78, g)	747	732	+15g
ADG-Finishing (D78-D127, g)	1023	997	+26g
Wean-Market ADG (D0-D127, g)	854	835	+19g

Table 2 - Comparative results of Study 1

Table 3

Group	FLEXcombo®	MNC-RTU	Difference
# pigs	110	113	
Initial weight（D0，Kg)	8.17	8.04	
Average weight at market（D171，Kg)	124.88	121.9	+2.98kg
Wean-Market ADG (D0-D171，g)	659	643	+16g

Graphs or Images 1

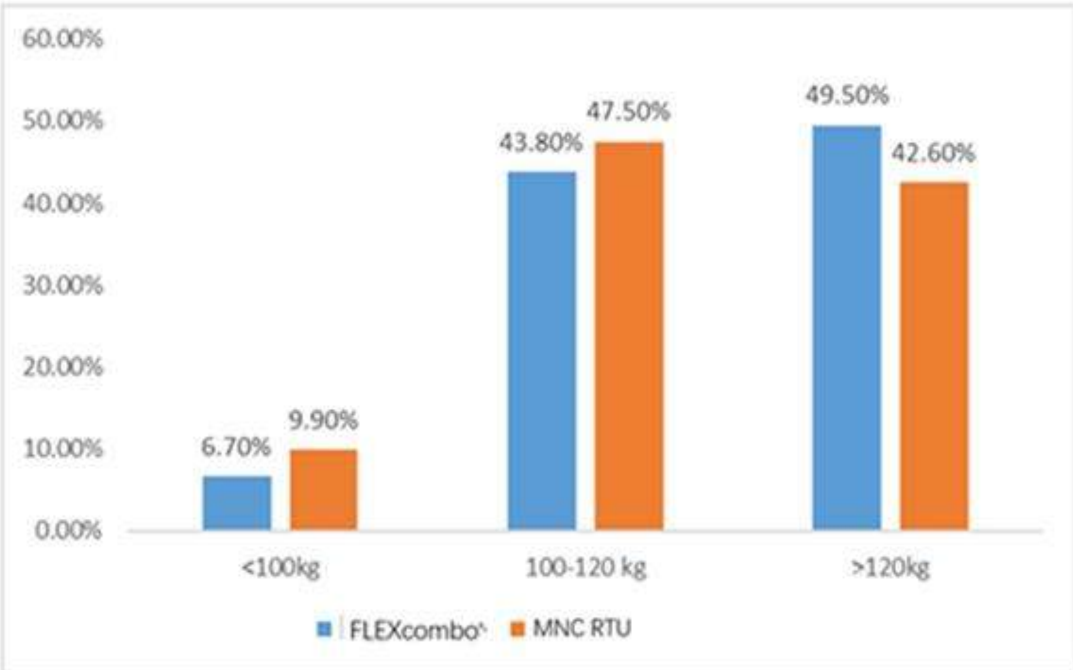

Figure 1 - Weight distribution at range of <100kg, 100-120kg and >120kg of Study 1

P-V-022

COMPARATIVE FIELD EFFICACY STUDY OF COST-EFFECTIVENESS OF TWO COMBINED VACCINES OF PORCINE CIRCOVIRUS TYPE 2 (PCV2) AND MYCOPLASMA HYOPNEUMONIAE

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[1]*Boehringer Ingelheim*

Introduction
Respiratory disease causes significant production losses in the swine industry, and pig producers vaccinate their pigs against respiratory diseases caused by porcine circovirus type 2 and mycoplasma hyopneumoniae. Previous studies showed that commercial vaccines could improve the pig health in terms of the mortality rate and average daily weight gain of a herd, but it is poorly understood that how much impact PCV2 and Mycoplasma hyopneumoniae vaccines would have on the production cost of a pig farm. We hypothesised that how fast a pig reach to the market weight would vary by vaccines, and therefore affect the feed cost at fattening house in a farm. In this study, we compared the efficacy of two commercial vaccines through the difference in not only the mortality rate after weaning, average daily weight gain, average market weight at day 167, but also the feed cost after day 167 for all the remaining pigs to reach the market weight.

Materials and Methods
The field trial was conducted on farrow to finish farm. A total of 240, 28 day-old pigs were randomly allocated to two different treatment groups, weighed, ear-tagged and vaccinated individually. Group A was vaccinated with freshly prepared mixture of Ingelvac CircoFLEX® and Ingelvac MycoFLEX (FLEX®combo, Boehringer Ingelheim Vetmedica GmbH) and group B was vaccinated with Porcilis® PCV M hyo (Intervet international B.V., Netherlands) containing PCV2a and Mycoplasma hyopneumoniae antigen in one bottle.

Results
As a result, there was no significant difference in the mortality rate, average daily gain, and the average market weight of the two groups. However, the number of pigs reached the market weight was higher in group A (26/116) than group B (19/115). The additional feed cost for the remaining pigs reached to the market weight calculated based on the market price show a significant difference in two groups: 3,584,000 KRW for the group A, and 4,627,840 KRW for the group B. We anticipate that a farm can save 8,385 KRW for feed per pig with FLEX®combo. Therefore, we believe FLEX®combo can reduce production costs better than Porcilis PCV M.

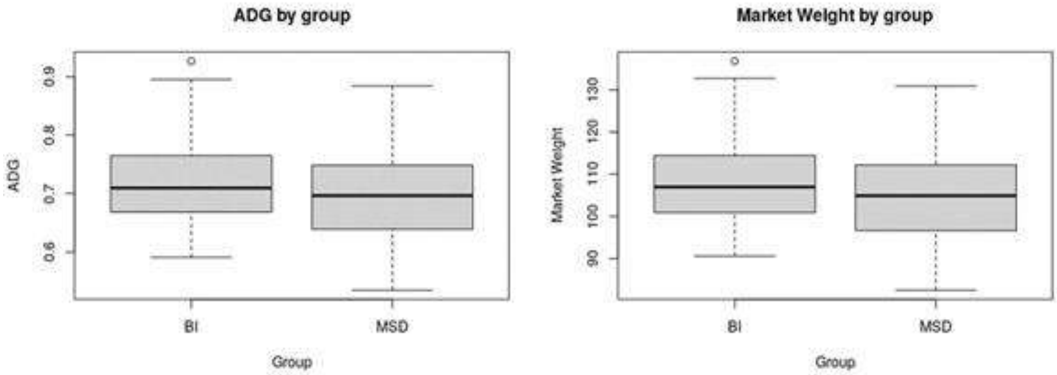
Conclusions and Discussions
As demonstrated before, PCV2 vaccines can have a significant effect on mortality in Korean pig production. But we should focus on the economic values. This comparative trial shows that the effect of PCV2 and M.hyopneumoniae vaccines on performance parameters and economic differences. This is critical for decision making on PCV2 and M.hyopneumoniae vaccines.

Acknowledgement and References
[1] Park et al. (2014) Swine disease report 2013, Korean Pork Producer Association
[2] M. Adam et al. (2013) Proc 5th ESPHM symposium, Edinburgh, United Kingdom, P124

Table 1

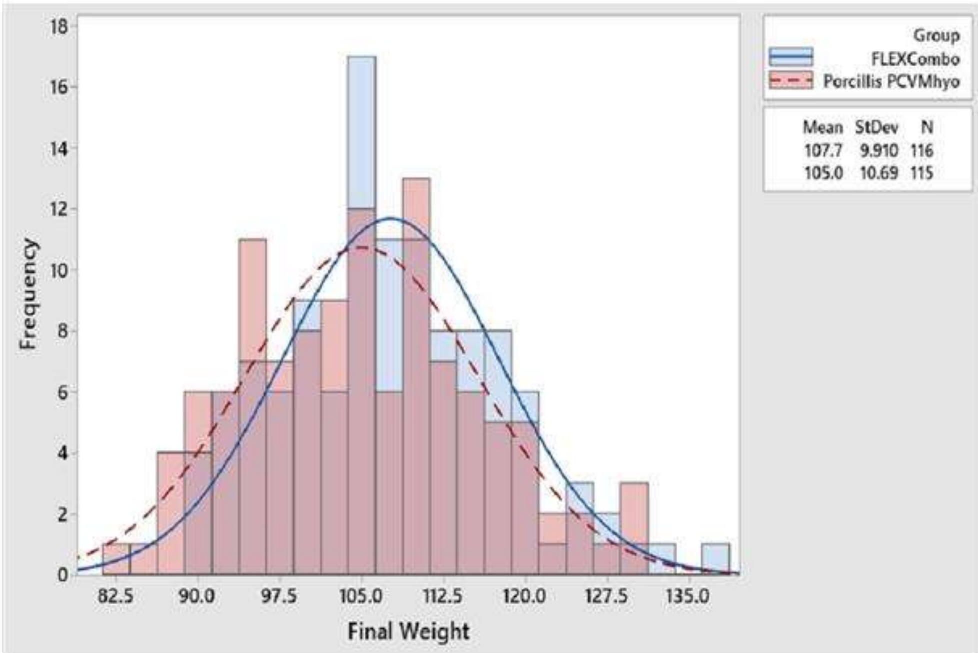
Table1. Pig health parameters of the two groups . The statistics done for the mortality rate by Mann-Whitney U test and the average daily weight gain and average market weight by Two sample T-test.

	Group A	Group B
Mortality rate (%)	3.3 ± 0.18	4.2 ± 0.2
Average daily weight gain (kg/day)	0.72 ± 0.07	0.7 ± 0.08
Average market weight (kg at day 167)	107.65 ± 9.91	104.99 ± 10.69



Graphs or Images 1

Fig1. Histogram of body weight at day 167 (market weight) between two groups. The number of pigs reached the market weight was 26 out of 116 (22.4%) in group A and 19 out of 115 (13%) in group B.



P-V-023

COMPARATIVE OF TWO VACCINATION PROGRAMS AGAINST PCV2 AND MYCOPLASMA HYOPNEUMONIAE IN THE GROWER-FINISHER FARM

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Introduction
Pigs are susceptible to PCV2 and Mycoplasma hyopneumoniae (M.hyo) infection after being transferred from one farm to another. These two pathogens represent the most significant global spread to pigs. This study's objective was to compare two vaccination procedures against PCV2 and M.hyo, one based on ImpranFLEX adjuvant by fresh mixing for both pathogens and the other based on a single dose of a ready-to-use vaccine.

Materials and Methods
The side-by-side study was conducted in a 2,000-pig grower-finisher farm in northeast of Thailand. The 8–9-week-old pigs were moved from sow farms with negative PRRS status and were tested for PRRS and PCV2 within the first week of arrival on finishing for monitoring. Upon arrival, 1,000 piglets were equally divided at random into two groups, T1 and T2. Two hundred pigs of each group were individually weighted, ear tagged and randomized, as well as 10 blood samples were collected from each group. Group T1 was vaccinated with one dose of freshly mixed PCV2 and M.hyo combination vaccines (FLEXcombo®), whereas Group T2 was vaccinated with one dose of a ready-to-use PCV2 and M.hyo vaccine. Both administrations were of 2 mL IM on the left side of the neck. Average daily weight gain (ADWG) and Feed Conversion Rate (FCR) at finishing stage, as well as the market weight, were chosen as parameters for measuring the efficacy of the vaccine. These factors were statistically evaluated (p 0.05) using the modelling Mann-Withney-Wilcoxon test approach and The Total losses was used by Chi-square test of independence. BECAL™ (Boehringer Ingelheim Economic Calculator) was used to perform an economic analysis.

Results
During finishing phase, ADWG in group T1 was 22.8g per day better than group T2 (p=0.006), resulting in a 2.4 kg increase in market weight (Table 1 and Figure 1). Group T1 had also better FCR (2.82 vs 2.92). On the first day of arrival, prior to vaccination regimen, ELISA blood test results revealed 95% PCV2 seropositivity and 5% PRRS seropositivity (data not shown).

Discussion and Conclusions
In this study, the ADWG of finishing pigs vaccinated with FLEXcombo® was increased by 2.4%, or 22.8 g/day compared to the one-dose ready-to-use vaccination protocol. Furthermore, the improvement on feed efficiency observed with FLEXcombo® resulted in an economy of 10.2kg of feed per pig during finishing phase. The economic analysis using BECAL™, taking in consideration the current feed and pork prices in Thailand, demonstrated that the improvement in ADWG and FCR resulted in a financial benefit of +$8.09 per pig. These results are in line with previous studies, reinforcing the consistent efficacy and safety profile of the freshly mixed vaccine combination1,2, as well as the advantages of market weight selling management and the maximization of producers' profits.

Reference

1. S. Figueras et al, IPVS 2018
2. S. Jamawat et al, IPVS2018

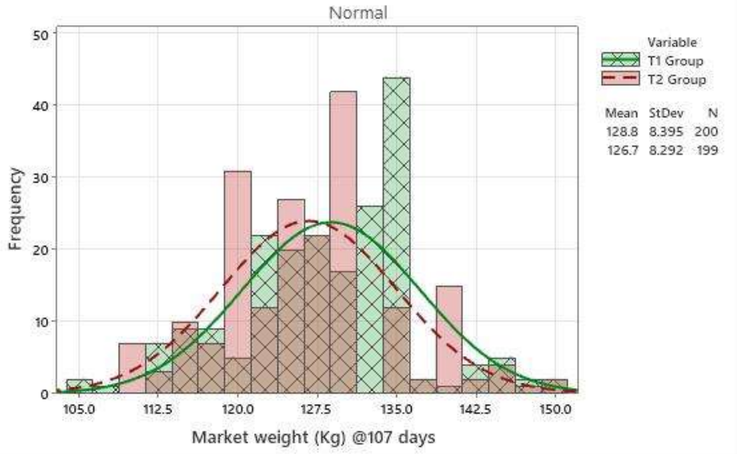
Table 1

Table 1 : Finishing performance parameters

	T1	T2	Diff	P-value
Amount Pig placed	500	500		
% Losses	1.6	2.0	-0.4	0.634
Start Weight (kg)	26.70	26.27	0.43	0.175
Weight gain (kg)	102.4	100	2.4	0.006
Market weight (kg)	130	127	3	0.148
FCR	2.82	2.92	-0.10	N/A
ADWG (g/day)	957.4	934.6	22.8	0.006

Graphs or Images 1

Figure 1. Comparison of market weight between group T1 and Group T2



P-V-024

COMPARATIVE TRIAL OF A NEW INTRADERMAL VACCINE (MHYOSPHERE® PCV ID) AND AN INTRAMUSCULAR BIVALENT VACCINE AGAINST MYCOPLASMA HYOPNEUMONIAE AND PCV2 IN NURSERY PIGS ON THAI FARMS

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Introduction

Porcine circovirus type 2 (PCV2) and Mycoplasma hyopneumoniae (M.hyo) are two of the main pathogens that cause the Porcine Respiratory Disease Complex (PRDC) which has a great impact on production performance in the pig industry worldwide1,2 and on Thai pig farms. Vaccines, together with management and environmental control, are recommended as a strategic programme to reduce the impact of these pathogen. MHYOSPHERE® PCV ID is a new intradermal (ID) vaccine from HIPRA for the prevention of both M.hyo and PCV2-associated diseases. Due to the recent launch of this new vaccine, the field efficacy data on this vaccine in Thailand are limited. The purpose of this study was to demonstrate the efficacy of MHYOSPHERE® PCV ID on a commercial farm in comparison with the current intramuscular (IM) bivalent vaccine in Thailand.

Materials and Methods

A farm with 2,000 sows positive for PCV2 (confirmed by PCR) and M.hyo (confirmed Mhyo-like lung lesions at slaughter) was selected. For the trial, a total of 7,472 piglets were randomly allocated into two groups. Briefly, Group 1 piglets (n = 3,888, 4 batches) received 1 dose (2 ml) of a bivalent vaccine IM (Vaccine A) administered at 5 weeks of age. Group 2 (n = 3,584, 4 batches) received 1 dose (0.2 ml) of MHYOSPHERE® PCV ID at 3 weeks of age following the manufacturer’s instructions. The productive parameters monitored were the losses (%losses, %mortality, %culling) and average daily weight gain (ADG) and feed conversion rate (FCR) of the nursery phase which were compared between groups by T-test statistical analysis (IBM SPSS statistics base 22.0). In addition, 25 lung samples were randomly selected from each group when the pig was in the fattening phase and sent to a slaughterhouse and then evaluated by artificial intelligence (AI) diagnostic software, AI Diagnos, following the MADEC-modified method.

Results

No systemic or local reactions were observed in either group after vaccination. The MHYOSPHERE® PCV ID group showed significant differences ($p<0.05$) in terms of losses (less mortality and culling), which were lower than with vaccine A (Figure 1). There was no difference in the lung lesion index between Vaccine A (1.62) and MHYOSPHERE® PCV ID (1.62), nor in the ADG or FCR (Table 1).

Conclusions

In the same production system under field conditions, MHYOSPHERE® PCV ID showed a significantly better performance in reducing % losses (mortality and culling). Therefore, MHYOSPHERE® PCV ID has been proven to be efficacious on a pig farm in Thailand in reduceing the impact of PCV2 and M.hyo infection.

Acknowledgement

The authors would like to thank the commercial swine farm in Nong Bua Lamphu province,Thailand co-operatives for the information.

References

1-Ellis J, Clark E, Haines D, et al. 2004. VET Microbiol 98:159-163.2.
2-Segalés J, Allan GM, Domingo M. 2005. Animal Health Res Rev 6:119-142.

Table 1

Parameter	Vaccine A	MHYOSPHERE® PCV ID
Entry number	3,888	3,584
Losses	284	113
Entry weight	$6.80^{a} \pm 0.40$	$6.99^{a} \pm 0.38$
Entry age	$23.78^{a} \pm 1.51$	$23.79^{a} \pm 1.02$
% losses	7.3^{a}%	3.3^{b}%
% Mortality	4.9^{a}%	2.1^{a}%
% Culling	2.4^{a}%	1.1^{a}%
ADG	$412.91^{a} \pm 27.7$	$421.51^{a} \pm 23.2$
FCR	$1.478^{a} \pm 0.076$	$1.482^{a} \pm 0.033$

Table 1. Productive parameters during the nursery period after vaccination (mean ± SD). Note: *Different letters (a, b) within the same column represent significant differences (p<0.05).

Graphs or Images 1

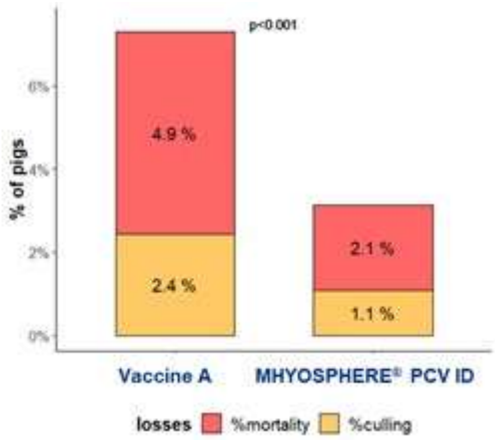

Figure 1. Percentage losses in each group

P-V-025

COMPARING PRODUCTION PARAMETER OF TWO DIFFERENT VACCINATION PROTOCOLS UNDER FIELD CONDITIONS

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Introduction
In the field, PRRS and PCV2 virus are the most widespread swine farm pathogens worldwide. Vaccination of both pathogens became the global routine to control the losses from infections. Mixing these two vaccines in one shot, reduced injections and help to saves labor. Safety and efficacy of PRRS MLVs reconstituted with a PCV2-subunit vaccine was confirmed1. The objective of this study is to demonstrate the efficacy of the reconstituted PRRS MLV vaccine with a sub-unit PCV2 vaccine in PRRSv negative pigs under field conditions.

Material and Method
The side-by-side trial was set up in a grower - finish farm (9-23 weeks) located in north-east of Thailand. The farm received PRRS negative pigs from the sow farm at 9 weeks of age. 1,000 pigs were randomly assigned to two groups of 500 pigs each. The treatment group (T1) received the mix of Ingelvac PRRS MLV with Ingelvac CircoFLEX (1ml) in one shot, while a control group (T2) received the two vaccines by separate injection. The measured parameters were, behavior 12-hour post injections, daily feed intake, total loss, growth performance, market weight distribution as well as serology profiles of 20 ear tagged pigs from each group of day 0 and day 28th following vaccination. The statistical analysis of serological results and market weights were performed using version17 of Minitab software.

Results
One day prior to vaccination (D0), all pigs in both groups were negative for PRRS by ELISA. On day 28, 100% of group T1 and 90% of group T2 were seropositive, as shown in Graph1. No significant differences regarding market weight distributions (Graph2) of 352 pigs in each group were demonstrated (standard deviation 8.46 vs 8.35; means 114.0 kg vs 114.5 kg)

The total loss between T1 and T2 differs numerically (0.2% vs. 1.2%), as does the growth performance; ADG (925 g. vs 917 g.) and the feed efficiency; FCR (2.59 vs 2.64)

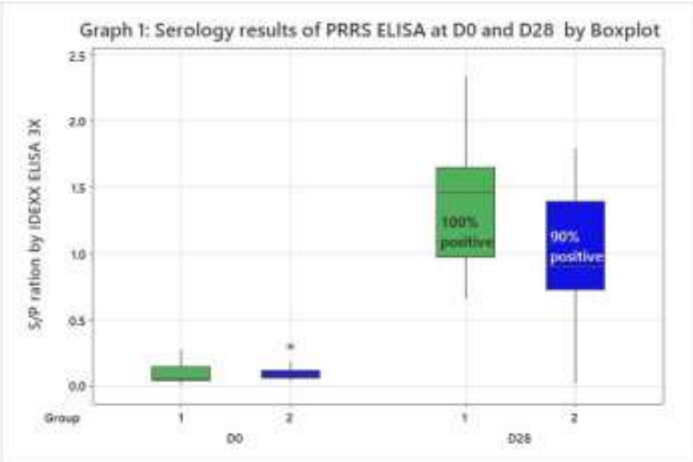
Discussion and Conclusion
The mixture of Ingelvac PRRS MLV and Ingelvac CircoFLEX administered as a single 1 ml injection was as safe and efficient as the conventional separate vaccination protocol. This mixing license not only gives protection against both viruses, but it also reduces the number of injections, pig stress, labor needs, and the risk of exposing pigs during times of high biosecurity concern like an ASF outbreak.

Table 1

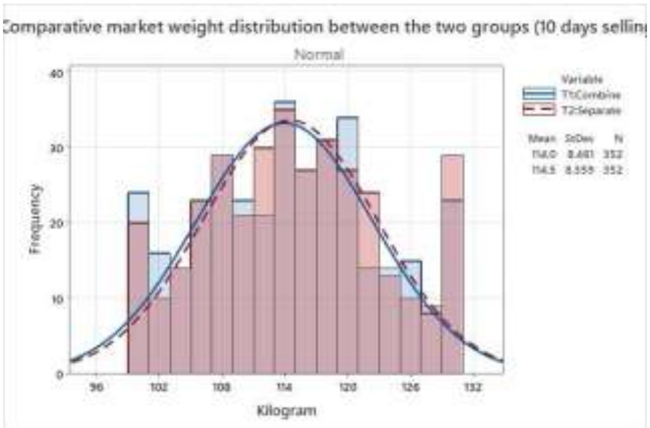
Table 1Fattening performance between combination and conventional group (no statistical analysis)

	Combine (T1)	Mono (T2)
Weight in	24.2	22.8
Market weight	114.0	114.5
% Total loss	0.2	1.2
ADG(g/day)	925	917
FCR	2.59	2.64
Day on feed	94.28	100

Graphs or Images 1



Graphs or Images 2



P-V-026

COMPARING THE PATHOGENICITY OF TWO FIELD STRAINS OF TYPE-2 PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS OF THE SAME GENETIC CLUSTER ISOLATED IN JAPAN

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Introduction
Porcine Reproductive and Respiratory Syndrome (PRRS) is a swine disease caused by the PRRS virus (PRRSV) and has caused huge economic losses to pig farming worldwide. The Genotypes of PRRSV are classified into type-1 (European) and type-2 (North American). Of these, type-2 PRRSV has widely spread in Japan, and field strains have been classified into five clusters (Cluster I-V) by phylogenetic analysis of ORF5 sequences [1]. Recently, the genetic diversity of PRRSV in Japan has increased, and in some areas, the proportion of cluster IV has been larger than before [2]. In this study, we compared the pathogenicity and antigenicity of two field strains (strain A and B) classified as cluster IV and isolated in 2018 (strain B) and 2020 (strain A) in the same region in the west of Japan.

Materials and Methods
We performed experimental infection of strain A or B and designated Experiment 1 or 2, respectively. In Experiments 1 and 2, five PRRSV-negative pigs were randomly allocated into two groups: challenge (n=3) or control (n=2). The challenge group was inoculated with 1 mL of a nasal spray containing 1×10^5 $TCID_{50}$ in each right and left nasal cavity. The control group was administered an equal volume of MEM in the same way as the challenge group. After inoculation, pigs were monitored daily for rectal temperature and clinical signs. Six weeks after inoculation, pigs were necropsied. Serums were collected to analyze the quantity of PRRSV RNA and neutralizing antibody titer and to isolate viruses. At necropsy, the lungs, pulmonary lymph nodes, and tonsils were collected to analyze the quantity of PRRSV RNA.

Results
The challenge group of strain A observed clinical symptoms such as inappetence and high rectal temperatures, while the challenge group of strain B observed no clinical signs. From the results of the quantity of virus RNA in the blood, strain A kept a high copy number of RNA up to 21 dpi with a peak between 4 to 11 dpi. However, strain B reduced the number of copies of RNA after a height of 11 dpi. Similarly, from the results of virus isolation, the virus titer of strain A was higher than strain B, between 18 to 28 dpi. RNA of strain A was detected in 2 of 3 lungs, 3 of 3 pulmonary lymph nodes, and 3 of 3 tonsils. However, RNA of strain B was detected in 0 of 3 lungs, 1 of 3 pulmonary lymph nodes, and 3 of 3 tonsils. Neutralizing activities were detected against only homologous strains.

Discussion
Comparing the pathogenicity of two field strains classified as cluster IV isolated in Japan, the clinical symptoms of each strain were different. The challenge group of strain A maintained high viremia, and at the end of the experiment, high levels of virus RNA remained in the lungs. Those suggested that the difference in viral replication in vivo is closely related to

differences in clinical signs. There were no neutralizing antibody responses against heterogeneous strains. In conclusion, our data reveal that the pathogenicity and antigenicity of strains differ between field strains classified as cluster IV widely spread in Japan.

References

1. Yoshii et al. (2005) Arch. Virol. 150, 2313-2324
2. Kyutoku et al. (2022) Epidemiologia. 3, 285-296

P-V-027

COMPARISON OF 10 COMMERCIAL PCR DIAGNOSTIC KITS FOR ASFV DETECTION: A FIELD STUDY IN THAILAND

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Introduction: African Swine Fever (ASF) has been the deadliest disease, causing high mortality in pigs. Rapid and accurate detection of ASF virus is extremely important for the effective management and prevention of disease. Portable PCR thermocycler can be used in a field laboratory, providing fast and sensitive molecular assay for field diagnosis of ASF (1). At present, there are many commercial PCR diagnostic kits available in Thailand. No assessment of PCR diagnostic kits had been carried out yet. The aim of this study was to compare the efficacy of 10 commercially available PCR diagnostic kits tested by portable PCR thermocycler in a field laboratory.

Materials and Methods: This study was conducted in the field laboratory in 2022. 10 commercial PCR kits including 9 Real-time PCR kits and an isothermal PCR kit, were selected based on availability during the study period. None of manufacturers were involved in the assessment and interpretation of the laboratory results. To determine the sensitivity of each PCR kits, the pig blood was confirmed positive ASFV using Real-time PCR assay as described previously (2). Afterward, the ASFV positive blood was diluted 10-fold serially in PBS with a range from 100 to 10-8. Viral DNA extraction of each dilution was performed. Serial of viral DNA amplification was carried out with portable PCR thermocycler according to the PCR detection method provided by the manufacturer. The same serial of viral DNA was transported to CPF Animal Health and Diagnostic Center for the confirmation by using Real-time PCR testing as a reference laboratory in this study.

Results: Different PCR kits showed variation in detection rate and Ct values. 7 of 10 commercial PCR diagnostic kits consisting of 6 Real-time PCR kit and an isothermal PCR kit, can detect ASF virus in diluted 1:10-5 compared with lower sensitivity of other 3 Real-time PCR kits as shown in the figure 1 and table 1.

Conclusions and Discussion: This field laboratory provided highly sensitive laboratory results, which was comparable to the reference laboratory. We concluded that there was difference in sensitivity among commercial PCR kits assessed in this study. 7 of 10 commercial PCR diagnostic kits can be used for field diagnosis of ASF. When performing ASFV detection in environmental swab samples that low viral load maybe expected, these commercial PCR kit might be able to detect the virus. In choosing an appropriate PCR kits for a field laboratory, the following parameters are necessary to be considered, including convenience of the kit usage, provided extraction process control for environmental swab sample, and laboratory cost.

Acknowledgement
Northern field laboratory and Animal health and diagnostic center, CPF (Thailand) Public Company Limited, Thailand

References

1. Daigle, J. Onyilagha, C. Truong, T. Le, V.P. To Nga, B.T. Nguyen, T.L. Clavijo, A. Ambagala, A. 2020. Rapid and highly sensitive portable detection of African swine fever virus. Transboundary and Emerging Diseases. 68(2): 952-959.
2. King, D.P. Reid, S.M., Hutchings. G.H., Grierson. S.S., Wilkinson. P.J., Dixon, L.K.. Bastos, A.D., Drew. T.W., 2003. Development of a TaqMan PCR assay with internal amplification control for the detection of African swine fever virus. J. Virol. Methods 107: 53–61.

Table 1

Dilution of ASF positive blood	Reference Lab	Kit 10
Diluted 1:10^{0}	21.76	Positive
Diluted 1:10^{-1}	24.54	Positive
Diluted 1:10^{-2}	24.81	Positive
Diluted 1:10^{-3}	28.34	Positive
Diluted 1:10^{-4}	32.68	Positive
Diluted 1:10^{-5}	35.74	Positive
Diluted 1:10^{-6}	Negative	Negative
Diluted 1:10^{-7}	Negative	Negative
Diluted 1:10^{-8}	Negative	Negative

Table 1. PCR results detected by an Isothermal PCR kits compared to the result of reference lab

Graphs or Images 1

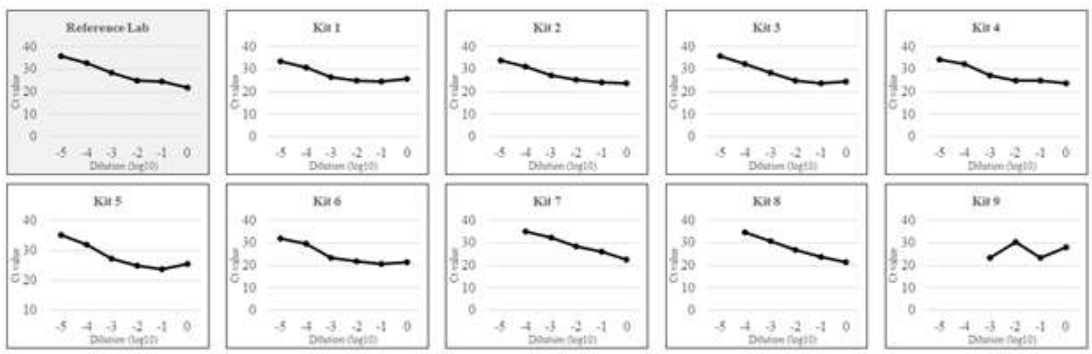

Figure 1. Ct value of serial dilutions of ASFV positive blood detected by each Real time PCR kits and Ct value tested by reference lab

P-V-028

COMPARISON OF MORTALITY IN A PRRS-ENDEMIC SITE 2 AND SITE 3 AFTER SWITCHING FROM A PRRSV2 MLV TO A PRRSV1 MLV IN KOREA

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[1]*HIPRA KOREA*, [2]*HIPRA*

Introduction
PRRS is a swine disease with a very important economic impact on the swine industry (1). The huge economic and productive losses due to its endemic distribution and the high levels of mortality caused by both types (PRRSV1 and PRRSV2) makes the immunization of pigs a necessity to minimize the impact on affected farms (2). Immunization with modified live vaccines (MLV) has proved to be effective in controlling PRRS infection (3). In experimental studies, high doses of neutralizing antibodies (NA) prevented transplacental PRRS infection of fetuses and provided sterilizing immunity to the sow and piglets in utero (4,5). The objectives of this trial were to evaluate if a PRRSV1 vaccine could better control coinfection (PRRSV1 and PRRV2) and the time required to detect NA after vaccination in a site 2 farm in Korea.

Materials and Methods
The trial was conducted in a site 2 and site 3 farm in South Korea where 70-days-old animals from a negative site 1 were introduced monthly and vaccinated with a PRRSV2 MLV (VR2332, 2 ml, IM). This farm was endemic to a PRRSV2 and in April 2021 suffered a severe outbreak of PRRSV1 causing high mortality in the pigs (Table 1) so the vaccine was changed to UNISTRAIN® PRRS ID (PRRSV1 vaccine, VP-046 BIS strain, 0.2 ml, ID, HIPRA) with an intradermal needle-free device (Hipradermic®, HIPRA). Blood samples were collected to perform ELISA (kit by IDEXX X3) and Neutralizing Antibodies (NA) in a local laboratory, which had the cell line adapted to UNISTRAIN® PRRS (VP-046) before vaccination (9 weeks of age) and at 6, 13, 18, 25, 35, 60, 80 days post vaccination (dpv). Mortality data were collected daily from all batches according to the date of entry of the pigs in the site 2.

Results
Regarding blood samples, it can be observed that from 13 dpv and 18 dpv high titers were found in ELISA (Figure 1) and NA (Figure 2), respectively.
On the other hand, mortality in the groups of animals vaccinated with UNISTRAIN® PRRS ID was significantly reduced as it can be observed in Table 1.

Discussion and Conclusion
Based on the results of this trial in a farm coinfected with PRRSV1 and PRRSV2 field strains, after vaccination with UNISTRAIN® PRRS ID the mortality was reduced significantly compared to the previous situation with a PRRSV2 MLV.
Additionally, Neutralizing Antibodies ,which has been demonstrated to be really important to eliminate the virus, shorten viremia, and further mitigate PRRSV-related syndromes (4,5), appeared 18 days after vaccination with UNISTRAIN® PRRS.

References
1. Holtkamp et al., 2013. J. Swine Heal. Prod. 21, 72–84.
2. Nan Y et al., 2017. Front. Microbiol. 1635, 1-17.

3. Kitchodok R et al., 2019. APVS Congress, 259.
4. Osorio, 2002.
5. López, 2007.

Table 1

	Number of animals entered	Mortality rate	Vaccine
Jan-21	2,052	21.9%	PRRSV2 vaccine
Feb-21	1,900	19.6%	
Mar-21	2,246	22.2%	
Apr-21	1,682	20.3%	
May-21	3,271	17.7%	
Jun-21	2,568	23.1%	
Jul-21	1,454	20.4%	
Aug-21	850	26.4%	
Sep-21	3,253	10.8%	
Oct-21	1,851	14.9%	
Nov-21	1,292	7.3%	UNISTRAIN® PRRS*
Dec-21	2,290	5.1%	
Jan-22	2,147	7.0%	
Feb-22	2,268	4.6%	
Mar-22	2,697	4.9%	

Table 1. Mortality data from each batch
p < 0,001***, a logistic regression was performed

Graphs or Images 1

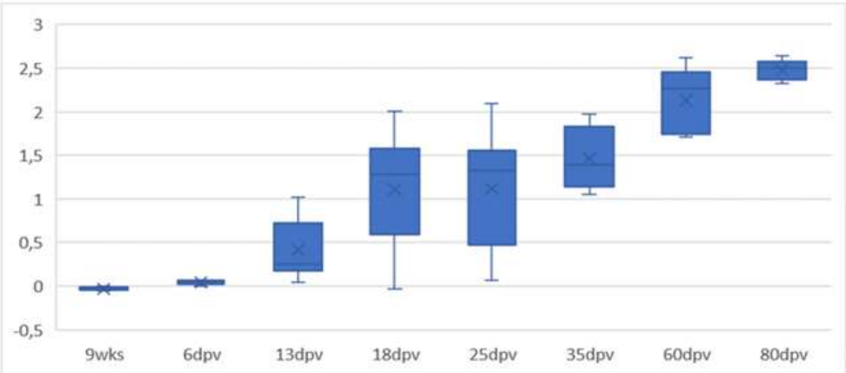

Figure 1. S/P ratio by ELISA test. Seroconversion after vaccination with UNISTRAIN® PRRS

Graphs or Images 2

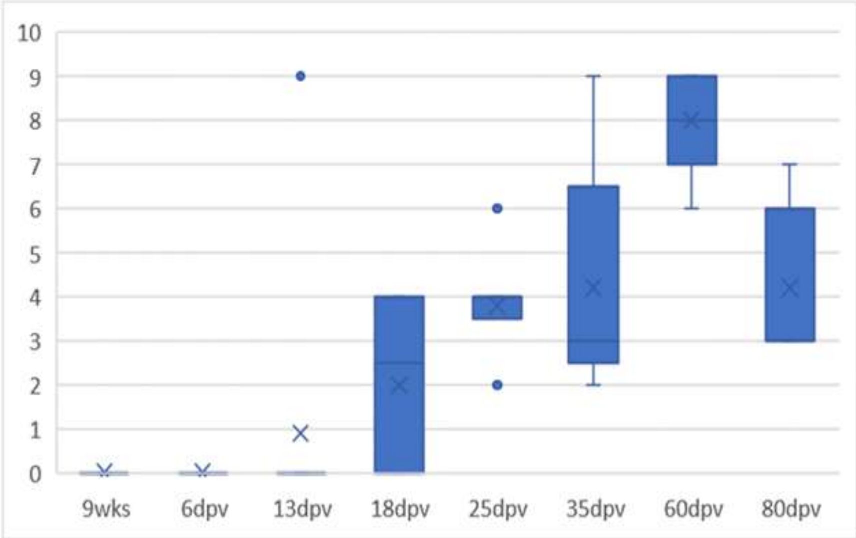

Figure 2. GMT of NAs titer (expressed as log2) against UNISTRAIN® PRRS

P-V-029

COMPARISON OF THE EFFECT OF FEEDBACK FEEDING AND LIVE VACCINATION ON OCCURRENCE OF PORCINE EPIDEMIC DIARRHEA IN KOREA

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Background

Porcine epidemic diarrhea virus(PEDV) is a highly contagious coronavirus disease that causes vomiting, diarrhea, dehydration in neonatal piglets. PEDV can also cause diarrhea, agalactia, and abnormal reproductive cycles in pregnant sows.

PEDV was first reported in Korea in 1992. After the outbreak of the PED in 2003, it gradually declined. But it has been showing a high incidence of diseases again since 2014, and in recent years, it has tended to occur throughout the year regardless of the season. In fact, we would like to share cases of feedback feeding and vaccination among PED management methods that occurred in farms in Jeollanam-do in 2020.

Materials and Methods

PED occurred in 6 farms from May to July 2020, and the outbreak area is a pig farm complex, with more than 10 farms located very close. The 6 farms tested were A to F in the order of the outbreak date. The farms of A, C, D, E were using feedback feeding(oral, use 10 suckling piglet intestines per 100 sows) and B, F were vaccinated the live PED vaccine(oral, vaccine 2ml+milk 3ml, 1×105.3 TCID50/dose, CAVAC, Korea). And all of farms accompanied with inactived vaccine(IM, 106.5 TCID50/dose, CAVAC, Korea) after feedback feeding or live PED vaccination. Clinical signs were classified from mild to severe based on the vomiting, diarrhea and mortality rate of suckling piglets. And to check the period of damage to farm due to the occurrence of PED, by analyzing the productivity of the farm. In all farms, feces and sludge were collected and genotype analysis was performed.

Results

On farms A, C and D, severe watery diarrhea and vomiting were observed in suckling piglets, and anorexia and diarrhea were observed in most sows. In the case of farms B, E and F, watery diarrhea and vomiting in suckling piglets were observed lower than those of other farms.

The mortality rate in live vaccination groups was significantly lower than that of in feedback groups. The duration of clinical signs in the live vaccination groups observation was shorter than in the feedback groups.

Clinical symptoms lasted for up to 4 weeks, and it took up to 6 weeks for the fertility rate to recover in the farms of using feedback feeding. However, in the farms vaccinated with live PED vaccine, clinical symptoms lasted for up to 2 weeks, and it took only up to 2 weeks for the fertility rate to recover.

As a result of the genotype analysis of A to F farms, it was confirmed as G2b, which was popular in 2014 in South Korea.

Discussion

As a result of using live PED vaccines against PEDV on farms, it is considered that live vaccines can replace feedback feeding methods.

And PED vaccination consistently in the farms will prevent PED outbreak and cut PEDV circulation on farms.

References
1. CH Lee et al. 2014. Emerg Infect Dis 20(7): 1223.
2. KI Jung et al. 2020. Virus Res 286:198045.

P-V-030

COMPARISON OF THE EFFICACY OF TWO READY-TO-MIX PCV2 AND MYCOPLASMA HYOPNEUMONIAE VACCINES BY LUNG LESION SCORING AT SLAUGHTERHOUSE IN CHINA

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Introduction

PCVD due to PCV2 virus and Enzootic pneumonia due to primarily Mycoplasma hyopneumoniae (M.hyo) are the most common diseases in current swine herds causing huge and economic losses. Vaccination against the two pathogens is widely used in the pig health control (1,2). Several commercial bi-valent vaccines are available in China. The aim of this study was to evaluate the efficacy of two vaccines by slaughter weights comparison and lung lesion scoring at slaughterhouse.

Materials and Methods

Three weeks-old piglets were randomly divided into two groups which vaccinated either with Circovac® and Hyogen® (DUO, Ceva, 2.5 ml/dose)-as Ready-To-Mix (RTM) or PCV2/M.hyo RTM vaccine A (2 ml/dose). The piglets were marked and fed in the same barn until slaughtering. The two groups' pigs were sent to slaughterhouse and implemented lung lesion scoring using Ceva Lung Program (CLP) at same day with double blind testing. The vaccine grouping information would be released after the scoring and weight data were sent to the farm manager.

Results

DUO group had less Enzootic pneumonia (EP)-like lesions compared with the vaccine A group following CLP methodology (Table 1).
DUO group had higher slaughter weigh compared with the vaccine A, consistent with the scoring results.

Conclusions and Discussion

According to the study of Straw (3), 1% of affected surface on lungs decreases ADG by 3.74g. So theoretically, the lower lung scoring, the better growth performance including the slaughter weight, consistent with this study. Lung lesion scoring at slaughter is a useful method to assess the efficacy of Mycoplasma hyopneumoniae vaccines. DUO vaccination reduced the extension of EP-like lesions compared to Vaccine A and also increased the slaughter weight.

Acknowledgement and References

1. Segales J et al. 2014. Expert Rev. Vaccines Early online. 1–15
2. Maes et al. 2017. Transbound Emerg Dis. 1–15
3. Straw BE. et al.1989. Am Vet Med Assoc. 195:1702–6.

Table 1

Table 1: EP-like lesion by CLP in different groups

	DUO (n=99)	Vaccine A (n=108)
Broncho-pneumonic lungs	48.49%	50.00%
Affected surface out of sick lungs	2.70%	4.13%
Lungs with scars	38.38%	47.66%
EP Index	1.02	1.42

Table 2

Table 2: The slaughter weight in different groups

	DUO (n=99)	Vaccine A(n=108)
slaughter weight kg	115.63	114.17

Graphs or Images 1

Fig.1 Lung lesion scoring at slaughterhouse by CLP

P-V-031

CONSTRUCTION AND EXPRESSION OF AFRICAN SWINE FEVER VIRUS CD2V IN MAMMALIAN PROTEIN EXPRESSION SYSTEM

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- Introduction

African swine fever (ASF) is an infectious swine disease that causes lethal hemorrhagic fever in domestic pigs of all ages and breeds bringing unmeasurable economic losses in affected countries. The causative agent, the African swine fever virus (ASFV), has large, double-strained, linearized DNA as the genome with at least 168 open reading frames. There are two phenotypes of virion of ASFV: the mature virions are completely enveloped, and the immature virions are without an outer envelope but still infectious. The only known viral protein on the outer envelope of mature ASFV is CD2v, which is named for its similarity with CD2 on T cells. However, the currently available diagnostic antibodies and few rapid tests for the detection of ASFV antigen in the market are mainly focused on the capsid proteins such as p72 or other early proteins like p30. In this case, those fully-enveloped, mature virions of ASFV may be neglected due to their nature shield and given a false negative result by using those tests.

- Materials and Methods

Therefore, in the present study, we are going to generate monoclonal antibodies specific for CD2v that can be further used in developing multiplex rapid tests accompanied with other ASFV antibodies. In order to mimic the natural structure and characteristics of CD2v, the mammalian protein expression was used to produce the ectodomain of CD2v of ASFV. The ectodomain of CD2v, representing 16-205 amino acids, was linked with tissue plasminogen activator (tPA) signal sequence, cloned into pcDNA3.1-His-Myc, and transfected to human embryonic kidney (HEK) 293 cell line.

- Results

As demonstrated by immunocytochemical staining (ICC) and western blotting, the ectodomain of CD2v linked with tissue plasminogen activator (tPA) signal sequence for secretory was successfully expressed by the HEK293 cell line. The ectodomain of CD2v expressed and purified will be subsequently used as an antigen to immunize mice and produce monoclonal antibodies.

- Conclusions and Discussion

For future works, those CD2v-specific monoclonal antibodies will be selected for multiplex rapid tests. Along with the use of capsid-specific monoclonal antibodies, the multiplex rapid tests developed in our study are expected to detect both enveloped and non-enveloped virions of ASFV.

- Acknowledgement and References

We thank Dr. Hui-Wen Chang and Dr. Yen-Chen Chang from National Taiwan University for kindly providing the HEK293 cell line in the present study.

P-V-032

CONSTRUCTION OF A NOVEL LINEAR INFECTIOUS CLONE OF PORCINE CIRCOVIRUS 3

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Introduction: Porcine circovirus type 3 (PCV3) is an emerging virus that was first identified in pigs suffering from porcine dermatitis and nephropathy syndrome (PDNS) in 2015. Since then, PCV3 has been detected in many countries and has been reported to be associated with respiratory diseases, multisystemic inflammation, digestive diseases, and reproductive disorders. PCV3 nucleic acid has been found in pig tissues, serum, semen, and even colostrum, suggesting that PCV3 poses a potential threat to the health of pigs worldwide. Therefore, the biological properties and pathogenic mechanism of PCV3 needs to be studied for disease prevention. Since the PCV3 virus is difficult to be isolated and cultured, the purpose of this study is to construct a PCV3 infectious clone and evaluate the infectivity of the PCV3 rescued virus for further studies of viral pathogenesis.

Materials and methods: Full length of the PCV3 genome was first amplified from the lymph-node tissues of sick pigs by PCR and then cloned into the pJET1.2 vector. The recombinant plasmid pJET1.2-PCV3 was amplified as a PCR template and the infectious clone was obtained as a linear DNA amplicon. Then, human embryonic kidney 293 (HEK293T) cells were transfected with the PCV3 linear infectious clone for 7 days, followed by frozen and thawed three times to collect the supernatant as a P0 PCV3 virus stock. The swine testis (ST) cells were further infected with P0 PCV3 virus stock for 10 days, followed by frozen and thawed three times to collect the supernatant as a P1 PCV3 virus stock. Viral replication was assayed by infection of ST cells with P1 PCV3 virus stock, and the viral RNA was extracted for real-time PCR to estimate the viral growth curve. In addition, the immunofluorescence assay (IFA) was performed to examine the expression and cellular distribution of viral capsid protein (Cap) in P1 PCV3-infected ST cells. Furthermore, three 4-week-old specific pathogen-free (SPF) piglets were inoculated intramuscularly with 2 ml of P1 PCV3 rescued virus and intranasally with 2 ml of the virus to study the pathogenicity of the virus in vivo. At the day 28 post-inoculation, various piglet organs were collected and the distribution of PCV3 virus was analyzed by real-time PCR. The distribution of PCV3 antigen was further confirmed by immunohistochemistry (IHC) with the monoclonal antibody specific to PCV3 Cap.

Results
PCV3 virus was successfully rescued by the linear infectious clone strategy, and the virus titer was measured as 106.8 TCID50/ml. The PCV3 Cap protein was showed to accumulate in the nucleus at hour 72 post-infection in IFA analysis. In the piglet experiment, PCV3 nucleic acid was detected in all tissues of PCV3 inoculated pigs with the highest copy number of viral load in lung and lymph node. The IHC results also showed strong positive signals in these tissues, and the PCV3 antigens were observed in the mononuclear cells. All these results indicated that the PCV3 virus rescued from the linear infectious clone was infectious and it could be used for subsequent studies on the pathogenic mechanism of the virus.

P-V-033

CROSS-FOSTERING: ASSESSEMENT OF STRATEGIES AT FARM LEVEL.

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INTRODUCTION
The need for litter equalization practices at farm level (cross-fostering) became increasingly vital in the hyper prolificacy scenario of the last two decades [1]. However, fostering practices present relevant detrimental risks [2], particularly on PRRSV-positive farms [3,4], and guidelines to reduce these practices to the minimum have been gradually adopted: only surplus piglets should be moved, and only within the first 48 hours of life of the piglet and farrowing of the dam [5]. The present study aimed to compare cross-fostering practices at farm level with the established guidelines.

MATERIAL AND METHODS
Ten (10) PRRSV-positive farms with “minimum cross-fostering” policies were assessed. The available litters in each farm were ear tagged with an individual number per piglet at the first day of life. The litter of origin was recorded (sow number) and, at weaning again, the litter number (sow number) was recorded. The procedure was repeated during 3 to 4 weeks in each farm. The percentage of surplus piglets (initial number of piglets minus the number of weaned piglets) was used as reference value for the expected percentage of cross-fostered piglets. The data were recorded and analyzed with Microsoft Excel®.

RESULTS
The initial number of piglets ranged between 15,3 and 12,4 piglets per litter, and the number of piglets weaned per litter ranged between 13,0 and 10,9.
The percentage of surplus piglets ranged between 17,1% and 6,5% per farm. However, the percentage of cross-fostered piglets ranged between 81,5% and 9,7%.
The percentage of cross-fostered piglets was highly above the percentage of surplus piglets in 90% of the farms, and in 50% of the farms the percentage of cross-fostered piglets was above 20%, although none of these farms had more than 17,1% of surplus piglets.

CONCLUSION AND DISCUSSION
The results show that, despite all farms had intentionally complied to the same guidelines for keeping cross-fostering at a level of the absolute necessary minimum, most of the farms moved a much higher percentage of piglets. Hence, increasing the risks associated with cross-fostering, particularly that of increased contamination with infectious agents, severely increasing the risk of disease losses and antimicrobial use [2,3,4].
Empirically, this seems to be a representative picture in commercial sow-herds in several, if not all, countries across the world. More comprehensive and detailed studies need to be performed to assess the reality of cross-fostering at farm level
However, the presented data clearly suggest a need to substantially improve cross-fostering practices and minimize the associated risks.

REFERENCES
1. Charneca R. et al. Effect of litter birth weight standardization before first suckling on colostrum intake, passive immunization, pre-weaning survival, and growth of the piglets. Animals (2021).

2. Nielsen C.L. et al. A field trial on the effect of cross-fostering on performance, clinical health and antibiotic usage during the suckling period of pigs. Prev Vet Med (2022).
3. Colomer M.A. et al. Improving the management procedures in farms infected with the Porcine Reproductive and Respiratory Syndrome virus using PDP models. Sci Rep (2019).
4. McCaw M.B. Improving the management procedures in farms infected with the Porcine Reproductive and Respiratory Syndrome virus using PDP models. Swine Health Prod (2000).
5. Zhang X. et al. Effect of Different Cross-Fostering Strategies on Growth Performance, Stress Status and Immunoglobulin of Piglets. Animals (2021).

P-V-034

DETECTION OF PORCINE CIRCOVIRUS TYPE 2, TYPE 3, AND MYCOPLASMA SUIS IN GILTS WITH PALE BODY USING REAL-TIME POLYMERASE CHAIN REACTION

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Introduction
Porcine circovirus (PCV) types 2 and 3 are significant pathogens that have harmed the global pig population. The virus is a single-stranded DNA virus that belongs to the Family Circoviridae and consists of two open reading frames (ORF), ORF1 and ORF2. PCV2 and PCV3 have been reported worldwide, including in Thailand (1). Mycoplasma suis is an important hemotropic pathogen in pig production. M. suis causes a variety of clinical signs, from acute (anemia) to chronic (delayed growth rate). Either PCV or M. suis infections can cause anemia in pigs. Farmers skipped all vaccinations to avoid needle injections during the African swine fever (ASF) outbreak and used female fattening pigs as dam lines, which may have carried PCV and spread it throughout the breeding unit. However, the detrimental effect on breeding stock performance was not noticeable.

Materials & Methods
This study included ten gilts aged 28–32 weeks with pale body skin and respiratory signs. Blood sample was collected in EDTA-treated tube and submitted to the swine health promotion laboratory. Geneaid™ DNA isolation kit (Geneaid Biotech ltd., Taiwan) was used to isolate DNA from EDTA-blood by following the instructions. The hydrolysis probe real-time PCR technique was selected to detect PCV2 (2), PCV3 (2), and M. suis (3). PCV2 and PCV3 were performed in duplexing. The cycle threshold (Ct) from QuantStudio3 was used to classify positive (Ct ≤ 40) or non-detection (Ct > 40).

Results
The results are presented in Table 1. Only three gilts were infected with PCV2 alone, while seven gilts tested positive for both PCV2 and PCV3. However, one gilt was found coinfected with M. suis and PCV2.

Conclusions & Discussion
The clinical signs were the same in all gilts: pale body and respiratory signs. Due to ASF prevention, the farm owner omitted the PCV2 vaccine and used female fattening pigs as a dam line. This measure may result in infections with PCV2 and PCV3 that are circulating within the pig farm. One gilt showed coinfection with PCV2 and M. suis. It is worth noting that the prevention protocol for ASF, such as skipped vaccination and using female fattening pigs as a dam line, may cause PCV2 and PCV3 outbreaks in breeding units. In conclusion, in a re-borne pig farm, not only the coinfection of PCV2 and PCV3 in the breeding unit should be of concern but also the coinfection with M. suis.

References
1. Khophloiklang, V. et al. (2021). Walailak J. Sci. Tech., 18, 6525
2. Kim, H.-R. et al. (2017). J. Virol. Methods, 250, 11–16
3. Guimaraes, A.M.S. et al. (2011). J. Appl. Microbiol., 111, 417–425

Table 1

Table 1 Results of PCV2, PCV3, and *M. suis* detection by TaqMan real-time PCR

Samples	PCV2	PCV3	*M. suis*
1	+	ND	ND
2	+	+	ND
3	+	+	ND
4	+	+	ND
5	+	+	ND
6	+	+	ND
7	+	ND	+
8	+	+	ND
9	+	ND	ND
10	+	+	ND

+: Positive (Ct ≤ 40), ND: Non-Detection (Ct > 40)

P-V-035

DETECTION OF PORCINE CIRCOVIRUS TYPE 3 IN SUCKLING AND FINISHER PIGS IN CENTRAL-SOUTHERN REGION OF TAIWAN

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Introduction
To date, four species of porcine circovirus (PCV) have been recognized. PCV2 is an important pathogen that causes significant economic losses in the swine industry. PCV3 is a newly emerging porcine circovirus with unknown pathogenesis and diverse clinical and pathological features. PCV3 induces a broad range of diseases similar to PCV2 and has been detected in sows, mummified fetuses and stillborn. Further, PCV3 has been also detected in invertebrates. Relevant research of PCV3 in Taiwan is rare, so the main objective of this study is to investigate the prevalence of PCV3 in suckling and finisher pigs in central-southern region of Taiwan.

Materials and Methods
A total of 52 oral fluid and 1245 fecal samples were collected from farms in Changhua, Yunlin, Chiayi, Tainan, Kaohsiung and Pingtung, and 152 tissue samples from cases were submitted to Yunlin-Chiayi-Tainan of Animal Disease Diagnostic Center from January 2022 to January 2023. PCV3 genome was detected by quantitative polymerase chain reaction (qPCR) using primers targeting cap gene [1]. PCR was performed to amplify the full-length genome for determining genotype in the PCV3-positive samples with Ct values under 25. Sequencing and analysis of cap gene of PCV3 were performed. Expression of two amino acid mutations (A24V and R27K) on the cap protein was used to divide all PCV3 strains [2].

Results
PCV3 positive rates were 17.3% (9/52) of the oral fluid samples, 10.5% (131/1245) of the fecal samples and 32.9% (50/152) of the tissue samples. All of the PCV3-positive samples showed Ct values under 25 and the cap genes of PCV3 strains were successfully sequenced. All of the strains from this study were clustered into PCV3c.

Conclusions and Discussion
In conclusion, our results confirmed the presence of PCV3 in central-southern region of Taiwan. PCV3c is the most prevalent genotype in Taiwan. Although no significant association was demonstrated between PCV3 infection and pathological changes, continuous surveillance will be performed and etiology of PCV3 will be investigated in future studies.

Acknowledgement
This work was supported by the Animal Disease Diagnostic Center of National Chiayi University, and I would like to express our thanks to all the members of the Bacteriology Laboratory in the department of Veterinary Medicine, National Chiayi University, Taiwan.

References
[1] Yuan et al., 2020. BMC Vet Res 16: 249.
[2] Fu et al., 2018. Transbound Emerg Dis 65: 296-303.

Table 1

Table 1 PCV3-positive rate in different samples.

Sample	Number of samples tested	Number of positive samples	Percentage
Oral fluid	52	9	17.3%
Feces	1245	131	10.5%
Tissue	152	50	32.9%

P-V-036

DEVELOPMENT OF A CLASSICAL SWINE FEVER VIRUS (CSFV) ERNS ANTIBODY ALPHALISA ASSAY FOR RAPID DETECTION AND CAPABLE OF DIFFERENTIATING INFECTED FROM CSFV-VACCINATED ANIMALS (DIVA)

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Introduction

Classical swine fever virus (CSFV) is an OIE-listed disease that requires highly effective diagnostic tools for its detection and control. The bead-based AlphaLISA® (Amplified Luminescent Proximity Homogenous Assay) antibody format reportedly achieves both high analytical sensitivity and rapid turn-around because of short incubation times and no wash steps [1]. The aim of this study was to develop a CSFV Erns AlphaLISA for serum and oral fluids that would complement the current CSFV E2 DIVA vaccines.

Materials and Methods

The CSFV Erns AlphaLISA was designed to detect IgG isotype-specific antibody. After assay development, its performance was evaluated using a panel of well-characterized serum (n = 760) and individual (n = 528) or pen-based (n = 30) oral fluid samples from 4 groups of animals: (1) unvaccinated and uninoculated; (2) unvaccinated and inoculated with wild-type CSFV (ALD strain); (3) uninoculated and vaccinated with a live CSFV (LOM strain) vaccine; and (4) uninoculated and vaccinated with live CSFV marker vaccine. The presence of CSFV antibody in samples from inoculated or vaccinated pigs was confirmed by the serum-virus neutralization test (VNT). The diagnostic performance of the CSFV AlphaLISA determined by receiver operating characteristic (ROC) analyses of the serum and oral fluid testing data.

Results

At a cutoff of $S/P \geq 0.7$, the diagnostic sensitivity of the CSFV Erns IgG AlphaLISA was 98.0% and 92.9% for serum samples and 96.4% and 96.7% for oral fluid samples from wild-type and vaccinated pigs, respectively. At the same cutoff, the diagnostic specificity was 100% for both serum and oral fluid samples from negative control and marker-vaccinated pigs.

Conclusions and Discussion

Because incubation times are short and no wash steps are needed, the assay combined DIVA capability with solid diagnostic performance, rapid turnaround, simplicity of use, and compatibility with both serum and oral fluid specimens.

Acknowledgement

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References

[1] Beaudet, L., Rodriguez-Suarez, R., Venne, M.-H., Caron, M., Bédard, J., Brechler, V., Parent, S., Bielefeld-Sévigny, M. 2008. AlphaLISA® immunoassays: the no-wash alternative to ELISAs for research and drug discovery. Nature Methods 5:8-9.

P-V-037

DEVELOPMENT OF A FIELD EFFECT TRANSISTOR BIOSENSOR FOR PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS DETECTION

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Introduction:
Porcine reproductive and respiratory syndrome virus (PRRSV) causes respiratory distress in nursery pigs and reproductive failure in sow which leads to tremendous economic loss in most pig-producing countries [1]. The traditional detection methods require a lot of time, trained personnel and specific laboratory equipment. To overcome these defects, many on-site rapid detection methods have been developed but the validity is usually unsatisfied. Field effect transistors (FET) can be used to detect protein-protein interaction, nucleic acid hybridization and antigen-antibody binding with a high validity [2]. Among them, extended-gate FET (EGFET) has a separated sensing area to avoid the influence of other factors and also with less effect of environmental changes [3]. Besides, EGFET can be mass-produced and thus cost-effective. Therefore, the aim of this study is to develop a biosensing FET for PRRSV detection.

Materials and Methods:
The FETs were immobilized with anti-PRRSV monoclonal antibody and then blocked by bovine serum albumin (BSA). PRRSV virions were lysed, and serial diluted into 10^2, 10^-1, 10^-4 and 10^-7 TCID50/mL； all dilutes were tested 3 times to evaluate the sensitivity of the FET. Pseudorabies virus (PRV) and porcine circovirus type 2 (PCV2) were used to test the specificity of the FET. To start the test, blank buffer was applied to setup baseline, then the analyte was added. After incubating for 10 minutes, the analyte was removed then washed with blank buffer to remove unspecific bindings. Blank buffer was loaded for 10 minutes for gaining the ID-VG curve. The threshold voltage differences (ΔVth) were detected to check whether the molecules bind with the antibodies. The results were analyzed with one-way ANOVA.

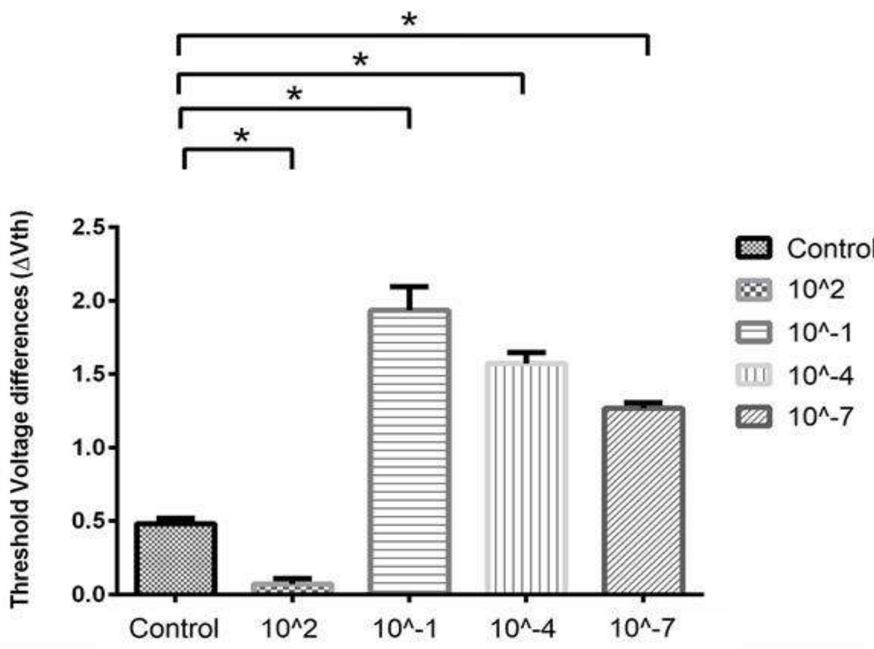
Results:
The sensitivity test showed that the ΔVth of 10^2, 10^-1 and 10^-4 TCID50/mL were significantly higher than the negative control ($p<0.05$). Furthermore, the ΔVth with 10^-4 dilution was 3-times higher than the negative control. The ΔVth of PRRSV was significantly higher than the ΔVth of PRV and PCV2 with 10^2 TCID50/mL ($p<0.05$). Therefore, the results show that the FET had low cross reactivity with PRV and PCV2.

Conclusions:
The bio-FET for detecting PRRSV had satisfying sensitivity and specificity. However, The clinical application of this assay under field conditions should be further investigated.

Acknowledgement:
We are gratefully acknowledge the technicians and veterinarians in NPUSTADDC and Novascope Diagnostics Inc. for their contributions to this study.

References:
[1] Zimmerman JJ, 2019. Disease of swine (11th ed.)
[2] Pullano SA et al., 2018. Sensors 18, 4042.
[3] Huang YC et al., 2014. Jpn. J. Appl. Phys. 53, 06JG02.

Graphs or Images 1



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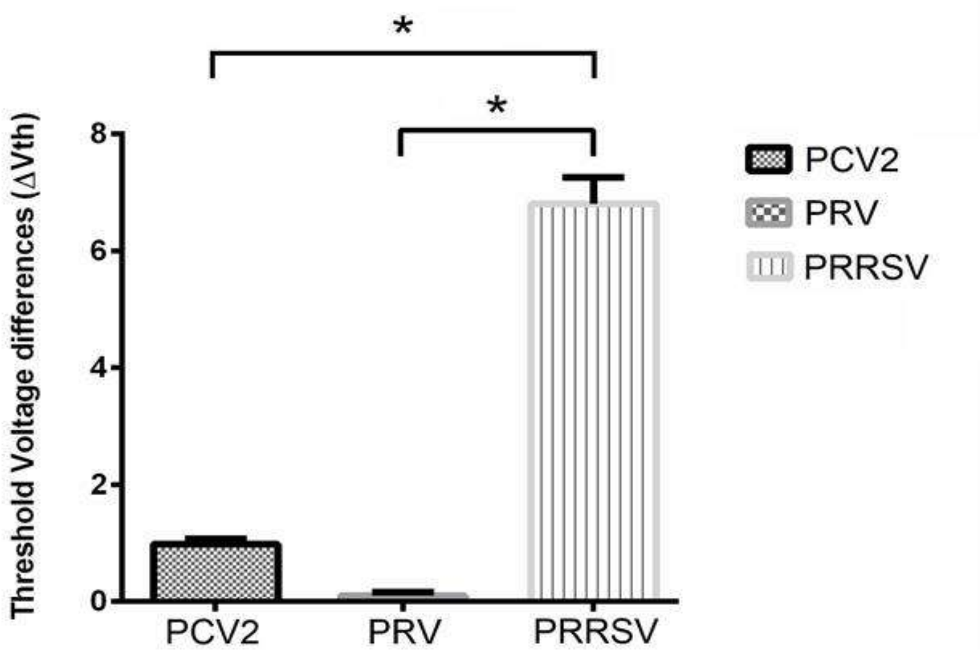

P-V-038

DEVELOPMENT OF A GENOTYPE 2B-BASED PORCINE EPIDEMIC DIARRHEA VIRUS (PEDV) VACCINE CANDIDATE

Student Dae-Min Kim[1], Ph.D. Sung Hyun Moon[2], Student Seung-Chai Kim[2], Professor Yeonsu Oh[3], Professor Ho-Seong Cho[2], Prof. Dong-Seob Tark[1]

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Introduction

Porcine epidemic diarrhea (PED) is a highly contagious enteric disease in pigs, which is characterized by watery diarrhea, vomiting, and severe hydration leading to huge economic losses in the swine industry. Since PED first emerged in South Korea in 1992, it has been annually reported. Vaccination is one of the most effective ways to control PED and is performed vigorously in PED endemic areas worldwide. Although the PED vaccines have been developed, these vaccines are less effective due to genetic diversity. Therefore, effective and safe vaccines are still required for controlling PEDV spread.

Materials and Methods

Schamatic diagram was represented in abstract image 1. Briefly, a PEDV was isolated from piglets suffering from diarrhea and serially passaged with 6 different media conditions in the cell culture system (180 passages). Then, the biological characteristics of the virus were analyzed by cytopathologic features and growth kinetics, and also pathogenicity was evaluated in 5-day-old piglets. Subsequently, the whole genome sequence of PEDV was determined by next generation sequencing (NGS) and the genomic alteration was performed in comparative analysis.

Results

The virus was successfully isolated and confirmed by cytopathic effects characterized by syncytium formation and fluorescent antibody (FA) test using PEDV-specific monoclonal antibody. The virus was adapted to 6 different culture conditions and the peaked titer was recorded by 8.67 ± 0.29 log10TCID50/ml. And the virus with different biological characteristics has been identified by the unique cytopathic effects without syncytium formation. In animal experiments, it was confirmed that the viruses were attenuated through 100 % survival rates, reduced clinical symptoms, and microscopic analysis of intestinal villi length. All animal experiment were summarized in table 1. Furthermore, the strains were included in a novel clade from genogroup 2 and the clade was classified as a sub-clade according to each strain.

Conclusions and Discussion

We successfully developed the new PED vaccine candidates carrying novel biological and genetic characteristics. The developed strains have been proven to be completely attenuated by comparing the parental strain challenge group in regard of not causing any mortality or any severe clinical diarrhea. Therefore, these strains could be useful live attenuated vaccine candidates for the control of PED.

Acknowledgment and References

This research was supported by a grant from IPET (Institute of planning and evaluation for technology in food, agriculture and forestry), the Republic of Korea (Project No.122004-2)

1. Y Kim et al. BMC Vet Res. 2017. 13(1):356
2. Wu Y et al. Virol J. 2019. 16(1):121
3. Y Hou et al. Int J Mol Sci. 2019. 20(21):5478

Table 1

Table 1. Summary of virulent studies of CKT-7 strains

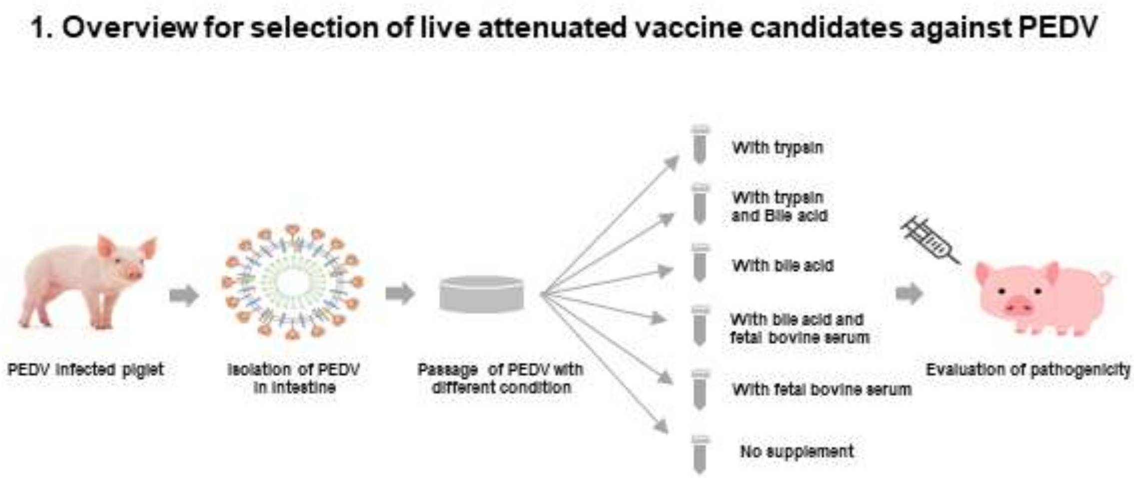
Group	Inoculum	Route	No.of pigs	Mortality rate* [% (no/total)]	Severe diarrhea rate [% (no/total)]	Clinical symptoms	Virus shedding	Peak fecal virus shedding titer [log10 copies/ul), dpi
1	Positive control	Oral	3	100 (3/3)	100 (3/3)	NA** (>4)	Started at 1 dpi	5.06 ± 0.24, 2
2	Negative control		3	0 (0/3)	0 (0/3)	No diarrhea	N/D***	NA
3	CKT-7 T15		3	0 (0/3)	0 (0/3)	Mild	Limited	NA
4	CKT-7 T15N		3	0 (0/3)	0 (0/3)	Mild	Started at 4 dpi	2.48 ± 0.13, 5
5	CKT-7 N		3	0 (0/3)	0 (0/3)	No diarrhea	N/D	NA
6	CKT-7 NF		3	0 (0/3)	0 (0/3)	Mild	Limited	NA
7	CKT-7 F		3	0 (0/3)	0 (0/3)	No diarrhea	N/D	NA
8	CKT-7 X		3	0 (0/3)	0 (0/3)	No diarrhea	Started at 2 dpi	2.68 ± 0.49, 3

* Fecal consistency scores : 0, solid; 1, pasty; 2, semi-liquid; 3, liquid; 4, death. Fc scores of 3 were regarded as severe diarrhea

** Not available

*** Non-detection

Graphs or Images 1



Porcine intestines were collected from PEDV-spreading pig farm in Republic of Korea. For isolation of PEDV, the samples were homogenized and inoculated into the monolayered Vero cells. Subsequently, isolated PEDV were incubated with six different culture conditions up to 180 passage numbers. Then, live attenuated vaccine candidates were evaluated to pathogenicity with 5 days old piglets.

P-V-039

ECONOMIC ASSESSMENT OF A SOUND-BASED PRECISION LIVESTOCK FARMING TOOL (SOUNDTALKS) BASED ON TIMING OF INTERVENTION AFTER A DUAL MYCOPLASMA HYOPNEUMONIAE AND PRRS VIRUS SEEDER CHALLENGE IN PIGS

Dr. Dale Polson[1], Dr. Carmen Alonso[1]
[1] *Boehringer Ingelheim*

Introduction
SoundTalks technology continuously records the sounds in pig production facilities and emits early warnings (yellow/red alerts) in the case of respiratory outbreaks triggering earlier caregiver awareness than via caregiver observations alone. Even though a capability for early warning has been established, further research is needed to understand the economic impact for producers. The objective of this study was to evaluate the performance and economic differences resulting from earlier intervention following the onset of a clinically detectable respiratory disease episode as measured by SoundTalks.

Materials and Methods
A total of 1655 11-week-old pigs (72 pens, 2 rooms) were continuously monitoring (i.e sound, water consumption, feed intake, treatments). All pens received 3 seeder pigs Mycoplasma hyopneumoniae-PRRS positive, and all animals received the same treatment. However, time of treatment after the first SoundTalks alerts (early vs late intervention) differed between treatment groups (randomly allocated): day zero (G0), 5 (G5) and 10 (G10). Mixed models were used to study production parameters associations with treatment groups after controlling for other independent variables. Economic effect differences between treatment groups were calculated using a “Standardized Economic Index” (SEI) based on a partial budget model using historical US monthly market prices and feed ingredient costs for the most recent 156 month time period (January2010-December2022). Performance differences of pre-defined contact challenged pigs that did not experience exceptional handling (e.g., snaring, bleeding, tracheal catheterization) were used for the SEI modeling.

Results
Two respiratory outbreaks caused by swine A influenza virus (IAV) were registered throughout the study and pigs were treated accordingly to group. Contact challenged pigs from G0 had a 12,7 and 20,4 gram higher ADG, a 23.4% and 10.1% decrease in individual treatments, and a 0.26% higher and 1.22% lower percent mortality compared to those from G5 and G10 respectively. All production variables were introduced into the SEI model and the resulting Benefit:Cost Ratio 156 month time series is shown in Figure 1.

Throughout the 156-month period, and using a CTO of USD $0.303/pig marketed (inclusive of installation, hardware and software subscription for a single site 1200 head grow-finish site) the mean monthly B:C Ratio was 3.51, ranging from 1.99 to 6.43 and exceeding 2:1 for 155 of 156 (99.4%) months (G0:G5). For the same 156 month period, the 48 month rolling B:C Ratio ranged from 2.62 to 4.02, exceeded 2:1 for 156 of 156 (100%) intervals (G0:G5).

Conclusions and Discussion
A 48 month rolling average was used to allow evaluation of the B:C ratio across the estimated lifespan of SoundTalks hardware. This study suggests that there can be a consistently favorable economic impact is achievable where relatively small performance differences are obtained using a technology that enables early detection and intervention.

References

1. Lopes Antunes AC, Jensen VF, Jensen D (2019) Unweaving tangled mortality and antibiotic consumption data to detect disease outbreaks – Peaks, growths, and foresight in swine production. PLoS ONE 14(10): e0223250
2. Polson, D. et al., 2018. Precision livestock farming (PLF) for pig health and production: Sound as a diagnostic sample. AASV, 2018.

Graphs or Images 1

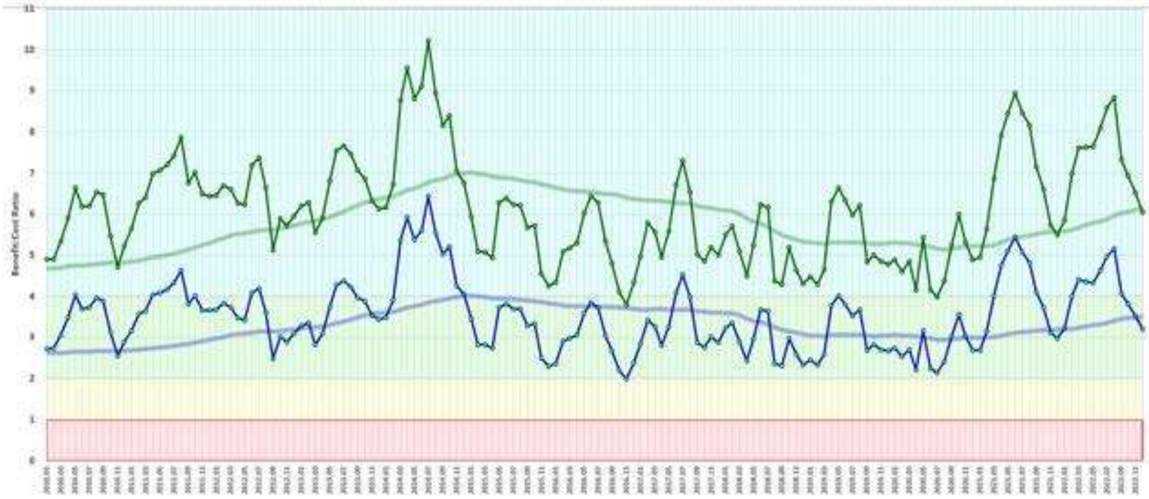


Figure 1. A 156 month time-series of benefit:cost ratio (green and blue dotted lines) as well as its 48 month rolling average (green and blue lines) for SoundTalks investment based on group differences (Group 0 vs 5 represented as blue lines; and Group 0 vs 10 as green lines) using North American monthly market price and feed costs
.

P-V-040

EFFECT OF FOSTERA® PRRS MLV AGAINST LINEAGE 3 PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS 2 IN A FARROW TO FINISH HERD IN TAIWAN

Ms. Yu-Hsuan Chen[1], Mr. Cheng-Han Yang[1], Mrs. Ya-Jong Lin[1], Ms. Chao-Wen Hsueh[1]
[1]*Zoetis Taiwan Limited*

Introduction
Porcine reproductive and respiratory syndrome (PRRS) is a single-stranded RNA virus has and will continue to evolve with new strains emerging with diverse capabilities of virulence, transmission, and persistence. Previous studies found lineage 3 of PRRSV2 can be found in most pig farms in Taiwan [1]. A challenge study showed Fostera® PRRS MLV, an attenuated lineage 8 strain can effectively against heterologous virulent lineage 3 PRRSV2 [2]. In this field report, we evaluated the protective efficacy of mass vaccination with Fostera® PRRS under field conditions.

Materials and Methods
The farm in evaluation study is a 1,300 sow capacity farm located in Southern Taiwan that managed in a 3-week batch system (1 farrowing every 3 weeks) and piglets are weaned at 28 days. This farm was routinely vaccinated with a PRRS lineage 5.1 MLV vaccine in both sows (mass vaccination every 3 months) and piglets (1-week-old) for many years. The farm noting a wave of severe PRRS-like clinical signs of coughing and high mortality in nursery and grow-finish pigs in March 2020. From the blood, lung, and lymph node samples of 11-week-old pigs sent to the veterinary diagnostic center, the diagnosis of PRRSV2 was confirmed. The PRRSV was then isolated and further categorized according to phylogenetic lineages based on ORF5 of the virus genome and confirmed as lineage 3.
Farm owner switched to Fostera® PRRS MLV from December 2020 and maintained the same timing of PRRS vaccination. Livability of pigs at nursery stage (4w-10w of age) was recorded during 2018/08-2022/12.

Results
The overall performance in nursery has improved and more stable than before from the livability record (figure 1). Even there was a dipping incidence at the batch in November 2021, other batches in the observed period sustained high livability till the end of observation period. The overall incidence and severity of clinical symptoms, mortality, and pneumonia in pigs of the farm from nursery to grow-finish herd were also lower.

Conclusions and Discussion
Isolates of PRRSV sequence were performed twice in 2020/12 and 2021/12 and both confirmed as lineage 3 PRRSV2, but the performance has been improved and remains stable after the farm started vaccinated Fostera® PRRS MLV. No record of severe PRRS outbreak until the end of observation period. The high mortality appeared in 2021/11 were confirmed a co-infection of PRRSV2 with other bacteria (Salmonella Typhimurium and E. Coli) in nursery, and the incidence resolved after the farm implemented feed medication and injection based on the antimicrobial sensitivity test report, also remedial action taken in their biosecurity strategy. There was no dipping of performance in nursery after that incidence. In summary, it is concluded that Fostera® PRRS MLV is more effective compared with previous PRRS vaccine at farm where pigs are exposed to lineage 3 PRRSV2.

Acknowledgement and References
[1] Deng MC, et al. 2015. Molecular epidemiology of porcine reproductive and respiratory syndrome viruses isolated from 1991 to 2013 in Taiwan. Archives of Virology 160:2709-2718 DOI 10.1007/s00705-015-2554-4.
[2] Hou F-H, et al. 2020. Evaluation of a type 2 modified live porcine reproductive and respiratory syndrome vaccine against heterologous challenge of a lineage 3 highly virulent isolate in pigs. PeerJ 8:e8840 http://doi.org/10.7717/peerj.8840

Table 1

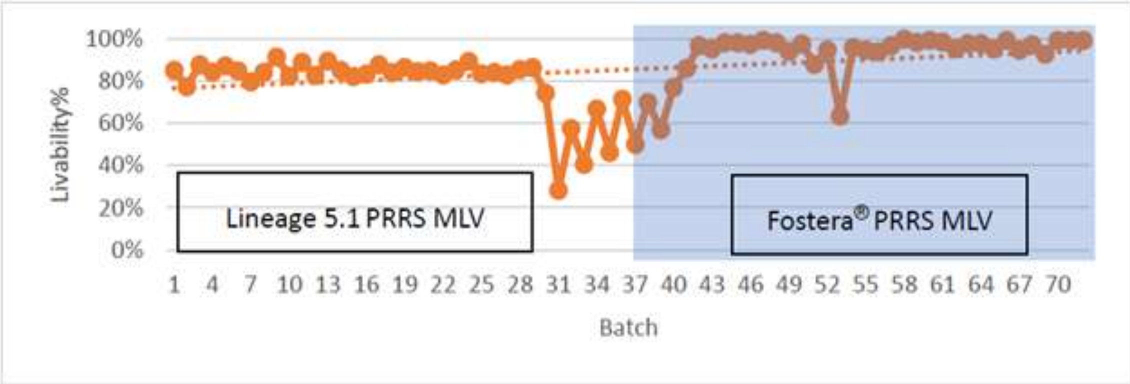


Figure 1. Livability at nursery (4w-10w) during 2018/08-2022/12

P-V-041

EFFECT OF FREEZE-THAW ON PRRSV RNA DETECTION BY RT-QPCR

Dr. Berenice Munguia-Ramirez[1], Dr. Betsy Armenta-Leyva[1], Dr. Alexandra Henao-Diaz[2], Fangshu Ye[1], Kent Doolittle[3], Silvia Zimmerman[3], Dr. Luis Gimenez-Lirola[1], Dr. Jeffrey Zimmerman[1]
[1]Iowa State University, [2]Pig Improvement Company Latinoamerica, [3]IDEXX Laboratories, Inc.

Introduction
Re-testing samples is common in diagnostic and research settings. This implies exposing samples to freeze-thaw cycles, but the effect on qPCR results is largely unexplored. Herein, the effect of freeze-thaw on the detection of PRRSV and a porcine internal sample control (ISC) by RT-qPCR was evaluated in serum, oral fluids, and feces.

Materials and Methods
Serum samples were from pigs experimentally inoculated with wild-type PRRSV (n = 5 pos; n = 5 neg). Oral fluids (6 pos; 4 neg), and feces (5 pos; 5 neg) were from individually housed pigs vaccinated with a PRRSV MLV (Ingelvac® PRRS MLV).

Samples were aliquoted into 2 ml tubes to create 4 sets (one per freeze-thaw "treatment"), and exposed to freeze-thaw cycles (2, 5, 10, and 15) by freezing at -80°C and thawing at 4°C overnight. Thereafter, samples (n = 40 per specimen) were tested in triplicates for PRRSV and ISC detection using a commercial PRRSV RT-qPCR (IDEXX Laboratories, Inc.). RT-qPCR Cqs were re-expressed in function of the PCR efficiency as “Efficiency standardized Cqs (ECqs)”. Freeze-thaw effect was analyzed using a mixed-effects regression model (MRM) in R 4.1.0 (R core team, 2020).

Results
In serum, no freeze-thaw effect was detected in PRRSV and ISC ECqs ($p > 0.05$; MRM). In oral fluids, the slope of the regression line showed a decrease of 0.027 (95% CI: 0.020, 0.033), and 0.052 (95%CI: 0.018, 0.085) ECqs per freeze-thaw cycle for PRRSV and ISC, respectively ($p < 0.05$; MRM). In feces, freeze-thaw cycles did not affect either PRRSV or ISC ECqs ($p > 0.05$).

Conclusions
Overall, freeze-thaw cycles had little impact on the detection of PRRSV and the ISC, albeit more so in oral fluids vs serum and feces. Notably, these results apply to PRRSV, and further studies are needed to address the effect of freeze-thaw in other pathogens and specimens.

Acknowledgements
PRRSV RT-qPCR testing was partially supported by donations from IDEXX Laboratories, Inc.

P-V-042

EFFECT OF HEATING OR DILUTING SWINE ORAL FLUID SAMPLES ON QPCR DETECTION

Betsy Armenta-Leyva[1], Berenice Munguía-Ramírez[1], Xue Lin[1], Fangshu Ye[1], Kent Doolittle[2], Silvia Zimmerman[2], Luis G. Giménez-Lirola[1], Dr. Jeffrey Zimmerman[1]
[1]Iowa State University, [2]IDEXX Laboratories, Inc.

Introduction:
Reports in the literature state that detection of nucleic acids (NA) in human oral fluids is improved by heating and/or diluting the sample. For example, "outstanding" direct RT-qPCR performance for the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in human oral fluid samples was reportedly achieved by diluting the sample with tris borate EDTA (TBE) and then heating at 95°C for 30 minutes (Ranoa et al., 2020). The objective of this study was to test the effect of heating (95°C × 30 m) or diluting (tris borate EDTA; TBE) swine oral fluid samples on the detection of RNA, i.e., PRRSV, influenza A virus (IAV), PEDV, or DNA, i.e., Mycoplasma hyopneumoniae (MHP).

Materials and methods:
In Experiment 1, oral fluid samples containing PRRSV (n = 8), IAV (n = 8), PEDV (n = 8) or MHP (n = 8) were 2-fold serially diluted (neat, 1:2, 1:4, 1:8) using oral fluid free of PRRSV, IAV, PEDV, and MHP as diluent (n = 32 aliquots per pathogen). Each aliquot was split into 4 and randomized to one of 4 procedures: (P1) heat (95°C × 30 m) and direct qPCR; (P2) heat, cool (25°C × 20 m) and direct qPCR; (P3) heat, cool, nucleic acid extraction, and direct qPCR; (P4, i.e., control) extraction and qPCR.
In Experiment 2, oral fluid samples containing PRRSV (n = 9), IAV (n = 10), PEDV (n = 10), or MHP (n = 10) were split into three aliquots: (D1) undiluted; (D2) diluted 1:2 with oral fluid free of PRRSV, IAV, PEDV, and MHP; (D3) diluted 1:2 with TBE (89 mM Tris, 89 mM boric acid, 2 mM EDTA). Samples were randomly ordered and then tested.
Nucleic acid extraction and qPCRs were done using commercial products (IDEXX Laboratories, Inc.).

Results:
In Experiment 1, standard procedures (P4, control) produced 32/32 aliquots positive for PRRSV, 32/32 for IAV, 32/32 for PEDV, and 32/32 for MHP. In contrast, the aggregated results for P1, P2, and P3, produced 7/96 aliquots positive for PRRSV, 14/96 for IAV, 13/96 for PEDV, and 65/96 for MHP, i.e., multiple false negatives. For positive results in all pathogens, standard procedures (P4) produced lower Cqs than procedures P1, P2, or P3.
The results (means) from Experiment 2 showed no gain with D2 or D3:
PRRSV - undiluted Cq = 32.3;D2 Cq = 33.5; D3 Cq = 34.8.
IAV - undiluted Cq = 28.9;D2 Cq = 30.0; D3 Cq = 29.8.
PEDV - undiluted Cq = 25.1;D2 Cq = 25.6; D3 Cq = 25.0.
MHP - undiluted Cq = 32.9;D2 Cq = 33.5; D3 Cq = 33.9.

Conclusions and discussion:
In conclusion, the heat and dilution treatments described in the literature were detrimental to the detection of PRRSV, IAV, PEDV, and MHP nucleic acids in oral fluid samples by qPCR. Interestingly, examination of the literature showed that these reports often did not include comparisons with standard methods. Therefore, we recommend that research along these lines include quantitative measures of the gain or loss in performance achieved by

alternative methods. In this study, the inclusion of comparisons showed that optimum results were obtained using standard extraction and amplification methods.

Acknowledgement:
Funding provided by the Swine Health Information Center (USA), IDEXX Laboratories, Inc. (USA), and CONACYT (Mexico).

References: Ranoa et al 2020. bioRxiv

P-V-043

EFFECT OF PCV2 VACCINATION IN SOW'S REPRODUCTION PARAMETERS IN A PRRSV NEGATIVE FARM

Mr. Jinan Ju[1], Mr. Jinyuan Liu[2], Mr. Yun Gao[2], Mr. Paul Li[1], Mr. Jun Zhang[1]
[1]*Ceva Sante Animale,* [2]*Pig industry department of Tyccon group*

Introduction
Porcine circovirus type 2 (PCV2) is ubiquitous in domestic pig farms causing the economically-important porcine circovirus diseases (PCVD). Experimental data shows porcine embryos are susceptible to PCV2 infection, which might lead to embryonic death, stillbirth, or foetus mummification (1). The aim of this study was to evaluate the effect of sow vaccination in their reproductive parameters in a PRRSV negative farm.

Materials and Methods
The trial was implemented in a large-scale pig farm with 4 weeks batch management in northwest China. The farm was PRRSV negative and PRV stable with gE positive in certain herds. Two consecutive batches of sows were selected randomly. Sows from batch 0908 defined as Group A were vaccinated with Circovac® (Ceva Hangzhou, 2ml/sow, I.M.) twice at 30 and 50 days of pregnancy. Sows from batch 1006 defined as Group B didn't apply any PCV2 vaccine as usual during pregnancy. The farm management procedures were all same between two groups. Total born, born alive, stillborn/mummies, and average birth weight were recorded and compared between two groups after farrowing.

Results
Born alive per litter of group A vaccinated with Circovac® was 0.57 higher than that of group B without PCV2 vaccination, statistically significant ($p<0.01$). Stillborn/ mummies of group A was 0.44 lower than that of group B, statistically significant ($p<0.01$). Total born and average birth weight were very close or same between two groups, no statistical difference.

Conclusions and Discussion
During this study, it could be observed an improvement of the sows' reproductive parameters in vaccinated animals, suggesting a positive impact of sow vaccination with Circovac®, in concordance with other study (2). As PCV2 contamination was widely spread around the world, farms could benefit from effective sow vaccination in the field. Vaccination procedure should be implemented for at least another parity to follow up the influence of long-term vaccination. Sow vaccination might also be applied at weaning to avoid interference of maternally derived immunity (MDI) with piglet's vaccination (3).

Acknowledgement and References
1. Kim J et al. Prevalence of porcine circovirus type 2 in aborted fetuses and stillborn piglets. Vet. Rec. 2004: 489–492.
2. Pejsak Z. et al.. Bull Vet Inst Pulawy 2009: 159-164.
3. Segalés. Expert Review of Vaccines Best practice and future challenges for vaccination against porcine circovirus type 2. 2015.

Table 1

Table 1: Reproductive parameters per litter of Group A and B

	Group A (n=428)	Group B (n=362)
Total born	12.63[a]	12.51[a]
Born alive	11.19[a]	10.62[b**]
Stillborn/mummies	1.44[a]	1.88 [b**]
Average birth weight kg	1.16[a]	1.16[a]

**Statistical significance between a and b ($p<0.01$)

P-V-044

EFFECT OF PEN SIZE AND NUMBER OF ROPES ON BEHAVIORS ASSOCIATED WITH ORAL FLUID SAMPLING

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[1]Iowa State University, [2]United States Department of Agriculture, Veterinary Services, Strategy and Policy, Center for Epidemiology and Animal Health, [3]PIC North America, [4]National Pork Board

Introduction.
Oral fluids are the sample of choice for routine surveillance on commercial farms. The method is well established in the swine industry because of the advantages it offers over individual samples, i.e., oral fluids are easily collected with no stress to workers or pigs and, overall, oral fluids are more likely to detect the pathogen of interest and at lower cost than individual pig samples. There are many refereed publications reporting the use of oral fluids for the detection of specific pathogens, but there are few studies elucidating the pig behaviors associated with oral fluid collection. In pens of ~25 pigs, White et al. (2014) reported that > 70% of pigs participated in OF collection in 30 minutes. In pens of 16 - 30 pigs, Graage et al. (2019) reported that 76% -82% of pigs contributed to the sample. However, given the wide range of pen sizes used in the US industry (20 - 1000 pigs), more extensive information about pig participation in oral fluid sampling is required for surveillance design.

Material and Methods.
Our long-term objective is to develop industry guidelines for OF collection across pen sizes. The aim of this study was to measure the effect of pen size and number of ropes on pig-rope interactions. The study was performed in 10-14 week-old pigs housed in pens of ~25, ~65, ~100, and ~130 pigs (32 pens for each size category) on commercial wean-to-finish production sites. In each pen, ~12% of pigs were individually marked for observation. Treatments consisted of placing 1, 2, 3, or 4 ropes in a pen (0.5-inch, 3-strand, twisted 100% cotton) and video recording pig interactions with the rope for 30 minutes. Each treatment (number of ropes) was randomly assigned to pens and repeated 8 times, i.e., once in each of 8 different pens. Video recordings were reviewed and rope contacts were counted for each marked pig by minute of the observation period, with "contact" defined as a video image of the pig with the rope in its mouth.

Results.
Pig behavior related to pig-rope interaction was affected by pen size and the number of ropes provided. For example, when one rope was placed in the 8 pens of 25 pigs, 71% (17 of 24) of the marked pigs interacted with the rope; when 4 ropes were provided, 92% (22 of 24) of the marked pigs interacted with the rope(s). In contrast, when one rope was placed in 8 pens of ~130 pigs, 23% (28 of 120) of the marked pigs interacted with the rope; 48% (58 of 120) when 4 ropes were provided.

Conclusions and Discussion.
This study documented changes in oral fluid sampling behaviors as a function of pen size and the number of ropes provided in the pen. Importantly, this work is strictly focused on pig behavior. Future work will expand to include the probability of detection as a function of pen size, number of ropes, and pig behavior.

Acknowledgements.
Our thanks and appreciation to Dr. J.F. Connor and Dr. T. Bauman for providing access to production facilities.

P-V-045

EFFECTS OF TWO DIFFERENT CIRCOVIRUS TYPE 2 AND MYCOPLASMA HYOPNEUMONIAE VACCINE COMBINATIONS ON ACUTE PHASE PROTEINS AND BODY TEMPERATURE IN PIGLETS

Dr. Yu-Sik Oh[1], Dr JongYeon Jeong[1], Dr GiJong Kang[1], Dr Chaewun Bae[1], Dr HeeJin Chae[1], Dr Carlo Magno Maala[2], Dr Luiz Leczniezki[3]

[1]Boehringer Ingelheim Animal Health Korea Ltd., [2]Boehringer Ingelheim Animal Health Philippines Inc., [3]Boehringer Ingelheim Animal Health GmbH

Introduction

Porcine circovirus type 2 (PCV2) and Mycoplasma hyopneumoniae (M. hyo) are two significant pathogens causing Porcine respiratory disease complex (PRDC). These two pathogens result in serious economic losses in the Korean swine industry (1). Two different kinds of commercial combination vaccines are available to mitigate financial losses, requiring fewer injections and providing better efficacy. Acute phase proteins (APPs) and body temperature have been proposed as suitable veterinary biomarkers to monitor stress for detecting inflammation and infections (2). In this study, the APPs and body temperature were evaluated for a better decision.

Material & Method

The studies were performed in four different commercial farrow-to-finish single-site farms, ranging from 180 to 650 sows in various provinces of South Korea. On weaning day, 1044 pigs were randomly divided into two groups equally and ear-tagged and vaccinated individually. Group 1 was vaccinated with 2 ml FLEXcombo® (freshly prepared mixture of Ingelvac CircoFLEX® and Ingelvac MycoFLEX®, Boehringer Ingelheim Vetmedica GmbH). Group 2 was vaccinated with 2 ml Porcilis® PCV M hyo (Intervet International B.V., Netherlands). Body temperature was measured prior to vaccination and 6 as well as 24 hours post vaccination in 10 animals from behind the ear using a contactless infrared thermometer. A subset of 10 piglets of each group was subjected to blood sampling before vaccination and 24 and 48 hours post vaccination to determine the acute phase proteins, haptoglobin and C-reactive protein using the Life Diagnostics ELISA kit. Statistical analyses were performed by two-way ANOVA.

Results

Both treatment groups showed an increase of acute phase proteins in serum compared to basal levels. However, this increase in acute phase proteins was more pronounced in group 2, vaccinated with Porcilis® PCV M hyo, and thereby significantly higher compared to group 1, vaccinated with a mixture of Ingelvac CircoFLEX® and Ingelvac MycoFLEX®.

Conclusions and discussion

FLEXcombo®, a highly efficacious vaccine against PCV2 and M. hyo, demonstrated to be more tolerable, evidenced by the lower expression of acute phase proteins and no observed increase in body temperature after vaccination. The findings of this study are consistent with other studies (3) and reinforce that the selection of vaccines should certainly be based on efficacy as well as on their effect on piglets' well-being.

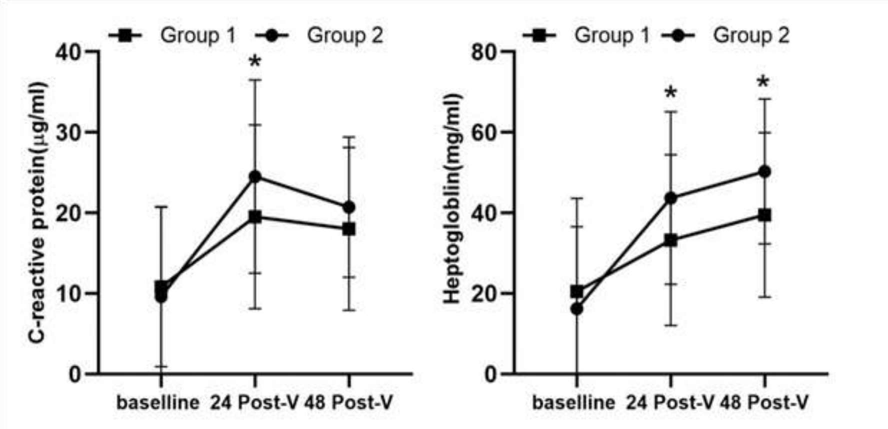
Acknowledgement and References

1. National swine disease surveillance 2016. KPPA
2. Piñeiro et al., (2007). The Veterinary Journal, 173, 669-674

3. Figueras et al., (2018). Proceedings of the 25th IPVS, Chongqing, China.

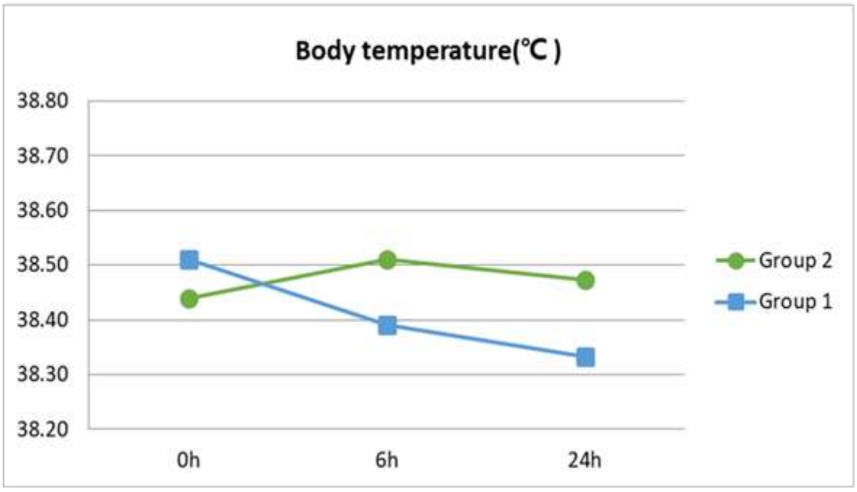
Graphs or Images 1

Figure 1. Mean serum concentrations of haptoglobin and C-reactive protein in piglets' baseline, 24 and 48 hours after vaccination, significant differences between groups 24- and 48-hours post-vaccination. (By Fisher LSD, P<0.05)



Graphs or Images 2

Figure 2. Mean body temperature in piglets: 0, 6, and 24 hours after vaccination.



P-V-046

EFFICACY AND SAFETY OF A TYPE 2 PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS MODIFIED LIVE (PRIME PAC™ PRRS) AGAINST CHALLENGE OF KOREAN TYPE 2 PRRSV FIELD ISOLATE (KU-N2001)

Dr Beomsoo Park[1], Dr Youngin Kim[3], Dr Jiyong Kim[3], Dr Sanghyun Noh[3], Dr Jeongyeon Shin[3], Dr. Hong-Yao Lin[2], Professor Young S Lyoo[1]
[1]College of Veterinary Medicine Konkuk University, [2]MSD Animal Health Innovation Pte Ltd, [3]MSD Animal Health Korea Ltd

Introduction
In Korea, porcine reproductive and respiratory syndrome outbreaks have been continuously reported since 1994, and PRRS control programs by vaccination are widely applied. The aim of this study is to confirm the safety and efficacy of the PrimePac™ PRRS vaccine against local isolates of Type 2 PRRS in Korea.
Materials & methods
Twenty-five 3-week-old, PRRSV-negative pigs were randomly assigned into 5 groups (Table 1).
After the treatment, animals were observed daily. Measuring the body temperature and daily weight gain with PRRS-related clinical scoring were performed for the vaccine safety evaluation. Four weeks after the treatment, Groups A, B, C, were intranasally challenged with PRRS isolate KU-N2001, P5 (105.01 TCID50/ml, 5mL). Blood was collected weekly throughout the test period for real-time PCR, antibody titer and PRRSV neutralizing antibody titers. For each group, 3 animals were necropsied at 1 week after challenge inoculation and 2 animals were at 2 weeks after challenge and pathological lesions in the lungs were quantified. Statistical analysis between experimental groups was compared using a multiple t-test, and a p-value of less than 0.05 was considered statistically significant.
Results
Significant differences in PRRSV viremia were found at 35 dpi or 42 dpi.($P<0.05$) between both vaccinated groups (A and B) and the control group C (Figure 1). From 13 dpi, Groups A and B showed higher neutralizing antibody titers than Group C, these were maintained until the end of the experiment. Especially, Group A showed the highest value of 5 (log2) at 42 dpi during the overall test period. Group A and B had significantly lower body temperatures at 14 days after challenge compared to the control. Both vaccinated groups (A & B) showed lower clinical symptom scores and fewer pathological lesions than Group C after challenge. With the comparison of average daily gain among the groups, daily weight gain of Group A and B was higher in overall test period, and a significant difference was found especially at 5wpi ($p<0.05$).
Conclusions
The findings in this study indicate that PrimePac™ PRRS effectively reduces PRRS viremia and induces neutralizing antibody and reduce clinical symptoms against challenge with a local Korean PRRS type 2 isolate. This vaccine may serve as a useful control tool for PRRS and increase productivity of pig farms in Korea.
References
1. Ladinig, Andrea, et al. "Pathogenicity of three type 2 porcine reproductive and respiratory syndrome virus strains in experimentally inoculated pregnant gilts." Virus research 203 (2015): 24-35.

2. Kwon, Taeyong, et al. "Genomic characteristics and pathogenicity of natural recombinant porcine reproductive and respiratory syndrome virus 2 harboring genes of a Korean field strain and VR-2332-like strain." Virology 530 (2019): 89-98

Figure 1

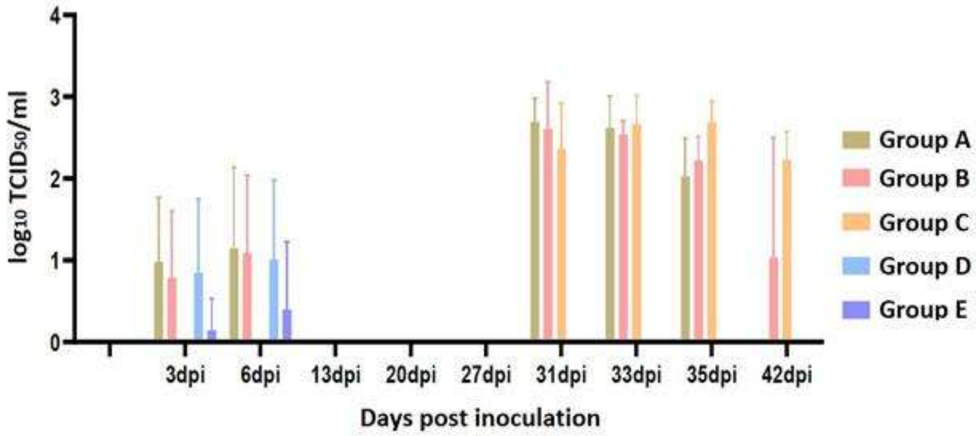


Figure 2

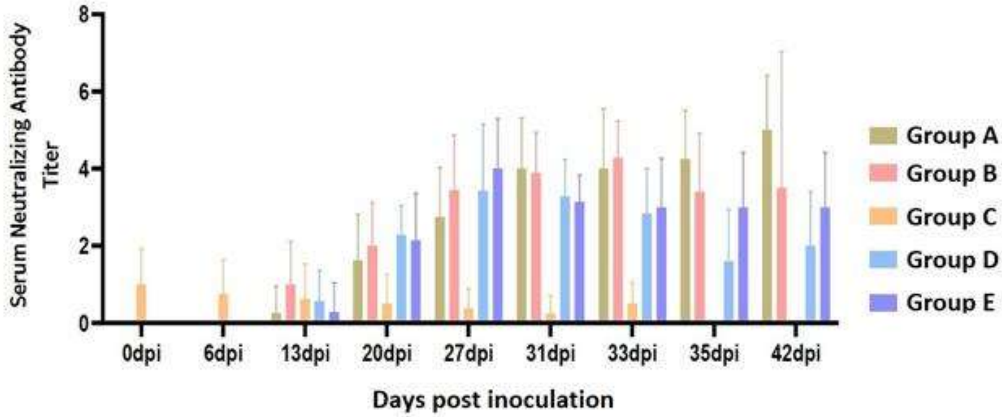
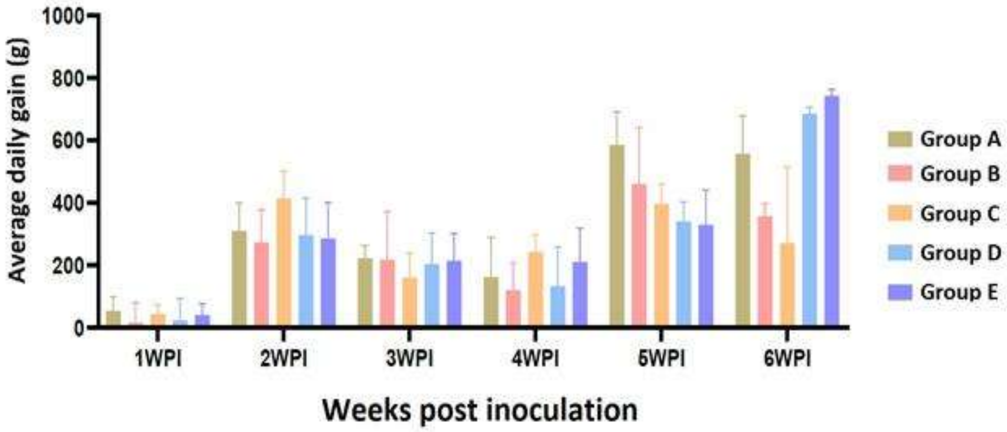


Figure 3



P-V-047

EFFICACY OF RECOMBINANT SUBUNIT VACCINE CANDIDATE AGAINST FOOT AND MOUTH DISEASE VIRUS SEROTYPE O AND A IN PIG

Prof. Jong-Soo Lee[1], Dr. Sung-Sik Yoo[2], Dr. Jong-Hyeon Park[3]
[1]College Of Veterinary Medicine, Chungnam National University, [2]Choong Ang Vaccine Laboratory Co., Ltd, [3]Animal and Plant Quarantine Agency

Foot-and-mouth disease (FMD) is a highly contagious and economically devastating disease in cloven-hoofed animals. To prevent the spread of FMD virus (FMDV), traditional inactivated vaccines are used to immunize susceptible animals in disease-endemic countries. However, the inactivated FMD vaccine has several limitations, including safety concerns. To overcome these limitations, subunit proteins have been studied as alternative vaccine candidates. In this study, we designed two multiepitope recombinant proteins (OVM and AVM), which containing anti-genic sites (residue of VP1 132-162 and residue of VP1 192-212) of three topotypes of FMDV serotype O or three topotypes of FMDV serotype A. Each recombinant protein was efficiently expressed in Escherichia coli with high solubility and the immunogenicity and protective efficacy of proteins as FMD vaccine candidates were evaluated. The results showed that OVM and AVM emulsified with ISA201 adjuvant induced effective antigen-specific humoral and cell-mediated immune responses and successfully protected mice from O/Jincheon/SKR/2014, O/VET/2013, and A/Malaysia/97 viruses. In addition, intramuscular immunization of pigs with the OVM and AVM emulsified with ISA201 elicited effective levels of neutralizing antibodies to the viruses with homologous epitopes. Importantly, OVM-AVM emulsified with CAvant®SOE-X adjuvant con-ferred 100% protection against O/Jincheon/SKR/2014 virus with homologous residues and 75% protection against A/SKR/GP/2018 with heterologous residues. The results presented in this study suggest that the combination of OVM and AVM protein with an effective adjuvant could yield an effective and safe vaccine candidate for the prevention and control of foot-and-mouth disease. In addition, our results provide a vaccine platform that can safely, cost-efficiently and rapidly generate protective vaccine candidates against diverse FMDVs. [National Research Foundation (2021R1A6A1A03045495) and Ministry of Agriculture, Food and Rural Affairs (318039-3), Republic of Korea]

P-V-048

EFFICACY OF A PSEUDORABIES VACCINE WHEN COMBINED WITH A CLASSICAL SWINE FEVER VACCINE IN SOWS WITH A NEEDLE-FREE DEVICE

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[1]DONGJING agriculture, [2]HIPRA CHINA, [3]HIPRA

Introduction
Since 2018, there have been a large number of African Swine Fever (ASF) outbreaks in China, so biosecurity has become one of the most important concerns for farms. Needles, an essential vaccination tool, are widely used on farms in China. However, due to the shared use of needles, many diseases are transmitted iatrogenically through them, such as PRRS(1) or ASF(2), among others. All pigs in China receive Pseudorabies (PRV) vaccine and Classical Swine Fever (CSF) vaccine, a combination of these two vaccines, administered with Hipradermic® (intradermal needle-free device, HIPRA) will improve farm efficiency and will reduce iatrogenic transmission of important diseases on pig farms. The objective of this trial was to evaluate antibody levels of this combination administered with Hipradermic®.

Materials and Methods
A field trial was conducted on a farm with 10.000 sows, a batch with a total of 100 sows was selected and it was divided into two groups. Group A, 0.2 ml was intradermally administered with the combination of the PRV vaccine together with a CSF vaccine through Hipradermic®. The PRV vaccine was AUSKIPRA® GN (PRV live vaccine, Bartha K-61 strain, HIPRA), and the CSF vaccine was a local one named Wenkang (Yangzhou Youbang). In group B, both PRV Vaccine and CSF vaccine were administered by intramuscular injection according to the manufacturer's instructions one week apart. PRV vaccine was an imported live vaccine of Bartha K-61 strain (Vaccine A), and the CSF vaccine was the same as in group A. At 28 days after CSF vaccination in group B, blood samples were collected to detect neutralizing antibodies against Bartha K-61 strain, and antibodies to CSF were detected by ELISA.

Results
In both groups the antibody levels of sows were significantly increased after inoculation with PRV vaccine (Figure 1).
We also tested post-vaccination CSF antibodies, and there was no significant difference between the two groups (data not shown).

Discussion and Conclusion
This trial demonstrated that a vaccination protocol with the combination of AUSKIPRA® GN and a CSF vaccine in one shot using Hipradermic® is at least as effective as the administration of the PRV vaccine and the CSF vaccine administered separately by intramuscular route. Moreover, Hipradermic® needle-free device has several advantages as: avoiding iatrogenic transmission of pathogens such as ASF and PRRS(3), lower stress and pain in pigs than with the use of needles (4) and finally you can also save the time spent changing needles so the efficiency of workers is also higher (5).

References

1, Hermann JR, Muñoz-Zanzi CA, Roof MB, Burkhart K, Zimmerman JJ. Probability of porcine reproductive and respiratory syndrome (PRRS) virus infection as a function of exposure route and dose. Vet Microbiol. 2005, 110:7-16.
2. Gallardo et al, Experimental Transmission of African Swine Fever (ASF) Low Virulent Isolate NH/P68 by Surviving Pigs. Transboundary and emerging diseases. 62. 10.1111/tbed.12431.
3. Dachrit Nilubol et al. Evaluation of ASF and PRRS virus transmission between pigs when using conventional needles and a needle-free device. IPVS 2022
4. Antoni Dalmau et al. Intramuscular vs. Intradermic Needle-Free Vaccination in Piglets: Relevance for Animal Welfare Based on an Aversion Learning Test and Vocalizations. Front. Vet. Sci. 2021 8:715260. doi: 10.3389/fvets.2021.715260
5. Imeah B, Penz E, Rana M, Trask C. Economic analysis of new workplace technology including productivity and injury: The case of needle-less injection in swine PLOS ONE 15(6): e0233599

Table 1

Titers	AUSKIPRA® GN(ID)	Vaccine A(IM)
1:8<	25.00%	20.00%
1:16	0.00%	10.00%
1:32	62.50%	40.00%
1:64	12.50%	30.00%

Table 1. Proportion of neutralizing antibody titers in each group before vaccination

Table 2

Titers	AUSKIPRA® GN(ID)	Vaccine A(IM)
1:16	10.00%	0.00%
1:32	0.00%	20.00%
≥1:64	90.00%	80.00%

Table 2.Proportion of neutralizing antibody titers in each group after vaccination

Graphs or Images 1

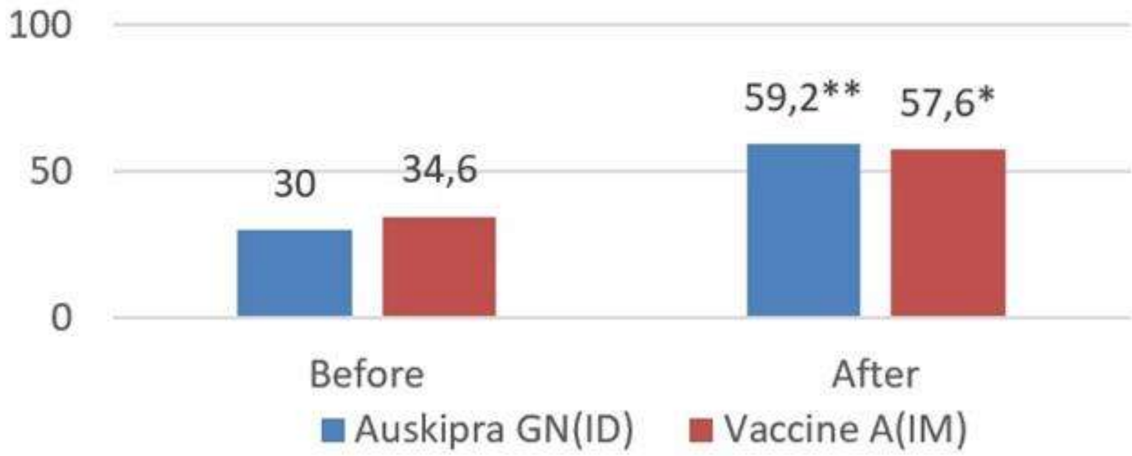


Figure 1. Mean PRV neutralizing antibody titers in gilts before and after vaccination
P-value 0,02* in IM group, p-value <0,01** in ID group.

P-V-049

EFFICACY OF PCV2 SOW AND PIGLET VACCINATION PROGRAM IN CONTROLLING EARLY PCV2 INFECTIONS

Mr. Roman Krejci[1], S Oliver[2], S Cárceles[2], L Garza[2], C Casanovas[2], D Espigares[2]
[1]Ceva, [2]CEVA Salud Animal

Introduction
PCV2 can be associated with reproduction failures in sows and early infections of piglets. The aim of this study was to evaluate the effect of PCV2 sow and piglet vaccination program during two years in two early PCV2 infection scenarios.

Material and Methods.
In 2019, 2 farms where piglets were vaccinated at weaning with a PCV2 subunit vaccine and sows remained unvaccinated were selected. Farm A showed PCV2 PCR positive blood samples at 6 and 9 weeks of age (woa) with loads of 1.2×105 and 2.3×107 PCV2 copies/mL, respectively. While, in farm B, PCV2 viraemic piglets were detected at 6 woa (1.5×106 PCV2 copies/mL). Given this situation, a new vaccination program was started using Circovac® in sows and piglets. Sows were vaccinated with one dose (2 mL) at the end of lactation and piglets were immunized at weaning with 0.5 mL until the first offsprings of vaccinated sows arrived. PCV2 infection dynamics in piglets born from vaccinated sows was evaluated by taking blood samples at 3, 6 and 9 woa (10 samples per age, sampling the smallest piglets from each pen). These samples were processed by PCV2 PCR by making pools of 5 samples. This sampling was repeated every 6 months during the two years of duration of the study. On the other hand, additional blood samples were taken at 3 and 5 woa in the first batch of piglets born from vaccinated sows to evaluate the levels of PCV2 maternally derived antibodies by ELISA.

Results
At 3 woa, the first piglets from vaccinated sows presented higher levels of antibodies than those observed at the beginning of the study in piglets from unvaccinated sows. At 5 woa, a decrease in antibody levels was observed, therefore it was decided to vaccinate at that age the piglets from vaccinated sows on both farms. Moreover, no PCV2 PCR positive samples at 3, 6 or 9 woa were detected in any of the samplings carried out throughout the study.

Discussion and conclusion.
Sow and piglet vaccination protocol with Circovac® has proven to be an effective tool for the control of early PCV2 infections.

References
Opriessnig, T et al. Clinical and vaccine immunology, 2008: 397-401
Dieste-Pérez, L et al. Preventive Veterinary Medicine, 2018: 42-46

Table 1

FARM A: PCR results of pooled serum samples from piglets at 3, 6 and 9 weeks of age

	BEFORE PCV2 SOW VACCINATION	AFTER PCV2 SOW VACCINATION				
	Piglets from unvaccinated sows	First sampling: first piglets born from PCV2 vaccinated sows	6 months after first sampling	1 year after first sampling	1,5 years after first sampling	2 years after first sampling
3 woa	NEG	NEG	NEG	NEG	NEG	NEG
6 woa	POS (1.2×10^5 PCV2 copies/mL)	NEG	NEG	NEG	NEG	NEG
9 woa	POS (2.3×10^7 PCV2 copies/mL)	NEG	NEG	NEG	NEG	NEG

Table 2

FARM B: PCR results of pooled serum samples from piglets at 3, 6 and 9 weeks of age

	BEFORE PCV2 SOW VACCINATION	AFTER PCV2 SOW VACCINATION				
	Piglets from unvaccinated sows	First sampling: first piglets born from PCV2 vaccinated sows	6 months after first sampling	1 year after first sampling	1,5 years after first sampling	2 years after first sampling
3 woa	NEG	NEG	NEG	NEG	NEG	NEG
6 woa	POS (1.5×10^6 PCV2 copies/mL)	NEG	NEG	NEG	NEG	NEG
9 woa	NEG	NEG	NEG	NEG	NEG	NEG

P-V-050

EFFICACY OF PCV2A VACCINES ADMINISTERED SEPARATELY OR MIXED WITH M.HYO COMPONENT IN THE PROTECTION AGAINST AN EXPERIMENTAL PCV2D INFECTION

Mr. Roman Krejci[1], István Kiss[2], Krisztina Szigeti[2], Zalán Homonnay[2], Han Smits[2]
[1]*Ceva,* [2]*SSIU, Ceva Phylaxia*

Introduction
PCVD (Porcine Circovirus Diseases) remain a common problem in most of swine farms. Strains of different genotypes of PCV2 are circulating in the herds. The PCV2d seems predominating particularly in farms with clinical PCVD problems. Different mono-valent or combined vaccines are available on the market. The aim of the study was to compare the efficacy of PCV2a vaccines against PCV2d infection.

Material and Methods
Conventional weaned piglets (20per group) were vaccinated at 3 weeks of age (WOA) either with Circovac®0.5ml(PCV2a vaccine, Ceva – Group C) separately or mixed with Hyogen®2ml (M.hyo vaccine, Ceva – Group DUO) or with PCV2a Vaccine A 1ml mixed with M.hyo vaccine B1ml (Group RTMA), a group of non- vaccinated pigs served as controls. All were challenged at 12 WOA (D0) with a PCV2d isolate. Pigs were sampled weekly and sacrified 4 weeks (D28) post-challenge (pch). Virus loads were measured by qPCR for efficacy evaluation.

Results
Viremia at 28DPI was 1,1; 1,3; and 1,7 log copy#/µl for groups C, DUO and RTMA respectively (p>0,05).
Fig 1.

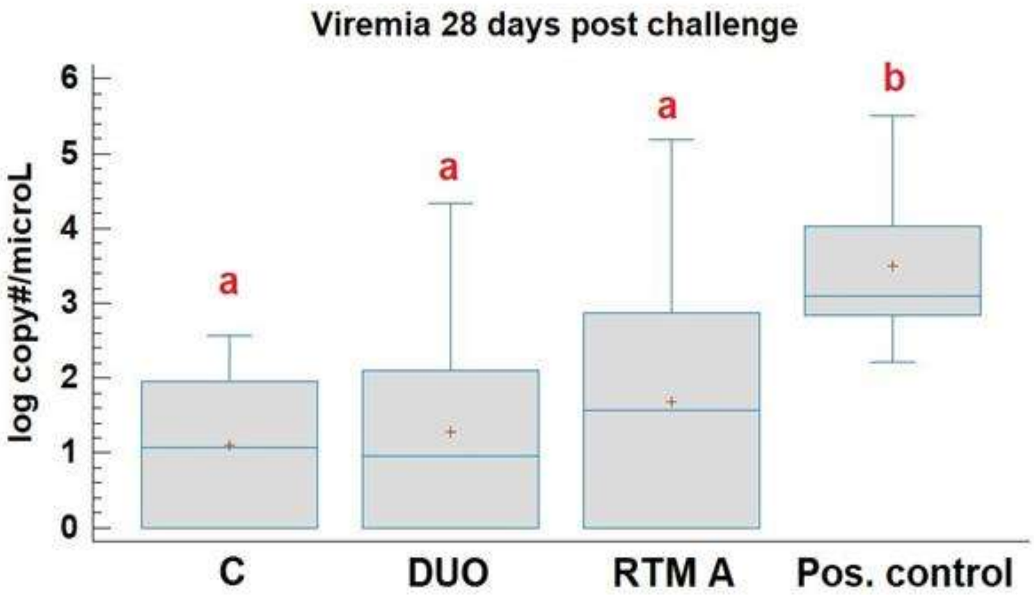
The PCV2 loads in mesenterial were 5,8; 5,5; and 5,5 log copy#/µl for groups C, DUO and RTMA respectively(p>0,05).
Fig 2.

The loads in mediastinal lymph nodes were 3,8; 3,5; and 4,4 log copy#/µl for groups C, DUO and RTMA respectively(p>0,05).

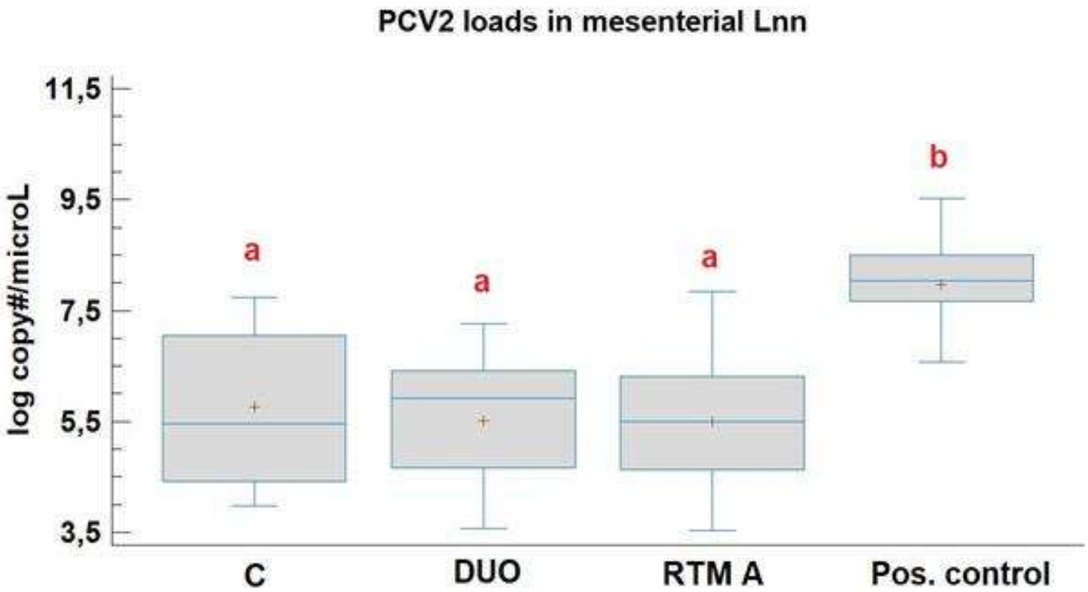
Conclusion
This study demonstrated that Circovac® administrated separately or mixed with Hyogen® provided equal protection against the epidemiologically most important genotype of PCV2. The reduction of viremia and viral loads in lymphoid tissues were also very similar to the other PCV2/M.hyo RTM products. These results confirm it's convenience being used in the control of PCVD single or together with the M.hyo component.

References
Segales J et al. 2014. Expert Rev. Vaccines Early online, 1–15
Opriessnig T et al. 2020. Vet Res. 286, 1-11

Graphs or Images 1



Graphs or Images 2



P-V-051

EFFICACY OF PHYTOGENIC FORMULATION (ANIMUNIN) FOR THE MANAGEMENT OF RESPIRATORY INFECTIONS IN PIGS

Dr. Shivi Maini[1]
[1]*Indian Herbs*

Introduction
Respiratory diseases of pigs include swine influenza, classical swine fever, the pneumonic forms of pseudorabies, porcine circovirus-associated disease, and porcine reproductive and respiratory syndrome (PRRS). The causal viruses may persist in a herd, but outbreaks of overt disease tend to be self-limiting.The most important syndromes in the second category are mycoplasmal pneumonia and pleuropneumonia. Atrophic rhinitis, once considered to be an important cause of respiratory disease in swine, has declined substantially as a result of eradication programs. In Taiwan, the widespread use of antimicrobial agents in primary care clinics and animal husbandry has lead to rapid emergence of resistant bacteria. Many measures to control resistance problems have already been instituted in Taiwan.
Alternate Strategies for management of respiratory diseases in pigs : There is resurgence of interest towards use of phytogenics as an alternative to conventional treatment of various respiratory diseases (Awad et al., 2019). Natural plant compounds have been found to be effective against a variety of respiratory infections. Some plant extracts, including Mentha piperita, Thymus vulgaris, Desmodium canadense extracts [Lelesius et al, 2019], Astragalus polysaccharides [Zhang et al., 2018], Glycyrrhiza radix [Li et al., 2009], and Forsythia suspensa (Li et al., 2011) have shown effectiveness against bacterial and viral respiratory pathogens.
Animunin- Natural Phytobitoic and respiratory tonic
Animunin is a phytogenic formulation (M/SIndianHerbs,India) constituted by principal phyto-bioactives having antibacterial, antiviral, antibacterial and anti-inflammatory activities. Animunin protects respiratory system from infections and maintain its functioning in a natural way.
Animunin
Researchers have reported antiviral efficacy of Animunin (Gangwaret al., 2022) against NDV and IBDV. In one of the study at Dept. at Veterinary Pathology College of Veterinary Science & AH, DUVASU, efficacy of natural respiratory antiseptic (Animunin) (M/S Indian Herbs Specialities, India) was evaluated for its antiviral and anti- inflammatory potential against NDV and IBDV. Researchers have determined differential expression of pro-inflammatory cytokines IL1ß, TNFα and oxidative genes SOD, Catalase in chicken embryo fibroblast culture (CEF) by using real time polymerase chain reaction (q-PCR). In this study, phytogenic formulation-Animunin was diluted to 5 concentrations (2.5, 5.0, 10.0, 15.0, 20.0 mg/ml) with culture media and 100 µl were added to each 96 well culture plates containing CEF cells. In one group, CEF cells were kept as negative control. Cytopathic effect (CPE) were determined and maximum concentration that caused no toxicity (TC0) was observed, and it was 10mg/ml.
Researchers (Neeraj Gangwar1, Ajay Singh2, Soumen Choudhary,DUVASU, Mathura) reported that cytopathic effect (CPE) was much pronounced (rounding of cells, granulation and separation) in IBD and IBDV virus infected cell lines. Animunin treated cell lines exhibited significant reduction in cytopathic effects.
Relative quantification of genes related to inflammation and oxidative stress (IL 1ß, TNF α ,SOD, Catalase) revealed that IL 1ß, TNF α are highly upregulated in positive / virus infected control cell lines, however normalization of genes were evident in Animunin treated

cell lines. SOD, Catalase activity is highly upregulated in Animunin treated cell lines than virus infected cell lines. The novel findings of the study are presented and published in IPSF, 2022 and study investigators concluded their findings that Animunin is a natural phytobiotic having antiviral, anti-inflammatory and immunomodulating properties (P240, IPSF, 2022)

P-V-052

EFFICACY OF PORCINE CIRCOVIRUS TYPE 2 AND MYCOPLASMA HYOPNEUMONIAE COMBINED VACCINE IN SPECIFIC PATHOGEN FREE PIGS CHALLENGED WITH PORCINE CIRCOVIRUS TYPE 2D

Dr. Jeong-Gyo Lim[1], Dr. Injoong Yoon[1]
[1]*Choong Ang Vaccine Laboratories Co., Ltd.*

- Introduction

Porcine circovirus type 2 (PCV2) and Mycoplasma hyopneumoniae (MHP) are economically important pathogens in pig industry. The use of a combined vaccine against PCV2 and MHP is one of the most effective ways of protecting pigs from both diseases and has become a part of general management.

PCV2 genotypes are classified as PCV2a, PCV2b, PCV2c, PCV2d, PCV2e, PCV2f, PCV2g and PCV2h based on the differences in ORF2 nucleotide sequences. Currently, PCV genotype 2d, known as mutant PCV2b, is the most common and dominant genotype in the USA, Russia, China,Thailand, Vietnam, and South Korea.

This study evaluated the efficacy of newly developing combined vaccines (Myco-X and Myco-XD) of PCV2 and MHP in specific pathogen free (SPF) pigs. Myco-X and Myco-XD are a combined vaccine of MHP with PCV2b and PCV2d, respectively.

- Materials and Methods

Sixteen SPF pigs were divided into 4 groups (4 pigs per group). These groups included; Myco-X-vaccinated challenged (G1), Myco-XD-vaccinated challenged (G2), unvaccinated challenged (2dC), and unvaccinated unchallenged (NC). Two milliliters of Myco-X were administered intramuscularly and 0.5ml of Myco-XD was injected intradermally at 3 weeks of age in accordance with the manufacturer’s instructions. The pigs were challenged with virulent PCV2d via intramuscular and intranasal route at 4 weeks post vaccination (Table 1). Each group was housed and raised separately under the same air condition-, ventilation-, and temperature-controlled facility, which included access to feed and water ad libitum. Blood samples were collected every week after vaccination until 3 weeks post challenge (WPC) and nasal swabs were collected at 0, 1, 2, and 3 WPC.

- Results

No PCV2 genomic copies were detected in the sera of all four groups at the time of challenge. The PCV2 levels of the G1, G2, and NC animals were significantly lower ($p < 0.05$) than that of the 2dC animals from 1 to 3 WPC. Genomic copies of PCV2 were not detected in the sera from the G1 and NC animals (Fig. 1).

No PCV2 genomic copies were detected in the nasal swab samples of the four groups before the challenge. The PCV2 levels of the G1, G2, and NC animals were significantly lower ($p < 0.05$) than that of the 2dC animals at 3 WPC. Genomic copies of PCV2 were not detected in the nasal swab samples from the G1 and NC animals (Fig. 2).

All vaccinated pigs showed effective reduction of the PCV2d load in the blood and nasal swab samples.

- Conclusions and Discussion

An experimental challenge study is essential for evaluating the efficacy of new vaccines. This study confirmed that two newly developing combined vaccines of PCV2 and MHP could effectively protect pigs from PCV2d challenge. In particular, Myco-X containing PCV2b demonstrates itself to cross-protect against PCV2d challenge, which is the most prevalent genotype worldwide.

On the whole, these two combined vaccines of PCV2 and MHP can protect pigs from PCV2d, assist pig practitioners and producers in reducing the number of vaccinations in pigs. On the other hand, further research on the efficacy evaluation of these new vaccines against the MHP challenge and PCV2d/MHP co-challenge is needed.

- Acknowledgement and References

1. Chae C. Vet J. 2005;169(3):326-336.
2. Kwon T. et al. Virus Res. 2017;228:24-29.

Table 1

Table 1. Experimental design with vaccination and challenge.

Group	No. of pigs	Vaccine	Vaccination (3 weeks of age)	Challenge (7 weeks of age)
G1 (PCV2b+MHP)	4	Myco-X	2.0 mL, intramuscularly	PCV2d
G2 (PCV2d+MHP)	4	Myco-XD	0.5 mL, intradermally	PCV2d
2dC	4	PBS	2.0 mL, intramuscularly	PCV2d
NC	4	PBS	2.0 mL, intramuscularly	ND

Graphs or Images 1

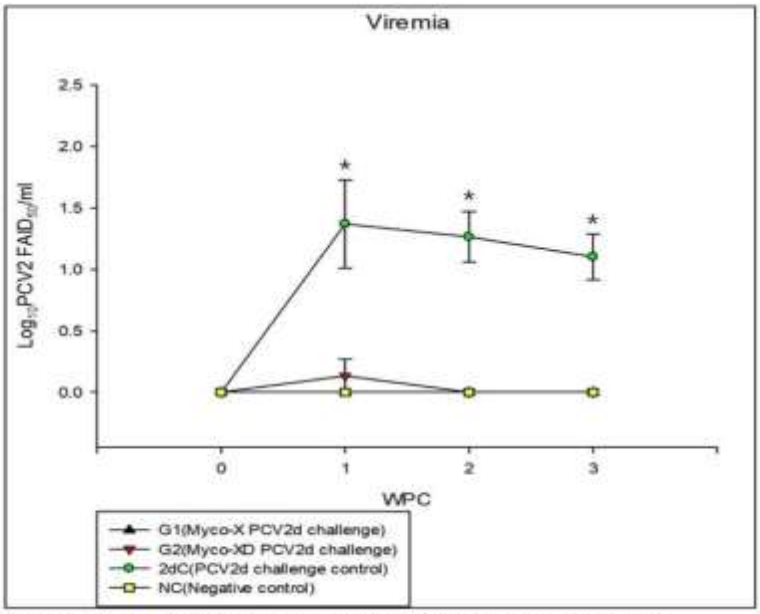


Fig. 1. Mean values of the genomic copies of PCV2 DNA in the serum samples.

Graphs or Images 2

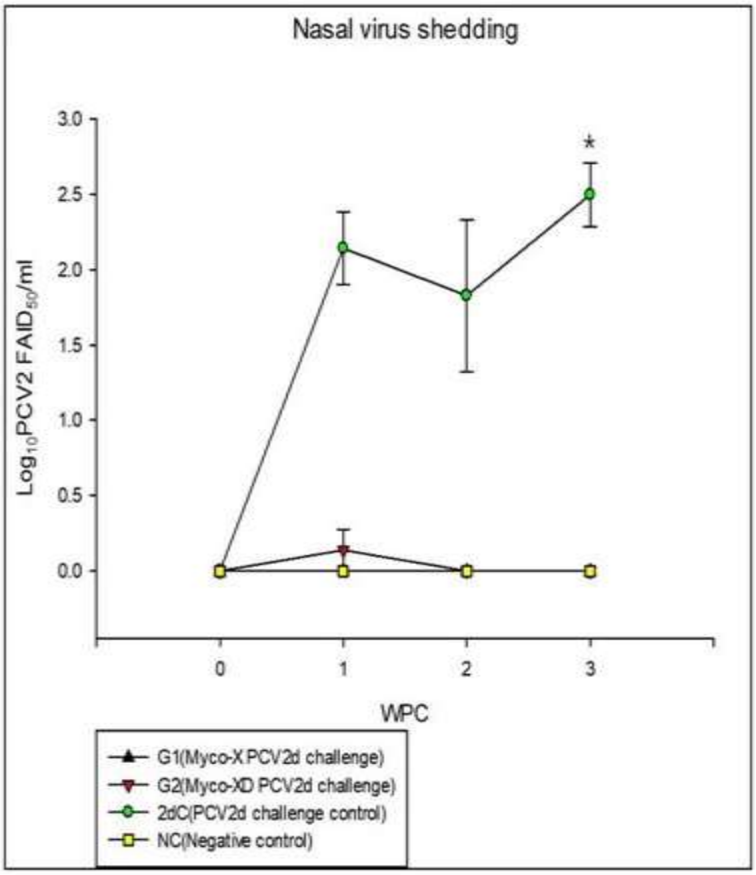


Fig. 2. Mean values of the genomic copies of PCV2 DNA in the nasal swab samples.

P-V-053

EFFICACY OF PSEUDORABIES VACCINE INOCULATED WITH A NEEDLE-FREE DEVICE

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Introduction
Since 2018, there have been a large number of African Swine Fever (ASF) outbreaks in China, so biosecurity has become one of the most important concerns for farms. Needles, an essential vaccination tool, are widely used on farms in China. However, due to the shared use of needles, many diseases are transmitted iatrogenically through them, such as PRRS(1) or ASF(2), among others. PRV is also a disease of great concern in Chinese farms, and many farms have developed eradication plans. This trial aims to test the efficacy of a PRV live vaccine to produce PRV antibodies in pigs by using a needle-free device.

Materials and methods
We conducted this field trial in a farm with 10,000 sows, a batch of 100 gilts was divided into two groups. Group A intradermal (ID) administration with 0.2 ml of AUSKIPRA® GN (live vaccine, Bartha K-61 strain, HIPRA) using Hipradermic® (needle-free device, HIPRA), group B intramuscular (IM) immunization with 2 ml of an imported brand of Bartha K-61 strain of PRV live vaccine (Vaccine A), in each group we selected 10 gilts and sampled them before vaccination (0DPV) and 28 days after vaccination(28DPV) to detect neutralizing antibodies to the Bartha K-61 strain.

Table 1. Groups and methods of the trial

Results
From Figure 1, it can be seen that the average Neutralizing Antibody titers of gilts in the two groups before vaccination was not high, however, after inoculation, the antibody titers of both groups increased significantly p.value < 0,001*** in both groups, ordinal regression was performed.

Figure 1. Mean PRV neutralizing antibody titers in gilts before and after vaccination

Table 2. Proportion of neutralizing antibody titers in each group before vaccination

Table 3.Proportion of neutralizing antibody titers in each group after vaccination

Discussion and Conclusions
This trial demostrated that the results for ID are as good as for IM. Moreover, Hipradermic® needle-free device has several advantages as: avoiding iatrogenic transmission of pathogens such as ASF and PRRS(3), lower stress and pain in pigs than with the use of needles (4) and finally you can also save the time spent changing needles so the efficiency of workers is also higher (5).

(1) Hermann JR, Muñoz-Zanzi CA, Roof MB, Burkhart K, Zimmerman JJ. Probability of porcine reproductive and respiratory syndrome (PRRS) virus infection as a function of exposure route and dose. Vet Microbiol. 2005, 110:7-16.

(2) Gallardo et al, Experimental Transmission of African Swine Fever (ASF) Low Virulent Isolate NH/P68 by Surviving Pigs. Transboundary and emerging diseases. 62. 10.1111/tbed.12431.
(3) Dachrit Nilubol et al. Evaluation of ASF and PRRS virus transmission between pigs when using conventional needles and a needle-free device. IPVS 2022
(4) Antoni Dalmau et al. Intramuscular vs. Intradermic Needle-Free Vaccination in Piglets: Relevance for Animal Welfare Based on an Aversion Learning Test and Vocalizations. Front. Vet. Sci. 2021 8:715260. doi: 10.3389/fvets.2021.715260
(5) Imeah B, Penz E, Rana M, Trask C. Economic analysis of new workplace technology including productivity and injury: The case of needle-less injection in swine PLOS ONE 15(6): e0233599

Table 1

Group	N° of sample	Sampling date
AUSKIPRA® GN (ID)	10	DPV 0 and DPV 28
Vaccine A (IM)	10	DPV 0 and DPV 28

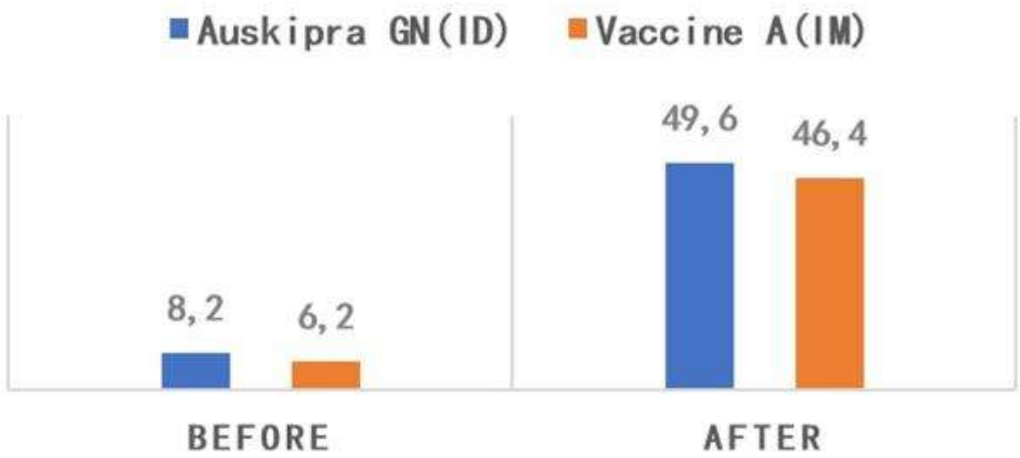
Table 2

Titers	Auskipra® GN(ID)	Vaccine A(IM)
1:4<	55.56%	50.00%
1:8	33.33%	20.00%
1:16	11.11%	30.00%

Table 3

Titers	Auskipra® GN(ID)	Vaccine A(IM)
1:16	10.00%	10.00%
1:32	40.00%	30.00%
≥1:64	50.00%	60.00%

Graphs or Images 1



P-V-055

ESTIMATING THE VALUE DIFFERENCE IN THE LIGHTEST 20% OF PIGS WHEN AVERAGE WEIGHTS AT MARKETING ARE ESSENTIALLY EQUAL IN A FIELD STUDY OF KOREAN PIGS RECEIVING DIFFERENT VACCINATIONS FOR PRDC

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Introduction

A Korean field study compared the results of using two different vaccinations for Porcine circovirus Type 2 (PCV2) and Mycoplasma Hyopneumoniae (M.Hyo)1. The study examined differences in mortality, average daily gain, levels of acute phase proteins and other physiological responses. The two vaccines were, FLEXcombo (Boehringer Ingelheim Vetmedica GmbH) and Porcilis PCV M hyo (Intervet international B.V., Netherlands). The study data (210 sows; Farrow-to-Finish, PRRS Stable, 102 trial animals divided into two groups) revealed differences in the distributions of weights for the lightest 20% of pigs in the two vaccinate groups but very little difference in average mortality or growth rate. This is a finding we have shown in four similar trials to result in profit differences2. We use the field study data to illustrate how very small differences in mean weight outcomes between groups can still hide significant differences in total revenue received at sale, and in many cases profitability3.

Materials and Methods

We estimate an individual growth curve for each pig using a Gompertz function and fit distributions to those growth rates. We simulated the revenue optimal marketing of 2,000 randomly selected observations (1,000 per group). Revenue is determined from five years of Korean price data and a typical Korean pricing matrix. We estimate the relationship between fat accretion and weight gain from a Korean publication4. We created three marketing groups, allowing slower growing pigs more time to achieve greater value. A top load of the heaviest 20% of pigs, a middle group comprised of 60% of the pigs and then the closeout group, the lightest 20%, were sold when the value of marginal gains no longer covered additional costs.

Results

The pathway of the lightest 20% of market animals through the Korean value matrix is shown in the Figure 1. The lightest weight 20% of animals receiving the FLEXCombo product reach the highest premiums to the base prices approximately eight days before the animals receiving Porcilis. Animals receiving the Porcilis product achieved more average revenue/head in the highest weights (top load) (₩433,725 vs ₩429,302). Animals receiving FlexCombo achieved higher revenue per head in the middle loads (₩428,459 vs ₩426,300) and the closeout (final) load (₩411,596 vs ₩348,227). The overall weighted average revenue per head was ₩425,235 for FLEXCombo, and ₩412,170 for Porcilis, or a difference of ₩13,065 (11,00 USD) per head. The group receiving FLEXcombo had also better weight homogeneity, improving the uniformity of the group.

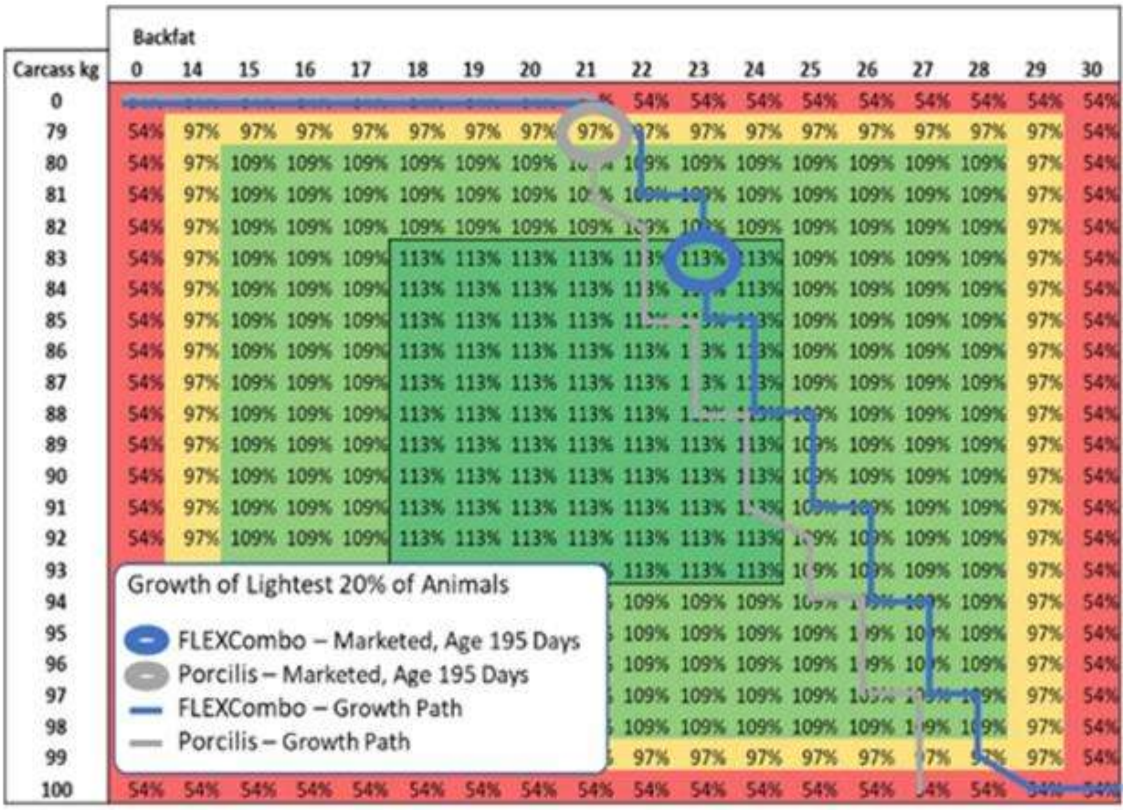
Conclusions and Discussion

Practitioners are strongly encouraged to go beyond an analysis of mean differences to differences in variance (weight variation) and kurtosis (skewness of the distribution) in trial data weight distributions as frequently these often-neglected measures contain key profit differences between groups.

Acknowledgement and References
1. Yusik Oh et al, “Comparative field efficacy study between ready to mix and ready-to-use vaccine in Korea”, Swine Technical Report, Proceedings Leman Conference, University of Minnesota, St. Paul, MN, June_2021
2. Dennis DiPietre and Lance Mulberry, “Exploring profitability differences when average wean-finish production metrics are essentially equal between groups”, Invited presentation Leman Conference, University of Minnesota, St. Paul, MN, June_2017
3. Eduardo Fano, et al, “Comparison of efficacy between two PCV2 vaccination protocols under PCV2 field exposure.” AASV. 2017;95-97 2.
4. Gye-Woong Kim and Hack-Youn Kim, “Effects of Carcass Weight and Back-fat Thickness on Carcass Properties of Korean Native Pigs”, Korean J Food Sci Animal Resources. 2017;37(3): 385–391. published online 2017 Jun_30. doi: 10.5851/kosfa.2017.37.3.385

Graphs or Images 1

Figure 1. Value measured at 195 days of age, 97% of base for Porcillis; 113% of base for FLEXCombo



P-V-056

EVALUATING OF CORRELATION OF THE ANTIBODIES AGAINST PORCINE CIRCOVIRUS TYPE 2 (PCV2) IN VACCINATED SOWS AND THEIR PROGENY

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Background
The control of Porcine circovirus type 2 is based on management strategies, control of coinfections, and vaccination. The benefits of sow vaccination on their progeny have been demonstrated in terms of reduction of PCV2-systemic disease prevalence, reduced virus load in the blood and tissues, increased PCV2 antibody titers in serum, and improved production parameters (Segalés, 2015). However, the impact of piglet vaccination is dependent on the level of maternally derived immunity (MDA) at the vaccination time (Feng et al., 2016; Martelli et al., 2016). Therefore, the objective of this study was to evaluate the correlation of PCV2 antibody titers between the vaccinated sows and their progeny. Hence, refining the vaccination schedule commonly established around weaning time could be necessary.

Materials and method
To assess the PCV2 infection before the start of the study, blood samples from ninety pregnant sows of different parity (1st, 2nd, and 3rd-5th parity) were taken at 84 days of gestation. A total of 42 non-viremia pregnant sows (processed by quantitative PCR (AniCon Labor GmbH, and Ct value > 42) were selected with 14 sows for each parity cluster.
These selected sows were randomly divided into two experimental groups. The pigs in group 1 were vaccinated intramuscularly (IM) with sub-unit ORF2-PCV2 vaccine (vaccine A) and those in group 2 were vaccinated (IM) with the whole inactivated PCV2 vaccine (vaccine B) at 90 days of gestation. Blood samples were collected in sows at 20 days post-vaccination to evaluate the PCV2 antibody and viremia. After farrowing, two piglets (< 1.2 kg and >1.5 kg body weight) per sow were bled at the first 24 hours and every week until 42 days of age (Table 1). The sera from sows and piglets were measured for the anti-PCV2 antibody (Ingezim Circo IgG, Ingenasa, Spain) and determined the PCV2 viremia by Realtime PCR.

Table 1. Study design

Results
All vaccinated sows were qPCR negative at 110 days of gestation and undetectable PCV2 viremia in their offspring in the first 24 hours of life.
A set of mixed-effect regression models was fitted to estimate the effect of the vaccine (experimental group), sow antibody, sow parity, piglet age (days), and piglet birth weight on the MDA. The result of mixed effect regression was established ($p<0.001$) in which the MDA was dependent on the sow antibodies and piglet age ($p < 0.001$). From this result, the regression equation was established as “Piglet antibody = 1.56 – (0.05*Piglet age) + 0.0005*(Piglet age)2* + (0.23*Sow antibody)”.

Figure 1. The correlation between PCV2-IgG levels in piglets and sows. High MDA (S/P > 1.2) could interfere with the vaccine (Martelli et al., 2016) and susceptible to PCV2 infection when S/P < 0.8 (Fort et al., 2009).

Conclusion and discussion

The appropriate vaccination time should be higher than 0.8 but lower than 1.2 (Figure 1). The application of the regression equation in this study could help determine the appropriate vaccination time for piglets.

In conclusion, the vaccination for pregnant sows has significantly improved their humoral immunity before farrowing and induced a high MDA level in the offspring.

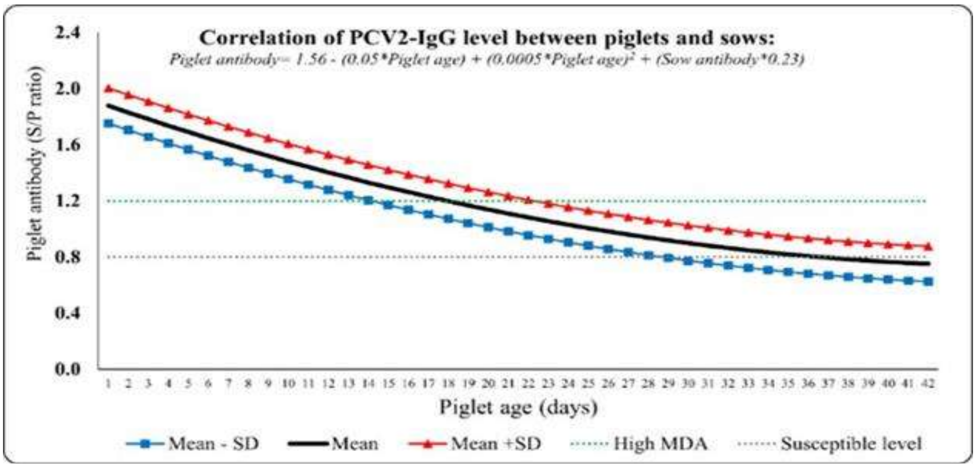
Acknowledgment and references

1. Feng H., Vaccine 2016.04.088.
2. Fort M., et al., Vaccine 27 (2009), 4031–4037.
3. Martelli P., et al., BMC Veterinary Research (2016) 12:77.
4. Segalés J., 2015. Vaccines 14(3), 473–487 (2015)

Table 1

Experimental group	Vaccination for sows			Blood samples			
				Pregnant sows		Piglet age (days)	
	(n)	The day	Vaccine	84 days	110 days	1st day	7, 14, 21, 28, 35 and 42
Group 1	21	90 days of gestation	Vaccine A	ELISA and qPCR		ELISA qPCR	ELISA
Group 2	21		Vaccine B				

Graphs or Images 1



P-V-057

EVALUATION OF A PCV2 MONOVALENT AND A PCV2 BIVALENT VACCINES AGAINST PCV2D INFECTION IN PIGS IN THAILAND

Ms. Pichanun Wongchanapai[1], Ms. Chonnatcha Muangpaisarn[1], Mr. Sakorn Chitmankwanyuen[1], Ms. Sunaree Nantakhruea[1], Mr. Wichian Navasakuljinda[1]
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Introduction
Porcine circovirus genotype 2d (PCV2d) is commonly found in pig farms in Thailand, while PCV2a and PCV2b remain present (1). A new PCV2 bivalent vaccine, containing two genotypes (PCV2a and PCV2b) may provide pigs with a broader protection against mixed PCV2 genotype infections happening within the same herd. The in silico study has indicated that the PCV2 bivalent vaccine can provide superior protection against heterologous genotypes infection when compared to PCV2 monovalent vaccines based on T-cell epitope content comparison (EpiCC) scores that estimate the degree of coverage of commercial vaccines against field isolates (2). This study was conducted to demonstrate the efficacy of the PCV2 bivalent vaccine , when compared to a PCV2 monovalent (PCV2a) vaccine in terms of the control of PCV2d infection in lymphoid tissues.

Materials and Methods
Eighteen crossbred male pigs 26-day old from PRRS negative herd were brought into the Animal Biosafety Level (ABSL)-2 room at the laboratory animal center, Chiang Mai University. They were divided into 3 treatment groups, 6 pigs/group, and were allocated to either one of the vaccines or to control saline; all treatments administered intramuscularly in the right side of the neck 7 days after all pigs were delivered to the laboratory animal center.

Table 1 Vaccination types by treatment group.

PCV2d challenge: 21 days after vaccination or saline, all pigs in each treatment group were inoculated with PCV2d (PCV2d/149/TH/2020) at 105 TCID50/ml, with a total of 4 ml divided into 2 mL intramuscularly in the left side of the neck, and 2 mL intranasally (1 mL each nostril) (3). All pigs were euthanized after raised for 30 days for evaluation.
Microscopic Lesions and Immunohistochemistry score: The tracheobronchial lymph nodes (TBLN) and superficial inguinal lymph nodes (SILN) from all pigs were collected. The microscopic lesion (ML) score of the lymphoid tissues from hematoxylin/eosin (HE) staining ranged from 0 [no pathological change] to 5 [severe lymphoid depletion and histiocytic replacement] (4). The immunohistochemistry (IHC) score of PCV2 antigens from all samples ranged from 0 [no signal] to 3 [strong positive signals] (5).
Results
The T02 group had the lowest scores when compared to the T01 and positive control. A significant difference in IHC score for TBLN was seen between T02 and the negative control group (p=0.037).

Table 2 Mean (SE) of ML and IHC score.

a, b Means with different superscripts were significantly different ($P < 0.05$).

Conclusion and Discussion

In summary, this study indicated that the PCV2 bivalent vaccine has superior efficacy to prevent PCV2d infection in lymphoid tissues of the pigs when compared to a PCV2 monovalent vaccine.

References
1. Thangthamniyom et al., 2017, Vet. Microbiol. 208: 239-246.
2. Bandrick et al., 2020, Vet. Immunol. Immunopathol. 223: 110034.
3. Kolyvushko et al., 2019, Vet. Sci. 6: 61.
4. Um et al., 2022, Vet. Med. Sci. 8: 578-590.
5. Fenaux et al., 2004, J. Virol. 78: 6297-6303.

Table 1

Group	Type of vaccine	Dose
T01	PCV2a monovalent vaccine (Fostera® PCV MetaStim)	2 ml, single dose, IM
T02	PCV2a-b bivalent vaccine (Fostera® Gold PCV)	
Negative control	Normal saline	

Table 2

Group	ML score		IHC score	
	TBLN	SILN	TBLN	SILN
T01	4.00 (0.26)	4.00 (0.26)	1.33 (0.21) [a, b]	1.17 (0.17)
T02	1.33 (0.33)	3.00 (0.52)	0.67 (0.42) [a]	0.83 (0.31)
Negative control	4.17 (0.31)	4.80 (0.20)	1.67 (0.42) [a, b]	1.40 (0.24)

P-V-058

EVALUATION OF THE DIAGNOSTIC ACCURACY OF AFRICAN SWINE FEVER ANTIGEN PEN-SITE TESTS IN NORTHERN AND NORTH-EASTERN THAILAND DURING 2022

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Introduction
African swine fever (ASF) is a devastating viral disease caused by the African swine fever virus (ASFV), leading to a high mortality rate of nearly 100% in domestic pigs and Eurasian wild boars(1). This had a massive impact on the swine industry's economy in various countries including Thailand. Currently, there is neither an effective vaccine nor an antivirus drug. Therefore, ASF control necessarily relies on accurate and rapid detection for the elimination of infected and contact pigs and movement control. Generally, Polymerase chain reaction (PCR) is a gold standard technique for ASF antigen detection(2). Unavoidably, being in a remote area or having insufficient resources can be barriers. Thus, the pen-side (PS) test is the choice for corrections. Nevertheless, the limitation of commercial PS tests was reported as low to moderate sensitivity by WOAH(3) and in other research (4). Hence, the PS tests were tested and evaluated for gathering data as a guideline for field application.

Materials and Methods
Four commercial ASF antigen PS tests were evaluated for sensitivity and specificity. The two kits are claimed only for the detection of P72 capsid protein (PS-1 and PS-2) and the other two kits are claimed for the detection of both capsid protein P72 and early expression P32 protein (PS-3 and PS-4). During 2022, the samples were collected from the pig farms located in northern and north-eastern Thailand, including breeder and finishing farms. Altogether 175 samples, composed of 36 serum samples (25 positive and 11 negative samples confirmed by PCR) and 139 whole blood samples (38 positive and 101 negative samples confirmed by PCR) were tested for ASF antigens to compare PS test to Real-time PCR by using primer and condition following King et al. (2013).

Results
The results showed that PS-1 had 20% sensitivity and 92% specificity compared with Real-time PCR. Likewise, PS-2 had 20% of sensitivity and 100% specificity. For the PS-3 and PS-4, an inferior result of 20% sensitivity and 78% specificity was found for PS-3, when compared with 45% sensitivity and 100% specificity of PS-4.

Conclusions and Discussion
The low sensitivity of PS tests might result in incorrect results (false negative), leading to mistaken decisions for a partial depopulation policy. Hence, the PS test should be used in specific conditions such as being in remote areas or with the inadequacy of tools or staff. In conclusion, the evaluation of the diagnostic accuracy of the ASF antigen PS test demonstrated a low sensitivity. The precision of sensitivity was mainly a weak point of PS tests with the immunochromatography technique. Thus, ASF PS tests should continuously be developed for high sensitivity and specificity.

References
1. Blome S, Franzke K, Beer M. African swine fever – A review of current knowledge. Virus Res. 2020;287(April).
2. Gallardo C, Fernández-Pinero J, Arias M. African swine fever (ASF) diagnosis, an essential tool in the epidemiological investigation. Virus Res. 2019;271(July).
3. Inui K, Gallardo C, Portugal R, Dixon L, Baton C, Williams D. The OIE ASF Reference Laboratory Network's overview of African swine fever diagnostic tests for field application. 2022;(February):1–13.
4. Matsumoto N, Siengsanan-Lamont J, Gleeson LJ, Douangngeun B, Theppangna W, Khounsy S, et al. Evaluation of the diagnostic accuracy of an affordable rapid diagnostic test for African Swine Fever antigen detection in Lao People's Democratic Republic. J Virol Methods [Internet]. 2020;286(September):113975. Available from: https://doi.org/10.1016/j.jviromet.2020.113975

P-V-059

EVALUATION OF THE EFFICACY OF A PORCINE EPIDEMIC DIARRHEA VIRUS VACCINE PEDQ

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- Introduction

Porcine epidemic diarrhea (PED) is a worldwide disease caused by porcine epidemic diarrhea virus (PEDV). Diarrhea, vomiting, and dehydration are the most common clinical signs in neonatal piglets, and the high mortality of piglets induces significant economic losses [1]. In Taiwan, the first outbreak of PEDV G2 strain was in 2013 [2]. Since that, PED became an intractable disease. Biosecurity and vaccine are effective strategies for PED prevention and control, but there is no commercial vaccine based on PEDV G2 strain in Taiwan. Therefore, the aim of this study is to evaluate the efficacy of a PEDV vaccine PEDQ.

- Materials and Methods

Total 10 sows were randomly separated into vaccine and control groups, including their piglets. Sows in vaccine group were intramuscularly injected with PEDQ vaccine at 8-, 5- and 3-week pre-farrowing, and control group were injected with 2 ml PBS as placebo. Before injection and at the day of farrowing, blood samples of sows were collected. Colostrum of each sow were also collected in 24 hours after farrowing. Piglets were challenged 10^3 TCID50 PEDV via oral route at 2-day-old. Blood samples of 5 piglets from each litter were collected at 2-day-old, and daily fecal scores of all piglets were recorded until 12-day-old. PEDV IgG of all blood samples were detected by ELISA. PEDV neutralization antibody titer of colostrum and 2-day-old piglets' blood were detected, divided by ten and transformed with base 2 log for analysis. All data were analyzed by t test and $p<0.05$ was considered statistically significant.

- Results

After vaccination, PEDV S1 IgG titers of vaccine group were significantly higher than control group ($p<0.0001$). The neutralization antibody titers in colostrum and suckling pigs' sera of vaccine group were also significantly higher than control group ($p<0.01$, $p<0.0001$). For clinical signs, fecal scores of control group were significantly higher than vaccine group.

- Conclusions and Discussion

The PEDV vaccine PEDQ can induce PEDV S1 IgG and neutralization antibody in sows which can be transferred to their suckling pigs through colostrum and help reduce the clinical symptoms induced by PEDV in pigs.

- Acknowledgement and References

We gratefully acknowledge the technicians and veterinarian in ADDC of NPUST for their contributions to this study.
[1] Zimmerman, J. J.,2019. Diseases of swine (11th ed.)
[2] Lin CN et al., 2014. The Journal of veterinary medical science, 76(9), 1297–1299.

Graphs or Images 1

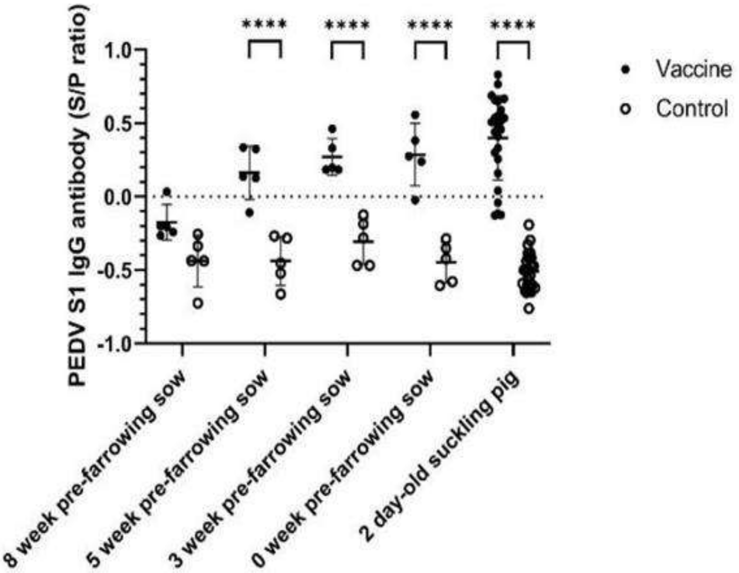

Figure 1. PEDV IgG antibody of sows and piglets

Data were analyzed by t test. **** means statistically significant difference ($p<0.0001$).

Graphs or Images 2

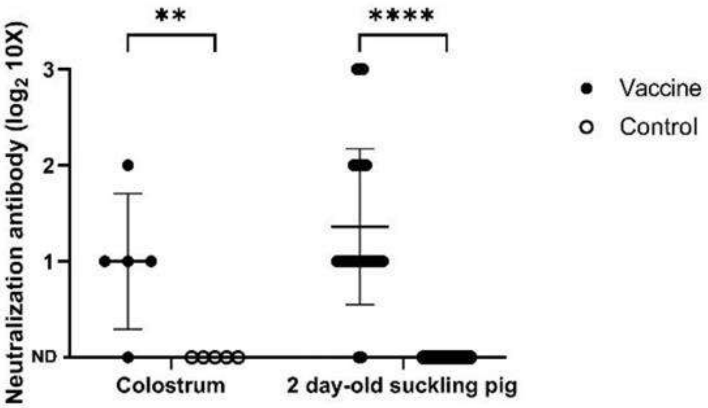

Figure 2. PEDV neutralization antibody of colostrum and serum samples

Data were analyzed by t test. ** means statistically significant difference ($p<0.01$). **** means statistically significant difference ($p<0.0001$).

P-V-060

EXPERIMENTAL RECOMBINANT VACCINE OF PORCINE CIRCOVIRUS (PCV) 2D GENOTYPE HAVING MORE EFFECTIVE PROTECTIVE ABILITIES AGAINST PCV2D VIRUSES AS COMPARED WITH PCV2A-BASED COMMERCIAL VACCINE

Dr. Young-Ju Jeong[1], Dr. Seok-Jin Kang[2], Dr. Hyang-Sim Lee[2], Dr. Bang-Hun Hyun[2], Dr. Nakhyung Lee[1]
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PCV2d is a major prevalent genotype in South Korea. Recently, shift in the PCV2 genotypes and the vaccine failure in the field necessitates to develop the currently circulating genotypes specific vaccine and to compare its protective efficacy with the commercially available vaccine which is commonly used in Korea.
In this study, a protective ability of recombinant VLP provided by PCV2d-ORF2 and PCV2a-based commercial vaccine were evaluated against PCV2d field isolate. The recombinant VLP of PCV2d-ORF2 was produced using baculovirus expression system. Experimental groups were divided to recombinant PCV2d-VLP (rVac), PCV2a-based commercial vaccine (cVac) and no vaccination (noVac). All piglets of each group were vaccinated at 3 weeks of age, dually challenged with PCV2 and PRRSV at 6 weeks of age and euthanized at 21dpc. The dually challenged viruses are PCV2d genotype and PRRSV-1 as European genotype obtained from swine farms affected by PMWS.
As a result, all piglets of three groups did not show any abnormally clinical signs after challenge. ADWG of rVac (0.67kg/day) was higher than that of cVac (0.44kg/day) and noVac (0.5kg/day). After challenge, ADWG showed the highest difference between rVac (0.54kg/day) and cVac (0.17kg/day) during 14-21dpc. PCV2 was effectively protected by rVac and cVac in blood and nasal swab. In the viral load, the genomic copies of PCV2 were completely decreased by rVac in lung, tonsil and lymph nodes whereas it was still detected in cVac. In the pathological analysis, lungs were normal in rVac whereas it showed a mild and severe pneumonia lesions in cVac and noVac, respectively.
Taken together, rVac provided by PCV2d-ORF2 is protected PCV2d more effectively than PCV2a-based commercial vaccine.

P-V-061

EXTENDED-GATE FIELD EFFECT TRANSISTOR BIOSENSOR FOR PORCINE EPIDEMIC DIARRHEA VIRUS DETECTION

Mr. Chia-Hung Lin[1,2], His-Teng Kao[3], Daisy Cheng[3], Shi-Wei Lin[3], Yi-Chan Lee[3], Wei-Hao Lin[1,2], Chao-Nan Lin[1,2], Ming-Tang Chiou[1,2]

[1]Animal Disease Diagnostic Center, College of Veterinary Medicine, National Pingtung University of Science and Technology, [2]Department of Veterinary Medicine, College of Veterinary Medicine, National Pingtung University of Science and Technology, [3]Sunplus Technology

Introduction:
Porcine epidemic diarrhea virus (PEDV) causes enormous economic loss worldwide [1]. Common detection methods require longer time, personnel and laboratory equipment. Many detection methods have been developed, but the validity of those may be unsatisfied. Semiconductor based biosensors can be used to detect extremely low concentrations of bio-targets [2-3]. Highly sensitive Extended-Gate Field Effect Transistors (EGFETs) that are compatible with standard semiconductor manufacturing processes, suitable for mass production and cost effective were developed. The extended gate component protects the FET from the liquid of bio-samples while conducting the electric signals with minimal signal loss. The aim of this study is to develop a biosensing EGFET for PEDV detection.

Materials and Methods:
The EGFETs were designed by Sunplus Technology and manufactured by United Microelectronics Corporation. The portable reader was designed and manufactured by Sunplus Technology. The sensing region was first prepared with an Epoxy coating and functionalized with PEDV DNA probes followed by blocking, then dried and packaged in a vacuum seal. PEDV samples were lysed and diluted before biosensing. The reader gave a constant bias voltage while the gate potential (VG) was swept from 1.5 to 3 V for each measurement. A blank buffer was used to establish a baseline. Each sample was incubated for 15min, then washed with blank buffer to remove unspecific bindings before measurement. Viral suspensions and other common pathogens causing diarrhea in pigs including rotavirus type A, B and C (RVs), transmissible gastroenteritis virus (TGEV), swine deltacorona virus (SDCoV), Escherichia coli (E. coli) and Salmonella Choleraesuis (Sal. C.) were tested. PEDV qPCR negative and positive rectal swabs (RC), feces (FC), ileal contents (CI) and small intestinal emulsions (SI) samples were also tested.

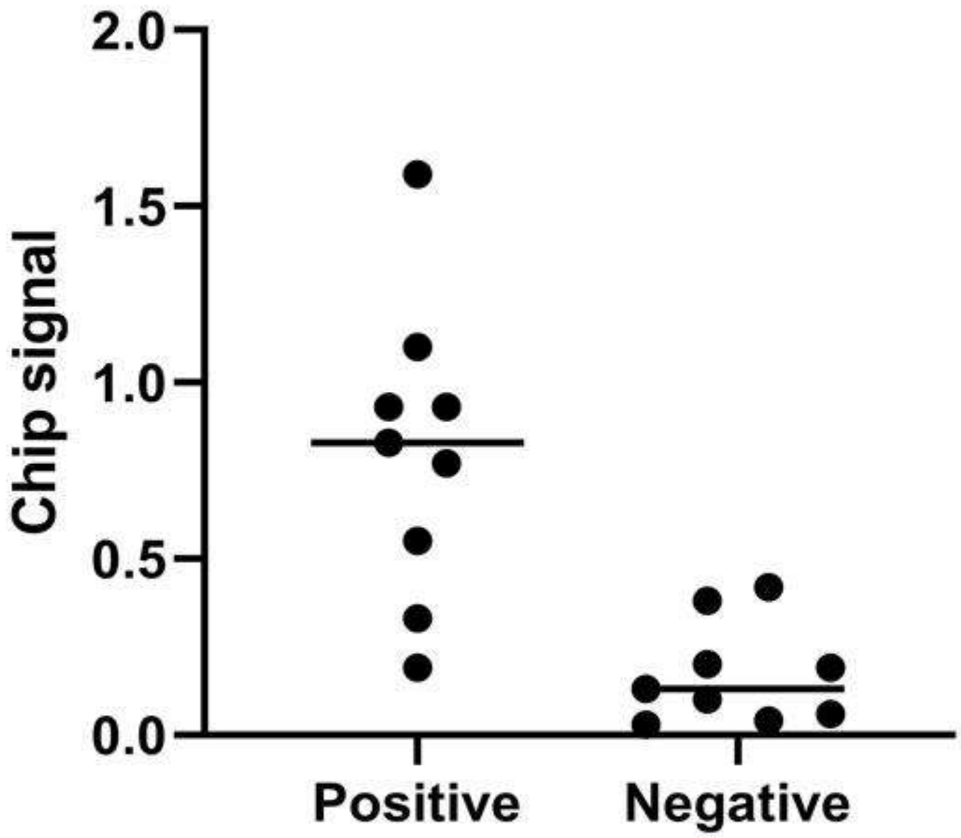
Results:
The extracted PEDV RNA showed the highest signal, follow by the PEDV lysate. The other common pathogens showed relatively lower signals; however, Rotavirus has an elevated reading. For the testing of clinical samples, PEDV negative samples' readings are lower than the PEDV positive ones.

Conclusions:
The EGFET may be used as a biosensor for PEDV detection in various samples. The results showed that RVs has an elevated reading, indicating non-specific binding with the current probe.

Acknowledgement:
We are gratefully acknowledging the technicians and veterinarians in NPUSTADDC and Sunplus Technology for their contributions to this study.

References:
[1] Zimmerman JJ, 2019. Disease of swine (11th ed.)
[2] Lin HC et al., 2005. IEEE Electron Device Lett. 26: 643-645.
[3] Chen CP et al., 2011. Anal. Chem. 83: 1938-1943.

Graphs or Images 2



P-V-062

FIELD EFFICACY BETWEEN TWO COMBINATION VACCINES OF PCV2 AND MYCOPLASMA HYOPNEUMONIAE IN A FARM IN NORTH OF VIETNAM

Dr. Vu Tran[1], Dr Huy Nguyen[1]
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Introduction
There have been several publications on the simultaneous control of Porcine Circovirus Associated Diseases (PCVD) and Enzootic Pneumonia in Asian Farms1. There are, however, only a few published studies comparing which combined PCV2 and Mycoplasma hyopneumoniae vaccine provides better protection as measured by improved performance parameters and acute phase protein (APP) outcomes. APP such as C-Reactive Protein (CRP) are accepted non-specific biomarker for evaluating the level of inflammation and stress on pigs and are appropriate tools for supporting the assessment of animal health and welfare2. This study compares two PCV2 and Mycoplasma hyopneumoniae vaccination protocols in a North Vietnam Farm.

Materials and Methods
The study was performed in a farrow-to-finish farm located in North of Vietnam. The farm has a history of PCV2 and Enzootic Pneumonia problems. The trial was started in October of 2021. A total of 804 pigs were divided into two groups, 398 pigs were assigned to the FLEXcombo group (Ingelvac CircoFLEX and Ingelvac MycoFLEX freshly mixed) while 406 pigs were allocated to the Porcilis PCV M Hyo group. Performance parameters were assessed, particularly mortality, average daily weight gain (ADG) and days to market. CRP was also measured before and after vaccination as an indicator of vaccine reaction (safety) and animal welfare.

Results
During the entire observation period, pig from FLEXcombo group had lower CRP levels compared to the Porcilis PCV M Hyo. This was more heightened 24 hours and 28 hours post-vaccination (Table 1).

Table 1.

Performance parameters are shown in Table 2. FLEXcombo group had lower mortality and higher ADG. Animals from this group achieved market weight 3 days earlier than Porcilis group as a result of the faster growth.

Table 2.

Discussion
Considering the findings on this study, FLEXcombo proved to be less reactive and could be perceived as the more animal welfare friendly vaccination scheme, besides supporting the better performance. Given the current prevailing pork price (54,000 VND/pig) the net benefit of vaccination is 32,113 VND/pig.
References
1. Toan et al, (2013). Proc. Of the APVS.
2. Hernández-Caravaca et al (2017). Res in Vet Sci, Vol 114, 2017, p 69-73.

Table 1

Table 1. C-Reactive Protein Levels before, 24 hrs and 48 hrs post-vaccination

Before vx		After vx 24h		After vx 48h	
FLEX	Porcilis	FLEX	Porcilis	FLEX	Porcilis
19.16	49.47	111.22	789.46	21.47	169.04

Table 2

Table 2. Performance Results of the 2 groups

Parameter	FLEXcombo	Porcilis PCV M Hyo	Dif.
Mortality %	2.02%	2.48%	-0.46%
Weigh gain (Kg)	84.71	84.23	+0.48
Growing period (days)	98	101	-3
ADG	0.864	0.834	0.030

P-V-063

FIELD EFFICACY BETWEEN TWO COMBINATION VACCINES OF PCV2 AND MYCOPLASMA HYOPNEUMONIAE IN A FARM IN SOUTH OF VIETNAM

Dr. Vu Tran[1], Dr Quy Tran[1], Dr Giang Pham[1]
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Introduction
There have been several publications on the simultaneous control of Porcine Circovirus Associated Diseases (PCVD) and Enzootic Pneumonia in Asian Farms1. There are, however, only a few published studies comparing which combination of PCV2 and Mycoplasma hyopneumoniae vaccines provide the better performance based on production parameters. This study compares 2 such vaccines in a Southern Vietnam Farm.

Materials and Methods
The farrow-to-finish farm is located in South of Vietnam. The farm has a history of PCV2 and Enzootic Pneumonia problems. The trial was started in April of 2022. A total of 649 pigs were divided into two groups, 323 pigs were assigned to the FLEXcombo group (Ingelvac CircoFLEX and Ingelvac MycoFLEX freshly mixed), while 326 pigs were allocated to the Porcilis PCVM Hyo group. Performance parameters such as % mortality, final weight, days to market, average daily weight gain (ADG), as well feed conversion rate (FCR) were measured to evaluate the differences between groups. The Return on Investment (ROI) was calculated for the economic analysis.

Results
Results are summarized in Table 1. The FLEXcombo group had faster growth, evident by the higher ADG (+57g) resulting in fewer days to reach market weight (-16.2 days). The FLEXcombo group also demonstrated lesser mortality, and more efficient feed conversion.

The feed economy due to the better FCR in the FLEXcombo group generated savings of 4.26 Kg of feed per pig. Moreover, the reduction on days to market contributed with 40.5 Kg of feed savings per pig. In total the feed economy associated to the FLEXcombo group was of 44.8 Kg per pig. The Return on Investment for the FLEXcombo group given all these benefits and considering the local market feed and pork prices is 6.3:1.

References
1. Toan et al, (2013). Proc. Of the APVS.

Table 1

Table 1. Performance Results of the 2 groups

Criteria	Unit	FLEX	RTU	Diff
# of pigs in	pig	323	326	
Ave day raising	day	154.3	170.5	-16
Slaughter day old	day	177.7	190.9	
Ave weight out	kg	111.8	112.9	
ADG	g/day	691	634	57
Mortality rate	%	1.5%	3.1%	-1.5%
Ave weight	kg	106.5	108.1	
Total feed intake/pig	kg	241	248	
FCR	kg feed/kg weight	2.26	2.30	-0.04

P-V-064

FIELD EFFICACY OF PORCINE CIRCOVIRUS (PCV) GENOTYPE 2D-BASED VIRUS-LIKE PARTICLES IN REPUBLIC OF KOREA SWINE FARMS

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Introduction
Porcine Circovirus type 2 (PCV2) causes massive economic losses to the swine industry worldwide by causing porcine circovirus associated disease (PCVAD). According to a recent surveillance study, there has been a genotype shift from PCV2a and 2b to PCV2d, which accounts for 95% of the PCV2 isolates in Korea. In a previous study, we demonstrated the potential of PCV2d ORF2 virus-like particles (VLP) in protecting animals from PCVAD in a controlled experiment. We are evaluating the field efficacy of the same vaccination in a farm setting on a large number of animal endemic with PCVAD possibility of natural infection.

Materials and Methods
The study included three farms (H, M and Y) with a history of PRRSV, PCV, Mycoplasma and E coli. We chose 90 three-week-old piglets housed in different rooms (n=30/room) to be vaccinated intramuscularly with PCV2d VLP (200ug/dose), while 30 animals were maintained as unvaccinated control in a single farm. Body weight gain, serum IgG (ELISA), neutralizing antibody titer (VN Assay), viremia and other parameters were monitored every four weeks until 20 weeks post-vaccination, in randomly selected 15 vaccinated and 5 control animals.

Results
The viral load of blood was not detected or remained at baseline throughout the investigation in the farm H, whereas it slightly increased in farms M and Y (8 wpv onwards), but the levels were significantly lower compared to the control group. Based on the data of viremia, PCV2 was naturally infected at the period of 4-8 wpv in farm H and 8-12 wpv in farm M and Y. In phylogenetic analysis, PCV2d was detected in all farm, and PCV2b was co-detected in farm M. Out of the two ELISA for evaluating the serum IgG, in-house ELISA coated with PCV2d VLPs was better in detecting the antibodies compared to the PCV2a-based commercial ELISA kit. In all farms, IgG levels of serum were peaked at 8 wpv in the vaccinated group and showed a statistically significant difference from the control animals. Up to 8 wpv, the vaccinated group from both the farm H and Y had higher neutralizing antibody titers than the control animals, which was statistically significant. In these two farms, from 12 wpv control group showed a notable rise in NA titer compared to the group that had received the vaccination. In contrast, there was no appreciable variation in the NA titer between the control and immunized group in farm M from 0-20 wpv. The average weight gain of the immunized groups in all three farms was higher from 0-12wpv than the control animals. It gained an average of 3.3kg, 4kg and 3.6kg at 12-week post-vaccination (wpv) in the vaccine groups of farm-H, M and Y, respectively, compared to their control group.

Conclusions

In conclusion, the piglets vaccinated with PCV2d VLP resulted in significantly different parameters between vaccinated and control animals in all three farms. This field trial was successful in protecting piglets from PCV2 naturally infection

P-V-065

FOOT-AND-MOUTH DISEASE VIRUS 3C ANTAGONIZES TYPE-I INTERFERON SIGNALING AND C142T SUBSTITUTION ATTENUATES THE FMD VIRUS

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3C protease (3Cpro), a chymotrypsin-like cysteine protease encoded by the foot-and-mouth disease virus (FMDV), plays an essential role in processing the FMDV P1 polyprotein into individual viral capsid proteins in FMDV replication. Previously, it has been shown that 3Cpro has involved in the blockage of the host type-I interferon (IFN) responses by FMDV. However, the underlying mechanisms are poorly understood. Here, we demonstrated that the protease activity of 3Cpro contributed to the degradation of RIG-I and MDA5, key cytosolic sensors of the type-I IFN signaling cascade in proteasome, lysosome and caspase-independent manner. And also, we examined the degradation ability on RIG-I and MDA5 of wild-type FMDV 3Cpro and FMDV 3Cpro C142T mutant which is known to significantly alter the enzymatic activity of 3Cpro. The results showed that the FMDV 3Cpro C142T mutant dramatically reduce the degradation of RIG-I and MDA5 due to weakened protease activity. Thus, the protease activity of FMDV 3Cpro governs its RIG-I and MDA5 degradation ability and subsequent negative regulation of the type-I IFN signaling. Importantly, FMD viruses harboring 3Cpro C142T mutant showed moderate attenuation of FMDV in a pig model. In conclusion, our results indicate that a novel mechanism evolved by FMDV 3Cpro to counteract host type-I IFN responses and a rational approach to virus attenuation could be utilized for future vaccine development. [National Research Foundation of Korea (2018M3A9H4079660, 2021R1A6A1A03045495)]

P-V-066

FOOT-AND-MOUTH DISEASE VIRUS NON-STRUCTURAL PROTEIN 2B DOWNREGULATES THE RLR SIGNALING PATHWAY VIA DEGRADATION OF RIG-I AND MDA5

Prof. Jong-Soo Lee[1], Ms. Asela Weerawardhana[1], Dr. Jong-Hyeon Park[2]

[1]College Of Veterinary Medicine, Chungnam National University, [2]Animal and Plant Quarantine Agency

Foot-and-mouth disease virus (FMDV) is a single-stranded, positive-sense RNA virus containing at least 13 proteins. Many of these proteins show immune modulation capabilities. As a non-structural protein of the FMDV, 2B is involved in the rearrangement of the host cell membranes and the disruption of the host secretory pathway as a viroporin. Previous studies have also shown that FMDV 2B plays a role in the modulation of host type-I interferon (IFN) responses through the inhibition of expression of RIG-I and MDA5, key cytosolic sensors of the type-I IFN signaling. However, the exact molecular mechanism is poorly understood. Here, we demonstrated that FMDV 2B modulates host IFN signal pathway by the degradation of RIG-I and MDA5. FMDV 2B targeted the RIG-I for ubiquitination and proteasomal degradation by recruiting E3 ubiquitin ligase ring finger protein 125 (RNF125) and also targeted MDA5 for apoptosis-induced caspase-3- and caspase-8-dependent degradation. Ultimately, FMDV 2B significantly inhibited RNA virus-induced IFN-β production. Importantly, we identified that the C-terminal amino acids 126-154 of FMDV 2B are essential for 2B-mediated degradation of the RIG-I and MDA5. Collectively, these results provide a clearer understanding of the specific molecular mechanisms used by FMDV 2B to inhibit the IFN responses and a rational approach to virus attenuation for future vaccine development. [National Research Foundation of Korea (2018M3A9H4079660, 2021R1A6A1A03045495)]

P-V-067
FOOT-AND-MOUTH DISEASE VIRUS VP4 INHIBIT IRF3 NUCLEAR TRANSLOCATION TO DOWNREGULATE HOST TYPE-I INTERFERON PATHWAY

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Foot-and-mouth disease (FMD) is a highly infectious virus and is a causative agent of acute vesicular diseases that affect pigs, cattle, and other domestic and wild hoof animals.VP4 is one of the 4 structural proteins involved in the formation of the viral capsid, which is also involved in evading the type-I interferon (IFN) pathway. Overexpression of FMDV-VP4 downregulates the IFN signaling in porcine (PK15, PAM, and LFBK) and human (HEK293T) cell lines, thus increasing Vesicular Stomatitis Virus (VSV-GFP) and Influenza virus (PR8-GFP) replication in the virus replication study. FMDV-VP4 transient transfection or stably over-expression exhibited a minimal secretion of pro-inflammatory cytokine and negatively regulated the transcription of IFN stimulatory genes. In the IFN-related luciferase reporter assay, VP4 causes downregulation of IFN promoter activity until the level of IRF3-5D. Studies revealed that VP4 does not inhibit the IRF3 dimerization and phosphorylation. The current study demonstrates the inhibition of IRF3 nuclear localization from VP4 by competitively interacting with KPNA4 and KPNA2 in vitro. KPNA molecules are the transporters of IRF3 to the nuclear compartment. Both KPNA molecules interacted with VP4 in overexpression and endogenous conditions, and the competition assay shows VP4 has a higher binding affinity to KPNA molecules than IRF3. These results collectively suggest that FMDV-VP4 negatively regulates the type-I IFN pathway and leads to severe FMDV infection. [National Research Foundation of Korea (2018M3A9H4079660, 2021R1A6A1A03045495)]

P-V-068

GENETIC CHARACTERIZATION OF PORCINE TESCHOVIRUS (PTV) ISOLATES FROM COMMERCIAL PIG FARMS IN SOUTH KOREA

Mr. Beom Soo Park[1], Mr. Ji Hyeon Hong[1], Mr. Jong Su Jun[1], Mr. An Kook Choi[1], Mr. Kyung Soo Yang[1,2], Mrs. Hye Kyung Yoo[2], Prof. Young Soo Lyoo[1]
[1]Immunopathology Lab, College of Veterinary Medicine, Konkuk University, [2]Farm&Pharm veterinary hospital

Introduction:
PTV is a single-stranded, positive sense RNA virus in the family Picornaviridae. Pigs infected with PTV mainly show clinical manifestation as febrile reaction followed by paresis with various mortality[1]. A recent Chinese study found strains genetically distinct from PTV-A, and it was named PTV-B along with strains found in Japan[2], [3]. Little information is known about PTV-B because so far little research has been done with these agents. An existence of the PTV-B has not been scrutinized in Korean pig population. In this study, the genotypes of PTV in Korean domestic pig farms were analyzed and its genetic characteristics were investigated.

Materials and Methods:
Fecal samples were collected from Korean domestic pig farms nationwide. PTV was isolated and serially propagated in PK15 and ST cell cultures and confirmed the virus replication after five passages. The virus adaptation in cell culture was determined by PTV specific RT-PCR. Sanger method was used to obtain the full viral genomic sequence. Maximum Likelihood (ML) trees were constructed from the aligned VP1 and 3CD amino acid sequences by using LG model with 100 bootstrap replicates in PHYML.

Results:
Two isolates were successfully propagated in cell lines originated from pigs and were named KU_PTV2201 and KU_PTV2202, respectively. Three nearly complete genomes were obtained, including two cell culture adapted isolates. The virus not adapted in vitro cell culture system was named KU_PTV2203. KU_PTV2202 and PTV2203 were identified in the sample derived from the same animal. Through ML tree of VP1 (Fig 1A), it was confirmed that KU_PTV2201 belongs to PTV-2, KU_PTV2202 belongs to PTV-23, and KU_PTV2203 belongs to PTV-19, respectively. In addition, it was confirmed that KU_PTV2203 belongs to PTV-B on the ML tree of 3CD (Fig 1B).

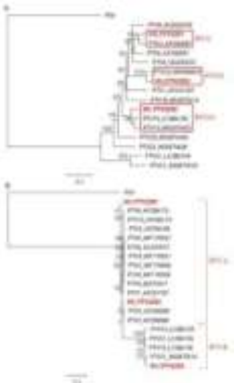
Conclusions and Discussion:
In this study, it was confirmed that various genotypes of PTV including PTV-B exist in Korean domestic pig farms. Based on our limited knowledge, it is the first identification of PTV-B in Korean pigs. As both KU-PTV2202 and PTV2203 co-exist in the same animal, it is speculated that there may be many cases of co-infection in the field. Since it is presumed that other new genotypes of PTV exist, further study is needed on the genotype distribution of PTV. In addition, further study is needed to see how PTV-B is differ from PTV-A in their pathogenicity and other characteristics in the susceptible animal.

Acknowledgement and References
[1] M. Y. Deng et al., Journal of Veterinary Diagnostic Investigation, vol. 24, no. 4, pp. 671–678, Jul. 2012
[2] T. Yang et al., Journal of General Virology, vol. 99, no. 9, pp. 1261–1267, Sep. 2018

[3] T. Yang, Y. Lu, L. Zhang, X. Li, and Y. Chang, Transbound Emerg Dis, vol. 67, no. 2, pp. 1015–1018, Mar. 202

Figure legend
Figure 1. Phylogenetic tree based on the VP1(A) and 3CD(B) amino acid sequences of PTV strains. Porcine sapelovirus (MN784122) was used as outgroup.

Graphs or Images 1



P-V-069

GENETIC DIVERSITY OF ORF5 OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS 2 IN TAIWAN FROM 2014 TO 2022

Mr. Kun-Lin Kuo[1,2], Mr. Wei-Hao Lin[1,2], Mr. Chao-Nan Lin[1,2], Mr. Ming-Tang Chiou[1,2]
[1]Department of Veterinary Medicine, National Pingtung University of Science and Technology, [2]Animal Disease Diagnostic Center, College of Veterinary Medicine, National Pingtung University of Science and Technology

- Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is responsible for respiratory disorders in nursery pigs and reproductive failures in sows. Since the outbreak of PRRSV2 was first reported in Taiwan, PRRSV2 has circulated in the field until now. Open reading frame 5 (ORF5) gene is the highly variable region of PRRSV. In recent years, the study of PRRSV2 lineages showed the significant turnover in Asia and North America, and this may be caused by the international spread of specific strains [1,2]. The introduction of foreign strains or evolution of Taiwanese strains may process the turnover of lineages of PRRSV2 in Taiwan. The aim of this study is to evaluate epidemiological status of PRRSV2 in Taiwan.

- Materials and Methods

Total 422 strains from Animal Disease Diagnostic Center of National Pingtung University of Science and Technology were sequenced and 62 Taiwanese reference sequences in Genbank of NCBI from 2014 to 2022 were included in this study. Phylogenetic tree and genetic distance of 484 Taiwanese sequences were constructed by the Tamura-Nei model of MEGA 11, then each Taiwanese strains were classified into different lineages based upon the phylogenetic tree.

- Results

Our results showed that Taiwanese strains during 2014 to 2022 were divided into lineage 1, 3 and 5. Lineage 3 strains were the most prevalent (86.6%), followed by lineages 1 (9.5%) and 5 (3.9%). Additionally, lineage 3 was subdivided into 7 sublineages (A-G), sublineage A (34.1%), C (28.9%) and D (17.4%) were more prevalent sublineages, and it showed the turnover of dominant sublineages. Lineage 1 strains were firstly discovered in 2018 and the proportion rapidly increased to 26.8% in 2022. On the contrary, lineage 3 proportion decreased from 96.2% to 73.2%.

- Conclusions and Discussion

We described the genetic diversity status of PRRSV2 in field over 9 years in Taiwan. Lineage 3 was the dominant lineage from 2014 to 2022, but it has been gradually replaced by lineage1 since 2020.

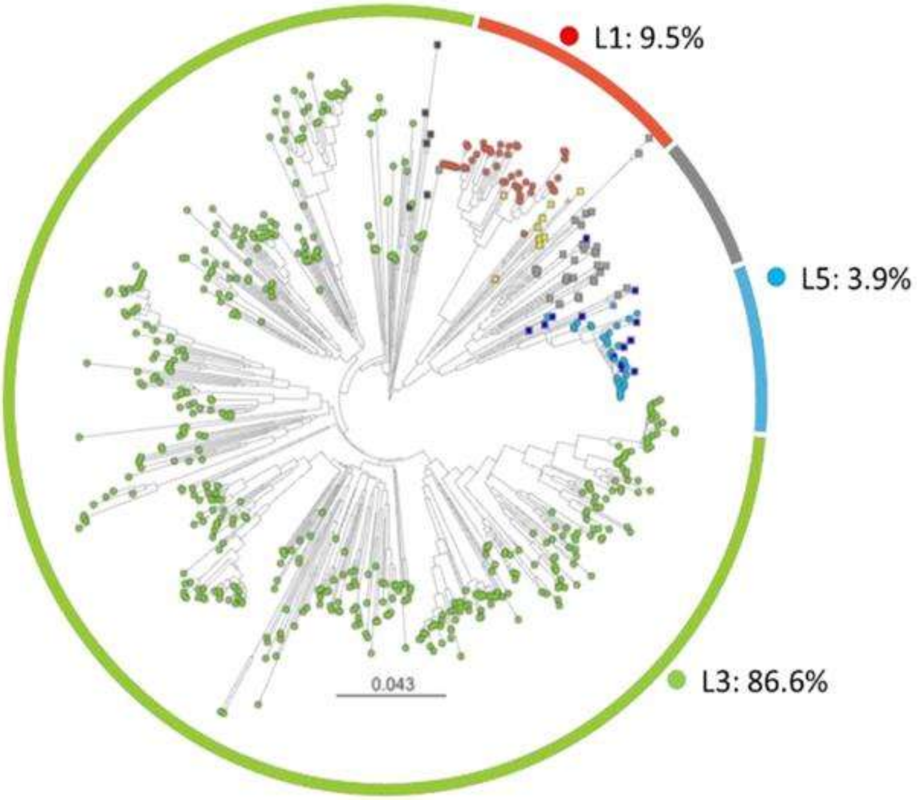
- Acknowledgement

We gratefully acknowledge the technicians and veterinarian in ADDC of NPUST for their contributions to this study.

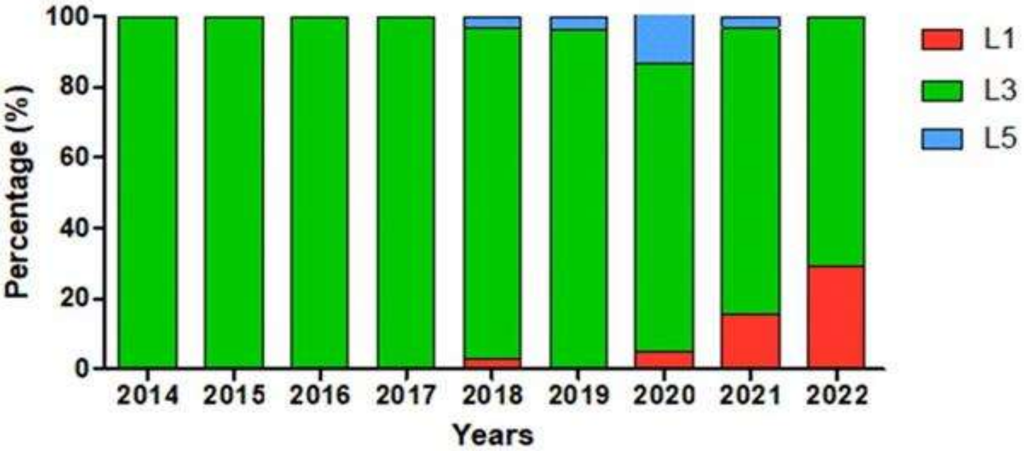
- References

[1] Paploski IAD et al., 2019. Front. Microbiol. 10: 2486.
[2] Gao J et al., 2017. Vet. Microbiol. 208: 164-172.

Graphs or Images 1



Graphs or Images 2



P-V-070

GLOBAL EPIDEMIOLOGY OF RESPIRATORY HEATH STATUS IN SWINE GROWING POPULATIONS SOUND-MONITORED: A 1-YEAR OBSERVATIONAL RETROSPECTIVE DATA BASE STUDY

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INTRODUCTION
SoundTalks®, a cloud-based 24/7 non-invasive technology, provides the Respiratory Health Status (ReHS) metric, the swine industry gold standard for objectively and continuously documenting clinical respiratory disease. Beyond the individual farm, there is a clear need for effective and ubiquitous disease monitoring that enables a much broader and even more effective early disease detection and intervention; as well as opening up the much greater opportunity for disease prevention and mitigation at a broader epidemiologic scale. To lay some essential groundwork for this broader health management application, the objectives of this study were to describe the epidemiological trends and seasonality of the respiratory health status measured by sound in some of the most swine dense regions a crossed the globe during a 1-year period.

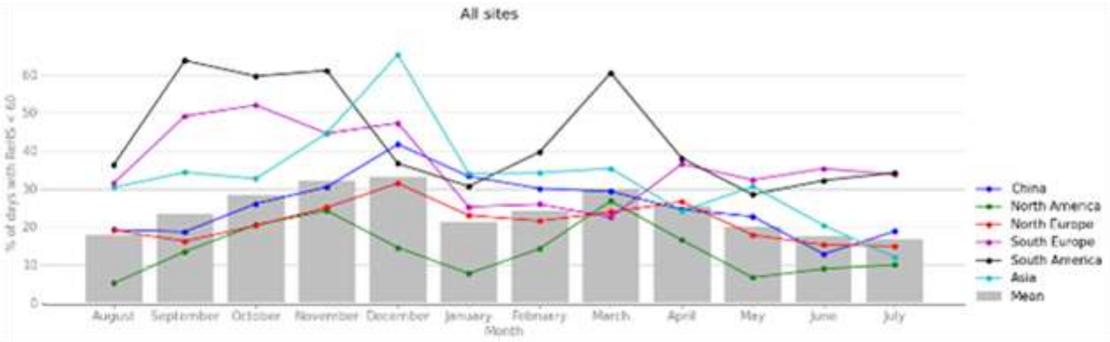
MATERIALS AND METHODS
This retrospective descriptive study was performed in 115 farms sound monitored by SoundTalks® for 1 year (August 2021 to July 2022). The inclusion criteria were commercial growing pig farms of at least 500 head capacity, with more than 120 days of monitored data, using industry standard management practices, and located in six major swine regions: North America, South America, Southeast Asia, China, and North and South Europe. All farm specific data were anonymized. Average ReHS was aggregated at room and farm levels. Farms were placed in health categories based on days with respiratory clinical signs, shown as yellow or red alarm-days (ReHS <60) over days without respiratory clinical signs (ReHS ≥60) shown as green days. Other sensor data (indoor temperature and relative humidity, outdoor temperature) were compiled and analyzed using a seven day rolling average and standard deviation.

RESULTS
A total of 115 farms from 15 countries were included in the study and grouped by corresponded regions. Average number of farms across regions were 20±9, with the most farms located in North America and North Europe (n=29) and fewest farms located in the South America (n=7) region. Farms were categorized in four groups by their ReHS status with 65.6, 32.3, 8.1 and 0.3 mean% of days with alarms during the study period. Farms from all but one region (i.e.North America) were present in the lower respiratory health status category, while only farms from North America, North Europe and Asia represented the healthiest category. When comparing overall means of environment sensor data, results demonstrated that farms within the lowest health status significantly differ from the medium and healthiest farms on max. temperature, as well as min. and max. humidity. The mean ReHS distribution by month of year was bi-modal, with peaks in December and March (Fig 1).

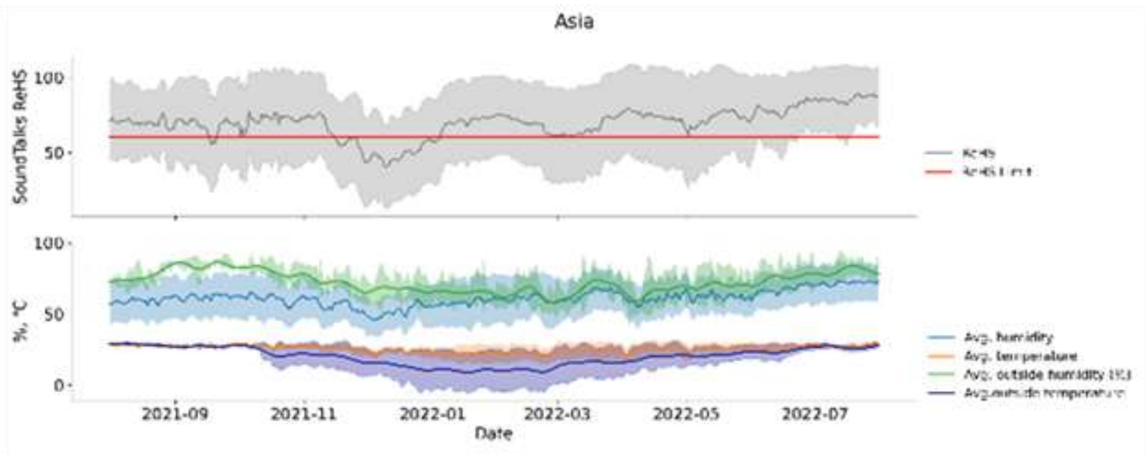
When considering the time series of ReHS and indoor and outdoor environment data, all regions showed the previously mentioned bimodal distribution of mean ReHS values following the trend of volatile weather conditions (i.e. typical fall and spring colder and warmer days). Specifically for the Asian region (Thailand, S.Korea and Japan, Fig 2), the first peak of respiratory problems starts in Nov and follows the trend of avg. outside temp/humidity. On the other hand, a second/third milder peak of respiratory problems were observed in March, when the temperatures start getting warmer and the outside humidity more unstable.

CONCLUSIONS
Study results demonstrate the respiratory health trends across the major swine regions over four calendar seasons. The large-scale disease detection and management potential of this sound-based system is clear when the technology is implemented at a system, regional and global scale. These initial results also highlight potential for supporting more effective global disease risk reduction and mitigation strategies for growing pigs and at-risk breeding herds. This potential will be realized as technology adoption broadens.

Graphs or Images 1



Graphs or Images 2



P-V-071

IDENTIFICATION OF PORCINE GLUCOSE REGULATED PROTEIN 78 AS A POTENTIAL CELLULAR CO-RECEPTOR PROTEIN FOR PORCINE EPIDEMIC DIARRHEA VIRUS

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Introduction

Porcine epidemic diarrhea virus (PEDV) could cause acute watery diarrhea, vomiting, and dehydration, and has led to high mortality rates, particularly in suckling piglets, and great economic impacts on the swine industry. Although the receptors of several coronaviruses have been confirmed but the receptor(s) of PEDV still remains unknown. In the present study, co-immunoprecipitation (Co-IP) with spike (S) protein, which is responsible for host affinity, virus-cell recognition and viral entry, and mass spectrometry analysis is performed to determine potential (co-)receptor of PEDV on pig enterocytes and Vero-E6 cells, which are permissive cells for PEDV. A membrane protein, porcine glucose-regulated protein 78 (pGRP78), was identified, and the role of pGRP78 in PEDV infection was investigated.

Materials and Methods

Cell membrane lysate was extracted from porcine intestinal epithelial cells and Vero-E6 cells for following co-IP. To identify the proteins after co-IP, the protein bands were cut from the protein gel for protein identification by using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). Final, to determine whether pGRP78 is essential for PEDV infection, pGRP78 was transfected to non PEDV-permissive swine cells such as intestinal porcine epithelial cell line 1 (IPEC-1), swine testicular cell line (ST) and PEDV-permissive human embryonic 293 cells (HEK 293). These cells were subject to PEDV inoculation using the 7th passage of the PEDV-Pintung-52 (PEDV-PT-P7) strain (GenBank: KY929405.1) and the viral infectivity and replicability were evaluated by real-time reverse transcription-polymerase chain reaction (RT-PCR).

Results

After the viral challenge, compared to wildtype HEK 293 cells, typical cytopathic effect (CPE) and an increased amount of cell-associated PEDV RNA were detected in pGRP78-expressing HEK 293 cells. However, no CPE and PEDV RNA was detected in IPEC-1 or ST cells with or without pGRP78 expression. Herein, we suggested that pGRP78 is not essential for PEDV entry but may interact with PEDV S protein and facilitate viral replication.

Conclusions and Discussion

This is the first report that identified pGRP78 as a potential co-receptor for PEDV in piglets' intestinal epithelial cells and Vero-E6 cells. Our results suggest that the pGRP78 might not serve as the functional cellular receptor for PEDV infection, but might involve in viral entry or replication. Understanding the interaction between PEDV S protein with pGRP78 will help to better understand the mechanism of PEDV pathogenesis and the functions of pGRP78 on viral infection and replication might provide novel strategies for designing antiviral interventions for PEDV prevention and control.

Acknowledgement and References
The authors are thankful for the support from the Mass Spectrometer Facility of Institute of Molecular Biology, Academia Sinica (Taipei, Taiwan) for conducting MALDI-TOF MS analyses.
1. Chu H, Chan C-M, Zhang X, Wang Y, Yuan S et al. Middle East respiratory syndrome coronavirus and bat coronavirus HKU9 both can utilize GRP78 for attachment onto host cells. Journal of Biological Chemistry 2018;293(30):11709-11726.
2. Li W, van Kuppeveld FJM, He Q, Rottier PJM, Bosch BJ. Cellular entry of the porcine epidemic diarrhea virus. Virus research 2016;226:117-127.

P-V-072

IMPROVEMENT OF GROWTH PERFORMANCE IN FINISHER HOUSE AFTER PRRS VACCINATION AT 3 WEEKS OF AGE

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Introduction

Porcine reproductive and respiratory syndrome is considered the most economically significant infectious pig disease worldwide. It is caused by a porcine arterivirus, which is further subclassified into two genotypes(1). PRRS is also common in South Korea and lots of swine farms have difficulty controlling it. At most PRRS positive farms in South Korea, using PRRS vaccine for reproductive problem control on sow herd is very common but using PRRS vaccine for respiratory problem control on grower-finisher is not common. For this reason, it is very valuable to evaluate PRRS vaccine efficacy on grower-finisher period. The objective of this study is to prove the efficacy of PRRS vaccination for respiratory problem control from 70 days to slaughter regarding mortality late.

Materials and methods

The field observation was conducted on a 900-sow PRRS positive farm with a 2-site system from Oct 2021 to May 2022. Pigs were transferred from nursery house to the grower/finisher house at 70 days of age and raised until slaughter. Before this study, sow farm didn’t vaccinate against PRRS to piglet and mortality rate in nursery house was normal. For PRRS positive result and high mortality rate in grower/finisher house, sow farm adjusted Ingelvac® PRRS MLV to piglets at 3 weeks of age from Sep 2021. Both PRRS type1 and type2 were detected in serum by RT PCR in finisher house and there was no PRRSV detection on sow farm. The performance in grower/finisher house(in J farm) was tracked to confirm the efficacy of PRRS vaccination to piglet, especially number of dead pigs and mortality rate from 70 days of age to slaughter were analyzed. For almost every pig was moved to J farm, other small grower/finisher farm’s performance were not tracked. This finisher was managed by the same employee during the evaluation period and there was no change in management practices except for the PRRS vaccines.

Result

A total of 10,802 pigs were monitored for this trial. Improvement in mortality was observed during the grower/finisher period after adjusting Ingelvac PRRS® MLV to piglet at 3 weeks of age. The number of dead pigs and mortality(%) decreased dramatically(Table 1) and this result was maintained for a long time(Figure 2). In aspect of economical approach, we could also get additional profit through PRRS vaccination. It was about 17.8$/pig when we assumed that average weight at death was 72.5kg(table 2). Differences of mortality rate obvious between non-vaccination and vaccination group. The results demonstrate that the PRRS vaccination to piglet at 3 weeks of age had a positive impact on mortality rate in grower/finisher house.

Discussion

The results from this study indicate that PRRS vaccination at 3 weeks of age can impact mortality rate in grower/finisher house. As there were no significant change in farm management and farm environment, this improvement is due to PRRS vaccination at 3 weeks of age. Further studies monitoring ages of slaughter should be done to evaluate the impact of PRRS vaccination in economical perspective.

Reference
(1) GJ Kang (2016) Efficacy of Ingelvac® PRRS MLV piglet vaccination in a large Korean farm, IPVS(2016)
(2) John Carr et al. (2018) PIG HEALTH 1st Edition

Table 1

Table 1. Additional profit of PRRS vaccination per pig. we assumed that average weight at death was 72.5kg. Pigs were raised in grower/finisher house from 30kg to 115kg.

Total improvement of mortality(%)	8.1
Average weight at death(kg)	72.5
Payment rate(%)	77
Meat price per kg($)	3.94
Additional profit per pig($) (8.1%*72.5kg*77% meat*3.94$)	17.8
Price of PRRS vaccination per pig($)	1.52
Return per pig($)	16.28

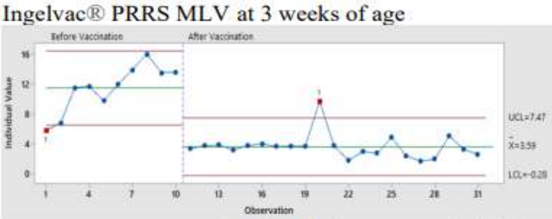
Table 2

Table 2. Mortality rate in grower/finisher(in J farm) between control and vaccination at 3 weeks of age

	Control	**Vaccination**
Period	Oct-Nov	Feb-May
No of batches	10	22
No of pigs at 70 days of age	3,545	7,257
No of dead pigs	412	252
Mortality(%)	11.6%	3.5%

Graphs or Images 1

Figure 1. Mortality of each group from 70 days of age to slaughter in grower/finisher house. 1-10 groups are controls and the other groups are vaccinated with Ingelvac® PRRS MLV at 3 weeks of age



*Horizontal axis means month of slaughter

P-V-073

INHIBITION OF MAVS-MEDIATED TYPE-I INTERFERON SIGNALING BY FOOT-AND-MOUTH DISEASE VIRUS VP3

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As a structural protein of Foot-and-mouth disease virus (FMDV), VP3 plays a imanortant role in virus assembly and escaping the host's innate immune response to promotes the FMDV replication. Previous studies demonstrated that FMDV VP3 blocks the type-I IFN response via inhibiting the mRNA expression of the mitochondrial antiviral-signaling protein (MAVS), however, the underline mechanism is poorly understood. Here we identified that the specificity of VP3 interaction with MAVS for the negative regulation of type-I IFN antiviral responses for effective replication of FMDV. Further, we describe that the transmembrane (TM) domain is the specific region of MAVS which interacts with the FMDV VP3. The TM domain of MAVS governs the mitochondria localization of MAVS and it is a key factor in type-I IFN signaling transduction via MAVS aggregation. Thereby, the interaction of FMDV VP3 with the TM domain of MAVS leads to the inhibition of MAVS mitochondria localization, self-association and aggregation, resulting in the suppression of type-I IFN response as shown in the results. Taken together, these data provide a clear understanding of a key molecular mechanism used by the FMDV VP3 for the suppression of IFN responses via targeting MAVS. [National Research Foundation of Korea (2018M3A9H4079660, 2021R1A6A1A03045495)]

P-V-074

ISOLATION AND GENETIC CHARACTERIZATION OF PORCINE SAPELOVIRUS (PSV)

Mr. Kyung Soo Yang[1,2], Mr. Ji Hyeon Hong[1], Mr. Beom Soo Park[1], Mr. Jong Su Jun[1], Mr. An Kook Choi[1], Mrs. Hye Kyung Yoo[2], Prof. Young Soo Lyoo[1]
[1]Konkuk University, [2]Farm&Pharm veterinary hospital

Introduction: PSV, a single stranded positive sense RNA virus, belongs to the genus Sapelovirus within the family Picornaviridae. The complete genome size of PSV is 7.5~8.3kb which consists of 5` untranslated region (UTR) called VPg (viral protein genome-linked), single large open reading frame (ORF), 3`-UTR, and a poly(A) tail. Since PSV was reported in UK in 1958 [1], PSV has been identified worldwide. In South Korea, PSV was detected in 2005 and then there has been little research on PSV carried out include epidemiology and genetic characteristics. PSV is known as an ubiquitous virus in pigs but pathogenesis is not well understood yet, and it has been detected on most commercial pig farms in South Korea [2]. Accordingly, it is important to obtain Korean PSV field isolate and to scrutinize their information on the viral genetics.

Materials and Methods: Fecal samples were collected nationwide from commercial pig farms. Positive PSV samples, as diagnosed by PSV-specific RT-PCR, were selected for isolating PSV by inoculating on PK 15 cell line. The PSV isolates were serially propagated in PK15 cell cultures over five passages and confirmed virus replication by PSV specific RT-PCR. The polyprotein genome sequences of the isolates were obtained by sanger method. We analyzed this isolated PSV with previously reported Korean PSV strains (KS04015, KS05151, KS055217) using Clustal omega method [2].

Results: Nine PSV isolates were successfully isolated in cell culture. Polyprotein sequence of these isolated PSV showed high nucleotide (84.4 ~ 97.9%) and amino acid (93.4 ~ 99.5 %) identities with the other Korean PSV strains (Table 2, 3). However, the VP1 had lower nucleotide (69.2 ~99.8%) and amino acid (78.2 ~ 100%) identities with other strains (Table 1).

Conclusions and Discussion: In this study, we compared newly identified Korean PSV isolates with previously characterized Korean PSV isolates. Based on result, we determined the high identities in polyprotein nucleotide and amino acid sequences between the Korean PSV isolates. However, there is relatively lower identities in the VP1 nucleotide and amino acid sequences, suggesting ongoing mutation in VP1 in the field. Thus, monitoring and investigating this mutation are essential, and further can be done with these isolates to understand these variations on the incidence of disease in susceptible animal.

Acknowledgement and References

[1] Lamont, P. H., and A. O. Betts. "Studies on enteroviruses of the Pig—IV: the isolation in tissue culture of a possible enteric cytopathogenic swine orphan (ECSO) virus (V 13) from the faeces of a pig." Research in Veterinary Science 1.2 (1960): 152-161.
[2] Son, Kyu-Yeol, et al. "Molecular epidemiology of Korean porcine sapeloviruses." Archives of virology 159 (2014): 1175-1180.

Table legend
Table 1. Genetic homology of VP1 nucleotide sequences between Korean PSV isolates

Table 2. Comparison of polyprotein nucleotide sequences homology between Korean PSV isolates
Table 3. Homology of polyprotein amino acid sequences between Korean PSV isolates

Table 1

Table 2

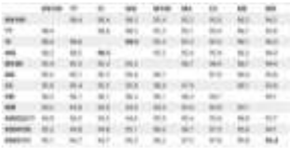
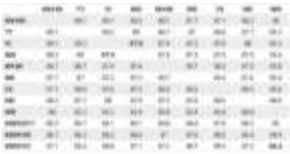


Table 3



P-V-075

MAMMALIAN CELL LINE AND METHOD FOR EXPRESSING SOLUBLE E2 RECOMBINANT ANTIGEN OF CLASSICAL SWINE FEVER VIRUS USING THE SAME AND APPLICATION THEREOF

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Introduction
Classical swine fever (CSF) remains one of the most important transboundary viral disease of swine worldwide with a past outbreak in Taiwan (Sandra et al., 2017; Pan et al., 2005). Development of vaccine about CSF contains live attenuated CSF vaccine and E2 subunit vaccines suggest an effective disease control and eradication (Bouma et al., 1999). The abolition of modified live virus (MLV) vaccines and the adoption of subunit vaccines are a critical strategy to choose for the possibility to declare Taiwan free from CSF (Zhou et al., 2019; Chen et al., 2021).

Materials and Methods
In this study, A mammalian cell line and method for expressing soluble E2 recombinant antigen of classical swine fever virus are created by Chinese hamster ovary (CHO) cell technology. The mammalian cell line with different cell passage numbers can stably express soluble CSFV-E2 recombinant protein in mass production, for reducing the manufacturing cost of the CSFV-E2 recombinant protein, as well as application on the production of a porcine CSFV-E2 subunit vaccine composition.

Results
We demonstrated that E2 protein can continuously be expressed in stable CHO cell lines up to twenty times of cell passage and the protein amounts attain 800 mg/L.
Conclusions and Discussion The widespread vaccine strategy for classical swine fever virus (CSFV) has been hampered by the cost of production. To address this need, we are developed stable clone cell lines to express the E2 protein of CSFV which induces a significant neutralizing antibody response. Here we have developed a platform to stably and continuously express the secreted and soluble E2 protein from the Chinese hamster ovary(CHO) cell line. We demonstrated that E2 protein can continuously be expressed in stable CHO cell lines up to twenty generations and the protein amounts attain 800 mg/L. This platform can produced low-cost and can be used in E2 subunit vaccine development.

Acknowledgement and References
We kindly thank all members in the RCAB, VMII207, and FS105 of NPUST, Taiwan, for providing computing, storage resources, and assisting the diagnostic works. We also thank ATRI (Agricultural Technology Research Institute, Taiwan) for providing animal house and assistance.
1. Sandra B.; Christoph S.; Julia.; Jolene C.; and Martin B. Classical swine fever-An update review. Viruses. 2017, 9(4). 86.
2. Pan, C.H.; Jong, M.H.; Huang, T.S.; Liu, H.F.; Lin, S.Y.; Lai, S.S. Phylogenetic analysis of classical swine fever virus in Taiwan. Arch. Virol. 2005, 150(6), 1101-1119.
3. Bouma, A.; De Smit, A.J.; de Kluijver, E.P.; Terpstra, C.; Moormann, R.J. Efficacy and stability of a subunit vaccine based on glycoprotein E2 of classical swine fever virus. Vet. Microbiol. 1999, 66(2), 101-314.

4. Zhou, B. Classical swine fever in China-an update minireview. Front. Vet. Sci. 2019, 6, 187.
5. Chen, J.Y.; Wu, C.M.; Chen, Z.W.; Liao, C.M.; Deng, M.C.; Chia, M.Y.; Huang, C.; Chien, M.S. Evaluation of classical swine fever E2 (CSF-E2) subunit vaccine efficacy in the prevention of virus transmission and impact of maternal derived antibody interference in field farm applications. Porc. Health Manag. 2021, 7(1), 9.

P-V-076

MEASURING THE EFFICACY OF PCV2 VACCINATION IN THE HERD PIGS UNDER THE NATURAL CO-INFECTION OF PRRSV AND PCV2 BASED ON THE DYNAMICS OF GENE, IMMUNE RESPONSES, AND RATE OF PCV2 ISOLATION

Dr. Ringo Rissar Siringo[1], Dr. Mazimpaka Eugene[1], Dr. Choonnasard Amonrat[1], Dr. Tasuku Hirooka[1], Mr. Taketo Wada[2], Dr. Eigo Sakamoto[3], Mr. Shunichi Honda[3], Dr. Hiroyuki Horiuchi[2], Dr. Tamaki Okabayashi[1]
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Introduction
PCV2 is the primary causative agent for Porcine Circovirus-Associated Disease (PCVAD). Coinfection with Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) was able to enhance PCV2 viremia and increase the incidence of PCVAD [1]. Vaccination against PCV2 is considered the most effective method to reduce the negative impact of PCV2 infection. However, there have been reported inconsistencies in the ability of the PCV2 vaccines due to several factors including co-infection and methods used to measure the vaccine efficacy [2]. This study aims to measure the PCV2 vaccine efficacy under natural conditions and a new method for PCV2 detection.

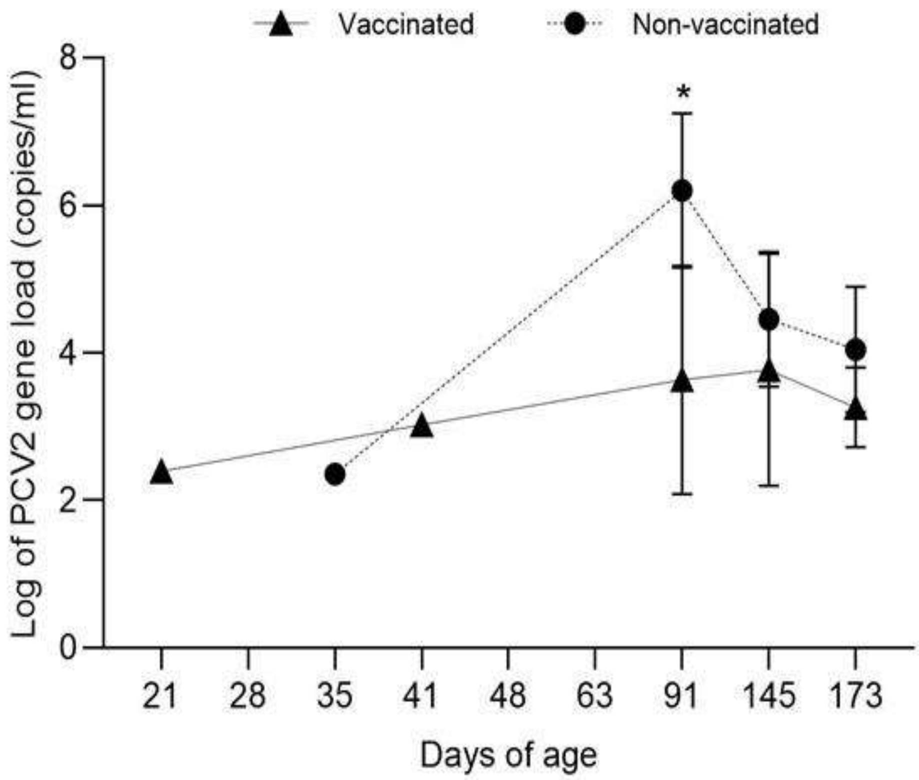
Materials and methods
This study was conducted in a one site farm with 250 sows located in West Japan during Apr. 2020 to Jan. 2021. The farm was seropositive to PRRSv at around 60 days of age, but post-weaning mortality was controlled without PRRS vaccination in piglets. On the day of weaning, suckling piglets were evenly divided into two groups by individual weight, sexes, and parities. A total of 44 piglets used for this study were divided into two groups, the vaccinated and non-vaccinated (22 piglets per group). Piglets in the vaccinated group were vaccinated with Ingelvac CircoFLEX® at 21 days of age. All piglets from each group were kept under the same management conditions on the farm. A total of 100 blood samples were collected randomly from the vaccinated and non-vaccinated groups at 21, 28, 35, 41, 48, 63, 91, 145, and 173 days of age. Blood samples were used for the serological test, qRT-PCR, Inverse PCR, PCV2 isolation, and IFA.

Results and Discussion
PCV2 gene load (Fig. 1) in the vaccinated group was only significantly lower ($P = 0.0095$) than in the non-vaccinated group at 91 days of age. PCV2 antibody titers (Fig. 2) in the vaccinated group at 91, 145, and 173 days of age were significantly lower ($P < 0.0001$) than in the non-vaccinated group. PCV2, genotype d, was isolated from the non-vaccinated group (isolation rate 50%) but not the vaccinated group (0%). These results indicate that PCV2 gene load in the sera of the non-vaccinated group is related to PCV2 viremia level, but not in the vaccinated group. Based on PCV2 isolation, Inverse PCR as a new method for circular DNA of PCV2 detection showed superior specificity (67%) over qRT-PCR (47%). Both groups were positive for PRRSV infection before 63 days of age (Fig. 2). Interestingly after PRRSV infection, increased PCV2 gene and PCV2 antibody titers in both groups were found at 91 days of age. These results suggest that PRRSV may enhance PCV2 replication in pigs [3][4] and influences the dynamics of PCV2 gene load in the sera of both groups. This study supports that PCV2 vaccination was able to reduce the negative effect of PCV2 infection under the natural co-infection of PRRSV and PCV2. Moreover, the PCV2 gene number

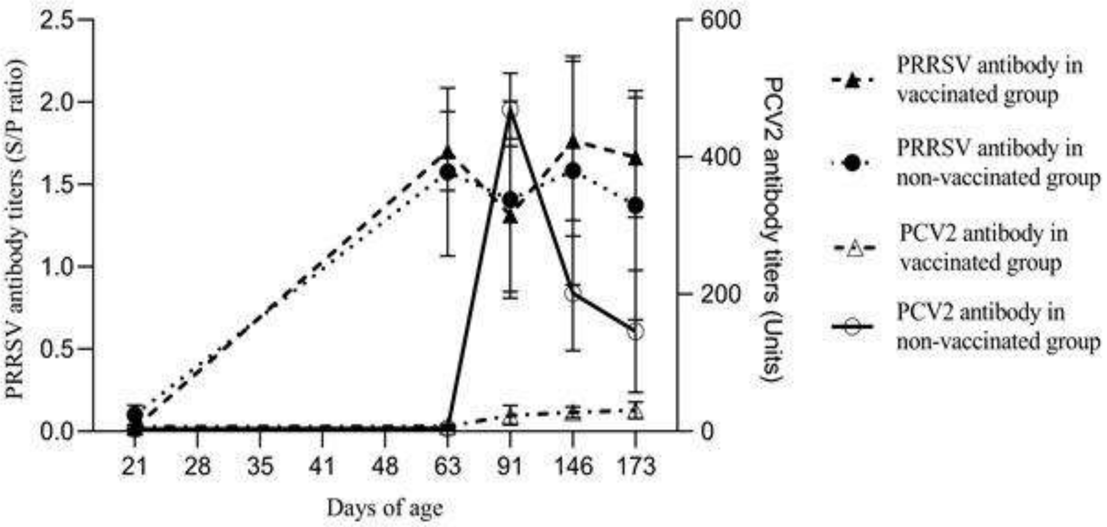
calculated using inverse PCR reflects a more accurate copy number of a potentially infectious virus than qRT-PCR results.

References
1. Kristensen, C. S., et al., 2011. Prev. Vet. Med., 98(4), 250–258.
2. Opriessnig, T., et al., 2007. J. Vet. Diagn. Invest, 19(6), 591–615.
3. Rovira, A., et al., 2002. J. Virol., 76(7), 3232–3239.
4. Park, C., et al., 2013. Clin. Vaccine Immunol, 20(3), 369–376.

Graphs or Images 1



Graphs or Images 2



P-V-077

MONITORING VIRAL GENETIC VARIABILITIES BY ANALYSIS OF SELECTIVE PRESSURE AND GLYCYLATION SITES IN PPV1-4 COLLECTIONS FROM COMMERCIAL PIG FRAMS IN SOUTH KOREA

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[1]*Konkuk University*

Introduction:
Porcine parvovirus (PPV) is prevalent worldwide, but research on its characteristics is limited, because no isolation of PPV have been succeeded except PPV1. Selective pressure in virus contributes to viral genetic variation and viral glycosylation sites are known to be involved in immune evasion mechanism in various viruses. This study analyzed selective pressure to determine which parts of the virus are undergoing the most mutations by comparing collected sequences of PPV1-4 from field samples with existing sequences of PPVs available in NCBI and confirmed whether any changes occurred in those regions using glycosylation site prediction.

Materials and Methods:
Using 6 sequences for PPV1, 10 for PPV2, 8 for PPV3 and 6 for PPV4, we detected the selected coding sequences of PPVs through DATAMONKEY (Datamonkey, http://www.Datamonkey.org/, 4 July 2020) [1]. Positive selection sites were detected using fast unconstrained Bayesian approximation (FUBAR) and mixed effects model of evolution (MEME). It was considered significant if $p < 0.05$ in MEME and the posterior probability > 0.9 in FUBAR. The selection pressure analysis of genomes was determined with Geneious Prime by calculating the differences between non-synonymous (dN) and synonymous substitution (dS) rates, for the aligned genes. Possible glycosylation sites were predicted using NetNglyc (http://www.cbs.dtu.dk/services/NetNGlyc/).

Results:
PPV1 showed a higher degree of amino acid sequence variation in NS1 and structural proteins compared to NS2 and NS3. PPV2 exhibited the highest selective pressure and most dynamic genetic and amino acid variations compared to other genotypes. PPV3 exhibited selective pressure in VP1 and VP2, while NS1 was conserved. PPV4 did not exhibit any selective pressure, and all proteins were relatively well conserved. PPV2 and PPV3 received more selective pressure than PPV1 and PPV4. All glycosylation sites were conserved, except for VP1 596aa of PPV3 (Table 1).

Conclusions and Discussion:
PPV2 and 3 showed more dynamic amino acid variation compared to PPV1 and 4, and mutations were observed in glycosylation sites at some sequences of PPV3. Although PPV1 and 4 were relatively conserved, being very small viruses, PPVs can exhibit significant antigenic differences with small amino acid change, and thus require close careful observation. It is important to monitor immune evasion due to the mutation and showing changes in pathogenicity. Emergence of the antigenically distinct virus by the mutations will be the prime interest of PPV research.

Acknowledgement and References

[1] Pond, Sergei L. Kosakovsky, and Simon DW Frost. "Datamonkey: rapid detection of selective pressure on individual sites of codon alignments." Bioinformatics 21.10 (2005): 2531-2533.

Table 1

Table 1. Results of selection pressure analysis and glycosylation site showing the position of codons in alignment of PPVs. MEME and FUBAR were used for selection pressure analysis, and NetNGlyc was used for glycosylation site prediction

Virus	gene	Selection pressure analysis		Glycosylation site prediction
		MEME	FUBAR	NetNGlyc
PPV1	[illegible]	-	[illegible]	[illegible]
	[illegible]	-	-	[illegible]
	[illegible]	-	-	[illegible]
	[illegible]	-	[illegible]	[illegible]
	[illegible]	-	[illegible]	[illegible]
PPV2	[illegible]	[illegible]	[illegible]	-
	[illegible]	[illegible]	[illegible]	[illegible]
PPV3	[illegible]	-	-	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
PPV4	[illegible]	-	-	[illegible]
	[illegible]	-	-	-
	[illegible]	-	-	[illegible]

396*a: An aa 396N appears as a glycosylation site only in PPV3KU/P22-4

P-V-078

NORMALIZATION OF PRRSV RT-QPCR RESULTS FOR SERUM AND ORAL FLUIDS (ECQS)

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[1]*Iowa State University,* [2]*Ohio State University,* [3]*PIC Latin America,* [4]*IDEXX Laboratories Inc.*

Introduction:
Normalization, the process of controlling for normal variation in testing, can be achieved in diagnostic PCRs by converting quantification cycles (Cqs) to “efficiency standardized Cqs” (ECqs). Simply put, ECqs represent the fold change of nucleic acids in a sample relative to a reference standard (RS), while accounting for amplification efficiency:
ECq = E^(-ΔCq)
where E = amplification efficiency and ΔCq = (sample Cq - RS Cq). This methodology has been used in basic research (Pfaffl, 2001), but not in routine diagnostics. Therefore, the objective of this study was to explore the application of ECqs to a commercial PRRSV RT-qPCR used for routine diagnostics.

Materials and methods:
Reference standards were created by rehydrating and then diluting (1 x 10^(-4) and 1 x 10^(-5) for serum and oral fluid, respectively) a PRRSV modified-live vaccine (Ingelvac® PRRS MLV) with serum or oral fluid to match the sample matrix to be tested. Sample ECqs were calculated using the mean amplification efficiency (E) and reference standard Cq calculated from the 4 reference standards run on each plate.
Serum (n = 132) and individual oral fluid (n = 130) samples from 12 pigs vaccinated with a PRRSV MLV from -7 to 42 days post vaccination (DPV) were tested and sample Cqs converted to ECqs.
Testing was performed using commercial reagents (IDEXX Laboratories, Inc.) and the MIC PCR™ Cycler (Bio Molecular Systems, Australia). Receiver operating characteristic analyses were conducted (R.4.2.1., package ‘pROC’) for each specimen type to estimate the area under the curve (AUC) and the diagnostic specificities and sensitivities for a range of ECq cutoffs.

Results:
Mean plate amplification efficiencies were 1.75 to 2.6 for serum and 1.7 to 2.3 for oral fluid. Mean plate reference standard Cqs were 29.1 to 31.3 for serum and 29.2 to 31.5 for oral fluids. Receiver operating characteristic analysis calculated the area under the curve for serum and oral fluid sample ECqs as 0.999 (95% CI: 0.997, 1.000) and 0.947 (0.890, 1.000), respectively. For serum, the diagnostic sensitivity and specificity of the commercial PRRSV RT-qPCR was estimated as 97.9% and 100% at a cutoff of ECq ≥ 0.20 and, for oral fluid, 82.6% and 100%, respectively, at a cutoff of ECq ≥ 0.45.

Conclusions and discussion:
Accounting for amplification efficiency (E) improves test accuracy because sample Cqs are directly related to E and assuming 100% E will lead to the over estimation of target concentration. In addition, all results have an ECq numeric value, including truncated or “indeterminate” Cqs. Therefore, it is possible to calculate cutoffs and evaluate diagnostic performance using receiver operating characteristic analysis.

Acknowledgements:
We thank IDEXX Laboratories, Inc. for providing reagents.
References: Pfaffl 2001. Nucleic Acids Res. 92:9.

P-V-079

ORGANOTYPIC AIR-LIQUID INTERFACE RESPIRATORY CELL CULTURE (ALI-REC) SYSTEM FOR STUDYING VIRAL COINFECTIONS WITH INFLUENZA A SUBTYPES H1N1 AND H3N2 IN PIGS.

Ms. Ana Fiorella Castillo Espinoza[1], BVSc & AH, MVM, PhD Rahul Nelli[1], MS Lu Yen[1], MS Gino Castillo, MS, PhD Juan Mora-Diaz[1], DVM, PhD, Diplomate ACVPM-Specialty Epidemiology Jeffrey Zimmerman[1], DVM, PhD Luis Gimenez-Lirola[1]
[1]*Iowa State University*

Influenza A virus (IAV) pose a threat to mammalian species due to their introduction and re-introduction into populations, primarily humans and pigs. The IAV subtypes H1N1, H3N2, H1N2, and their variants, are the major subtypes circulating in swine, acting as mixing vessels for IAV of human and avian origin. Two-dimensional culture models have played an important role in IAV-related research. However, these models lack the morphological, structural, and functional complexity otherwise inherent to the pseudostratified respiratory lining epithelia, thereby hindering the respiratory epithelium's faithful morphological and physiological reproduction. To overcome this limitation, air-liquid interface (ALI) respiratory epithelial cell (REC) culture systems has been described for studying viral infections, including IAV. ALI-REC culture system resembles morphologically (basal, ciliated, and secretory cells), structurally (cell interactions between cellular components), and functionally (mucociliary system) the respiratory airway in vivo. This is the first report describing the use of a porcine-derived ALI-REC culture system (ALI-PREC) to model and characterize H1N1 and H3N2 single vs. coinfection in vitro.

Tracheas collected from seven-days-old CD/CD piglets (n=3) were dissected, washed, and digested to isolate PRECs, which were seeded into pre-coated transwells and cultured under ALI conditions for 4-5 weeks to allow them to differentiate. Differentiated ALI-PREC cultures were then inoculated with H1N1 (A/Swine/Minnesota/37866/1999), H3N2 (A/Swine/Texas/4199-2/1998), or both (MOI 0.1 and MOI 1), along with mock uninfected controls. ALI-PRECs were exposed for 6 h with viral/mock inoculum at 37°C, 5% CO_2, followed by removal of inoculum, washing, and incubation for 24, 48, 72, and 96 h post-inoculation (hpi). ALI-PRECs were assessed microscopically, fixed, and stained for immunocytochemistry (ICC), and the subnatants from the basolateral compartment were collected and used to analyze IAV viral RNA by RT-qPCR.

The microscopic evaluation showed active ciliary motility in both virus- and mock-inoculated ALI-PRECs up to 24 hpi. By 48 hpi, ciliary motility was significantly reduced in virus-inoculated ALI-PRECs, and the characteristic cytopathic effects (CPE), including dead/lifting cells, started to appear, and became more pronounced by 72 hpi. At 96 hpi, the CPE was clearly observed in all virus treatments, i.e., cytoplasmic stranding, vacuolation, rounding of cells, clusters of rounded cells, cell shrinkage, and cell detachment. ICC further demonstrated that ALI-PRECs were permissive to initial virus entry and replication with IAV nucleoprotein detection at 24 hpi in fixed and stained cells.

RT-qPCR results showed that ALI-PRECs co-inoculated and single inoculated with H3N2 (MOI 1) started to release the virus steadily into the bottom subnatants of platewells at 24 hpi, while virus release in H1N1-inoculated ALI-PRECs was detected by 48 hpi. Overall, microscopic evaluation, ICC, and PCR results suggest that ALI-REC model is a suitable organotypic culture system for studying IAV single and co-infections. To further investigate the applicability of this model for immunological research, this study will pursue the characterization of the expression profile of genes associated with the innate immune response during H1N1 and H3N2 co-infection as well as the crosstalk between innate and adaptive immunity mediated by macrophages and dendritic cells via co-culture of immune cells and ALI-RECs in vitro.

P-V-080

PATHOGENICITY EVALUATION OF A NOVEL VIRULENT PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS IN TAIWAN

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Introduction:
Porcine reproductive and respiratory syndrome (PRRS) is one of the main pathogens in worldwide modern swine industry which causes reproductive failure and respiratory distress that lead to economic loss. Clinical severity of PRRS can be mainly influenced by virus pathogenicity [1]. Several highly virulent PRRSV had been reported. In Taiwan, a comparably highly virulent strain, TSYM which is able to induce high fever, high mortality (40-100%) and high viremia titer that is similar to HP PRRSV through animal challenge experiment [2]. In this study, a novel suspected highly virulent PRRSV, 108-929 was isolated and its pathogenicity was evaluated through animal challenge experiment.

Materials and Methods:
Twenty-three PRRSV-free 4 weeks old piglets were divided into 5 groups, control (C) (n=3), low titer intranasal inoculation (LIN) (n=5), low titer contact (LC) (n=5), high titer intranasal inoculation (HIN) (n=5), high titer contact (HC) (n=5). Piglets in LIN and HIN inoculated with 2 ml of low titer (10^3 TCID50) or high titer (10^4 TCID50) of 108-929 strain while the piglets of contact group stayed in the same pen with corresponding inoculation group for contact infection. Sera of all piglets were collected at 0 day post-inoculation (dpi) before inoculation, 1, 3, 7, 10 and 14 dpi for PRRSV antibodies and viremic load detection. All piglets were weighted on -7, 0, 7 and 14 dpi. Body temperature and clinical signs were measured and observed daily. All the piglets were euthanized at 14 dpi. Duration of high fever, average daily weight gained, PRRSV antibodies titer and viremic load were analyzed by Two way ANOVA and multiple comparison was analyzed by Tukey's method. Gross and interstitial pneumonia score were analyzed by Kruskal Wallis test with Fisher LSD procedure.

Results:
The duration of high fever of all the challenge and contact groups showed significantly longer than C group. At the first week after inoculation, average daily weight gained (ADWG) of HIN group presented negative and was significantly lower than C and LC group. At the second week after inoculation, ADWG of all the groups except C group presented negative. The gross lung lesion score of HIN, LIN and LC group were significantly higher than C group. All the challenge groups' interstitial pneumonia score were significantly higher than C group. At 7 dpi, PRRSV antibody positive rate of LIN group was 20%. At 14 dpi, HIN group was 100% positive rate and the titer was significantly higher than C and LC group. In HIN, LIN and LC group, PRRSV viremic rate firstly showed positive at 1 dpi as 40%, 20% and 20% while HC group was detected on 7 dpi. All the challenged and contact groups reached 100% PRRSV viremia positive rate at 10 dpi. HIN group's viremic load reached the peak value (7.17 ± 0.81) at 7 dpi and significantly higher than C group while the other groups reached their peak (LIN, 6.84 ± 0.63; HC, 6.96 ± 0.7; LC, 6.75 ± 0.74) at 10 dpi. All the challenged and contact groups' viremic load were significantly higher than C group at 10, 14 dpi. There were only 2 piglets in

HIN group reached above 8 log^{10} copies/µL viremia titer which also the two piglets who dead during the experiment.

Conclusions:
The novel PRRSV strain, 108-929 found in Taiwan showed similar pathogenicity with other highly virulent strains and should be defined as a high virulent strain.

Acknowledgement:
We gratefully acknowledge the technicians and veterinarians in ADDC of NPUST.

References:
1. MEC W. 1992. Pig Vet J. :62–8.
2. Hou FH, Chia MY, Lee YH, Liao JW, Lee WC.2019. Comp Immunol Microbiol Infect Dis. Elsevier; 2019;65:96–102.

Table 1

Table 1. Gross, interstitial pneumonia score mortality and high fever day of all groups. Asterisk (*) represents statistical difference compared to C group.

Group	Gross score	Interstitial pneumonia score	Mortality (%)	High fever day
C	3.5	0.83	0	0
LIN	41.3*	2.9*	0	7.6*
LC	36.2*	2.2	0	5.4*
HIN	45.67*	3.0*	40	6.67*
HC	32.0	2.1	0	5.4*

P-V-081

PERFORMANCE IMPROVEMENT AFTER A PCV2 AND MYCOPLASMA HYOPNEUMONIAE COMBINATION VACCINE (TRIVALENT VACCINE) IMPLEMENTATION IN NORTHEAST THAILAND: A CASE REPORT

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Introduction
Porcine circovirus genotype 2d (PCV2d) is commonly found in pig farm in Thailand, while genotypes PCV2a and PCV2b are still present (1). The use of a PCV2 bivalent vaccine containing PCV2a and PCV2b genotypes may provide broader protection to pigs confronting mixed PCV2 genotype infections within the same herd (2). On the other hand, Mycoplasma hyopneumoniae (Mhyo) remains an important primary bacterial respiratory pathogen, especially when pigs are co-infected with other secondary bacteria and viruses, including PCV2 (3). According to these reasons, the unique combination vaccine of bivalent PCV2 and Mhyo (trivalent vaccine) was developed to prevent clinical signs and reduce the negative impact that these infections have decreasing growth performance (4). This study was conducted to assess the efficacy of the trivalent vaccine in farrow-to-finish herds with PCV2 and Mhyo endemic infections, and a PRRS stable condition in the Northeast region of Thailand.

Materials and Methods
Pigs from seven farrow-to-finished herds totaling 9,000 sows were followed, assessing performance and economic impact after use of Fostera® Gold PCV MH (Zoetis), a combination vaccine including two PCV2 antigens (Chimeric PCV1-2a and PCV1-2b) and Mycoplasma hyopneumoniae (trivalent vaccine) for 1 year. Piglets were intramuscularly vaccinated from 3 weeks of age, following the approved indications, with a 2 ml single dose, or splitting the dose in two 1 ml administrations three weeks apart, depending on farm circumstances. Sera were collected to detect for PCV2 DNA by real time PCR and to compare with the previous program (Negative cut-off of CT is more than 40 cycles), i.e. before Fostera® Gold PCV MH implementation. Performance parameters, including market weight and % mortality, were collected and compared: before (previous program) and after Fostera® Gold PCV MH implementation (current program).

Results
The administration of Fostera® Gold PCV MH resulted in a reduction of PCV2 viremia (positive samples) in herds monitored by real time PCR. (Table 1 and Table 2) Only one out of the seven herds still found viremia positive samples after implementation of the Fostera® Gold PCV MH vaccination program, while the other herds were negative in every group of age tested.
The result from farm no.1 indicated that the health status improvement from the reduction of PCV2 infection, confirmed by real time PCR, lead to the better performance after Fostera® Gold PCV MH implementation when compared with previous program. Although, farm no.2-7 had no evidence of PCV2 infection both before and after new vaccine implementation, the performance improvement was shown after new vaccine implementation as well.

Conclusions and Discussion

In summary, this study indicates that the PCV2 and Mycoplasma hyopneumoniae combination vaccine (trivalent vaccine), Fostera® Gold PCV MH can reduce the occurrence of PCV2 viremia and improve the performance including market weight and % mortality, reducing the pressure of infections and improving the health status in the herd.

References

1. Thangthamniyom et al., 2017, Vet. Microbiol. 208: 239-246.
2. Bandrick et al., 2020, Vet. Immunol. Immunopathol. 223: 110034.
3. Garcia-Morante et al., 2022, Vet. J. 288: 105877.
4. Mancera Gracia et al., 2021, Vaccine. 9: 834.

Table 1

Table 1. PCV2 viremia measured with real time PCR in a herd before the new vaccination program.

Farm	Age (week)					
	4	7-8	11-12	15-16	19-20	23-24
1 (study1)	Neg	Neg	Pos	Pos	Pos	Pos
1 (study2)	Neg	Neg	Pos	Pos	Pos	Pos
2	Neg	Neg	Neg	Neg	Neg	Neg
3	Neg	Neg	Neg	Neg	Neg	Neg
4	Neg	Neg	Neg	Neg	Neg	Neg
5	Neg	Neg	Neg	Neg	Neg	Neg
6	Neg	Neg	Neg	Neg	Neg	Neg
7	Neg	Neg	Neg	Neg	Neg	Neg

*Neg was negative result or viremia was not found in this sample and Pos was positive result or viremia was found in this sample.

Table 2

Table 2. PCV2 viremia measured with real time PCR in a herd after the new vaccination program.

Farm	Age (week)					
	4	7-8	11-12	15-16	19-20	23-24
1 (study1)	Neg	Neg	Neg	Neg	Neg	Neg
1 (study2)	Neg	Neg	Neg	Pos	Neg	Neg
2	Neg	Neg	Neg	Neg	Neg	Neg
3	Neg	Neg	Neg	Neg	Neg	Neg
4	Neg	Neg	Neg	Neg	Neg	Neg
5	Neg	Neg	Neg	Neg	Neg	Neg
6	Neg	Neg	Neg	Neg	Neg	Neg
7	Neg	Neg	Neg	Neg	Neg	Neg

*Neg was negative result or viremia was not found in this sample and Pos was positive result or viremia was found in this sample.

Table 3

Table 3. The average market weight and % mortality from 7 farms in this study, $\bar{x}$ (SE)

Parameters	Previous program	Current program	p-value
Market weight (kg)	106.73 (0.18)	108.43 (0.5)	0.39
% Mortality	4.22 (3.62)^{a}	2.62 (3.99)^{b}	0.02

a,b Means with different superscripts were significantly different ($P < 0.05$).

P-V-083

PHYLOGENETIC ANALYSIS OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS IN PIG HERDS IN CHINA FROM 2014 TO 2022 BASED ON THE CLASSIFICATION OF OPEN READING FRAME 5 GENE

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Introduction:
Porcine reproductive and respiratory syndrome (PRRS) continues to cause severe economic losses to the pig industry worldwide. The difficulty of controlling the disease is largely related to the genetic diversity of its causative agent, PRRS virus (PRRSV). The Open reading frame 5 (ORF5) gene of PRRSV is highly variable and used to assess the variation and evolution of PRRSV in epidemiology survey [1-2]. The objective of this study is to reveal the prevalence of diverse lineages of PRRSV-2 in China over an 8-year period (from 2014 to 2022) by sequencing the PRRSV ORF5 gene.

Materials and Methods:
From January 2014 to July 2022, a total of 909 tissue or serum samples from 434 farms in 20 provinces of China were collected and confirmed to be positive for PRRSV ORF7 by RT-PCR. Sequencing of PRRSV ORF5 gene was conducted from these samples. The analysis for genetic evolution of the ORF5 sequences was performed by using MEGA7.0 software and some typical strains as anchors.

Results:
All 909 ORF5 sequences analyzed belonged to PRRSV-2. Based on their deduced amino acid sequences, the field isolates belonged to 4 lineages: the lineage 1, 3, 5 and 8 (Fig. 1). All strains within lineage 1 (L1) belonged to sub-lineage 1A (L1A), 1B (L1B) and 1C (L1C) further. The strains of lineages 1, 3, 5 and 8 in the samples accounted for 369, 121, 72 and 347, respectively.

As showed in Fig. 2, the proportion of lineage 8 (L8) PRRSVs appeared in the field noticeably decreased from 58.22% during 2014-2016 to 26.54% during 2019-2020, but slightly increased to 28.74% during 2021-July 2022. The proportion of lineage 5 (L5) PRRSVs slightly and continually increased from 4.0% during 2014-2016 to 7.41% during 2019-2020, and then, escalated to 13.03% during 2021-July 2022. The proportion of lineage 3 (L3) PRRSVs continually increased from 12.89% during 2014-2016 to 17.90% during 2019-2020 but declined to 9.58% during 2020-July 2022. The proportion of L1 PRRSVs continually increased from 24.89% during 2014-2016 to 48.66% during 2020-July 2022, and L1C strains within L1s were predominant over eight years of the investigation, but interestingly, dropped from 47.53% during 2019-2020 to 40.61% during 2021-July 2022 as L1A and L1B strains emerged in the past two years.

Conclusions and Discussion:

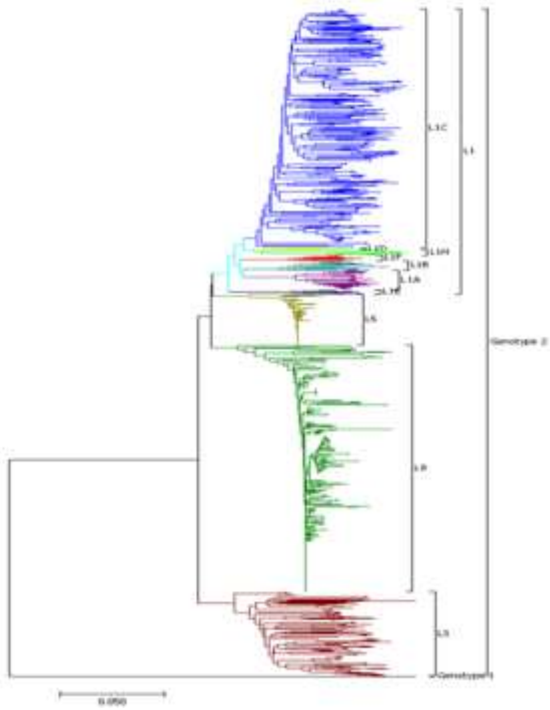
Lineage 1 PRRSVs showed a clear tendency to spread widely with new sub-lineages 1A and 1B emerged over the past two years. Controlling L8 and L1 PRRSV strains in the field should be substantially considered for China . This study provides an important epidemiology reference for the prevention and control of PRRSV.

References:

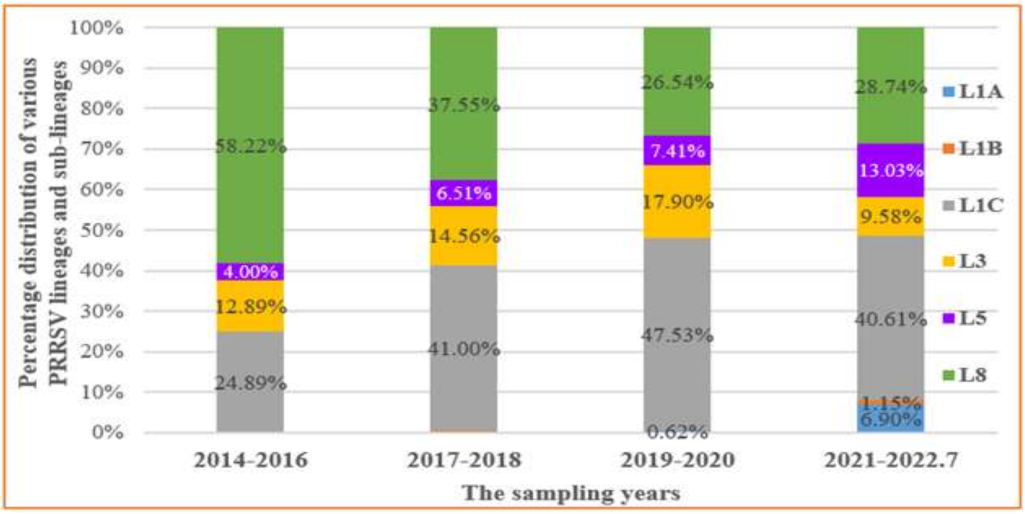
[1] Kewen Wang, et al., IPVS 2020 Proceedings, P750

[2] Pamornchainavakul N., et al. Front. Vet. Sci. (2022) 9:846904

Graphs or Images 1



Graphs or Images 2



P-V-085

PORCINE PARVOVIRUS SEROLOGY SURVEY ON JAPANESE FARMS

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Introduction

The protective role of specific antibodies against Porcine Parvovirus (PPV) enhanced via vaccination is key to controlling infectious reproductive problems in breeders and the goal is to maintain homogeneous herd immunity against PPV via vaccination (1). Therefore, the presence of seronegative gilts and sows or those with a low immune level to PPV on vaccinated farms causes great concern among farmers, and this is generally considered a marker of poor vaccination practices or low vaccine efficacy (2).
The present study was carried out to survey the serological status of mature gilts and sows on Japanese farms implementing vaccination against PPV, considering possible differences between the type of vaccine.

Material and Methods

A total of 802 samples (sows from parity 0 to 9) were collected from 75 farms (sized 35 to 3,000 sows) located in 17 different provinces in Japan from September 2021 to January 2022. The number of samples collected by each farm was in correlation to the size of the farm. Samples were shipped to Spain and analysed at HIPRA Diagnos. Antibody titer against PPV infection was determined by using haemagglutination inhibition assay (HI). The type of vaccine used in the 75 farms was variable: PPV monovalent live (PPV-L; n=126) or killed (PPV-K; n=8); PPV + Japanese encephalitis combo live (PPV+JE-L; n=251) or killed (PPV+JE-K; n=361); PPV+JE+Getah virus combo live (PPV+JE+G-L; n=42). In summary, a total of 383 animals were vaccinated with a vaccine containing PPV killed antigen and 419 a PPV lived antigen.

Results

Samples with a result of 1:8 were classified as negative, 1:16 and 1:32 as risk due to them having a low immune level, and titers ≥1:64 were considered protective (3). The results showed the presence of 9% seronegative animals and 16% at a risk range of antibodies (Table 1), the percentage being higher in the gilts compared to the multiparous. Regarding, the PPV live or killed antigens, the percentage of susceptible animals was lower in those vaccinated with a killed one (Table 2). Comparing the antigens nº in each type of vaccine, the monovalent and the bivalent types had the highest percentage of susceptible animals (negative and those categorized as risk) (Table 3). 34 of the 75 sampled farms (43.3%) had at least one seronegative animal, and 16 of the remaining 41 farms (39%) had at least one animal within the risk protective range level, so 50 of the 75 sampled farms had susceptible animals suffering PPV related problems.

Table 1. Antibody response against PPV in gilts and sows

Table 2. Antibody response against PPV by type of antigen

Table 3. Antibody response against PPV by type of vaccine

Discussion & Conclusion

The results observed might suggest weaknesses in current implemented PPV vaccination protocols in the Japanese pork industry. Therefore, vaccine protocols and/or vaccines should be revised or changed to increase the herd immunity level for PPV. Furthermore, it needs to be considered that it is not possible to know if the animals with titers ≥1:64 were protected or not before natural exposure. So, in those farms where there is a presence of animals with negative or risk level of titers, and at the same time positive animals, it cannot be elucidated that the animals were protected before contact with field virus causing reproductive disorders (4).

References

1. Christopher-Hennings J et al. 2019. In: Diseases of swine 11th edition. NJ, USA. pp. 75-97
2. Giménez-Lirola LG. et al. 2012. J Microbiol Methods, 91: 73-79
3. Andre F. Streck et al., 2011. Berliner und Munchener Tierarztliche Wochenschrift 124, Heft5/6 (2011), Seiten 242-246
4. Truyen U et al. 2009. Journal of General Virology, 90, 2437-2441

Table 1

	Negative	Risk (1:16 – 1:32)	Positive (≥1:64)
Gilts (n=192)	23%	21%	56%
Sows (n=610)	4%	14%	82%
Total (n=802)	**9%**	**16%**	**75%**

Table 2

	Negative	Risk (1:16 – 1:32)	Positive (≥1:64)
Killed (n=383)	7%	11%	82%
Lived (n=489)	11%	20%	69%
Total (n=802)	**9%**	**16%**	**75%**

Table 3

	Negative	Risk (1:16 – 1:32)	Positive (≥1:64)
Monovalent (n=134)	12%	29%	59%
Bivalent (n=612)	9%	14%	77%
Trivalent (n=56)	2%	5%	93%
Total (n=802)	9%	16%	75%

P-V-086

PREVALENCE AND MULTIPLE INFECTION PATTERN OF PPV1 THROUGH PPV4 IN SOUTH KOREA FROM 2016 TO 2022

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Introduction:
Porcine parvovirus (PPV) is a widespread virus globally and new genotypes, in addition to the well-known genotype PPV1, are continuously being discovered. PPV1, the most well-known porcine parvovirus, is a significant cause of reproductive failure in pigs [1]. PPV2 was first identified in Myanmar in 2001 and is believed to contribute to pneumonia in pigs. PPV3, first discovered in Hong Kong in 2008, does not show pathogenicity alone, but further research is required to determine if it may trigger infection of other pathogens, such as PCV2. PPV4 was first detected in the United States in 2005, and while its infection may persist, little is known about its pathogenicity. The objective of this study was to investigate the prevalence rates and infection pattern of PPV1 through PPV4.

Materials and methods:
From 2016 to 2022, 120 oral fluid samples were collected from domestic pig farms in South Korea. These samples are divided in 3 breeding stage (weaning, growing, and finishing stage) and submitted for laboratory diagnosis. For the diagnosis of PPV 1-4, a known multiplex primer sets were used [2]. The multiplex PCR (mPCR) conditions were as follows: pre-denaturation at 95°C for 10 min; 30 cycles of denaturation at 95°C for 30 s, varied annealing temperature for 40 s, and extension at 72°C for 1 min; and a final extension at 72°C for 5 min. The PCR products were evaluated with 2% agarose gel electrophoresis.

Results:
PPV1 showed a gradual increase in prevalence with age. PPV2 and PPV3 had similar prevalence in the weaner and grower stages but decreased in the finisher stage. PPV4 had high prevalence without significant differences across all stages. In the weaner stage, PPV4 had the highest and PPV1 had the lowest prevalence. In the grower stage, PPV2 had the highest and PPV3 had the lowest prevalence. In the finisher stage, PPV4 had the highest and PPV3 had the lowest prevalence (Figure 1).
Although there were many cases of single infections (30%), several genotypes were often co-infected, such as dual infections (30%), triple infections (23.3%), and quadra infections (8.3%). The most frequent co-infections pattern includes both PPV2 and PPV4 (35%), followed by PPV1 and PPV4 (29.2%) and PPV3 and PPV4 (26.7%) respectively. The lowest co-infection was between PPV1 and PPV3 alone or with other genotypes (19.2%) (Table 1).

Conclusions and Discussion:
For the prevalence of parvovirus infection on Korean commercial pig farms, PPV4 had the highest overall occurrence. PPV4 was also most frequently found to be co-infected with other genotypes of the virus, PPV1, 2, and 3. It is not yet well understood whether PPV4 influences the infection of other genotypes, so it appears that further research needs to be conducted. The prevalence of PPV2 and PPV3 decreases as the pigs grow up, while the prevalence of PPV1 increases with age. This suggests that the susceptibility of the viruses may differ depending on the age of the pigs.

Acknowledgement and References

[1] Mengeling, W. L., K. M. Lager, and A. C. Vorwald, 2000: Sci. 60–61, 199–210
[2] Kim S-C, Jeong C-G, Nazki S, Lee S-I, Baek Y-C, Jung Y-J, et al. https://doi.
org/10.1371/journal.pone.0245699

Table 1

Table 1. Infection pattern of PPV genotypes in Korean domestic pig farms

PPV1	PPV2	PPV3	PPV4
5 (4.2%)	12 (10%)	5 (4.2%)	14 (11.7%)
PPV1/2	**PPV1/3**	**PPV1/4**	**PPV2/3**
5 (4.2%)	3 (2.5%)	10 (8.3%)	1 (0.8%)
PPV2/4	**PPV3/4**	**PPV1/2/3**	**PPV1/2/4**
14 (11.7%)	3 (2.5%)	2 (1.7%)	7 (5.8%)
PPV1/3/4	**PPV2/3/4**	**PPV1/2/3/4**	**Negative**
8 (6.7%)	11 (9,2%)	10 (8.3%)	10 (8.3%)

Graphs or Images 1

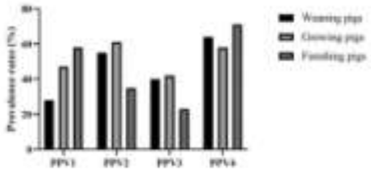

Figure 1. Prevalence rates of PPV type 1, 2, 3, and 4 at breeding stage.

P-V-087

RESULTS OF PORCINE PARVOVIRUS VACCINATION PLANS IN CHINA. WHICH OPTION PROVIDES THE BEST PROTECTION?

Mr. Joaquín Miguel[1], Mr. Wentao Fei[2], Mr. Tianning Yang[2], Mr. Ming Hsun[1]
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Introduction
Porcine Parvovirus (PPV) is a significant cause of reproductive failure, but in correctly vaccinated herds, the reproductive losses are low (1). In order to control it, the goal is to maintain homogeneous herd immunity against PPV via vaccination (1). However, in some Asian countries, partial vaccination plans which consist of only immunoprophylaxis of gilts and primiparous animals are commonly implemented. The objective of the present study was to investigate the current situation of porcine parvovirus infection in relation to vaccination schedules in gilts and sows in China.

Materials and Methods
From January 2021 to June 2022, a total of 6516 sera from gilts (G), primiparous (P), and multiparous (M) were collected from 171 farms located in 19 different Chinese provinces. The immune level to PPV was determined by inhibition of haemagglutination assay (IHA) at HIPRA DIAGNOS. The vaccination plan was categorized into 3 categories: Partial vaccination (only gilts and parity 1), Complete (gilts and sows every cycle), and Non-vaccinated. The animals were grouped into gilts, primiparous and multiparous. Samples with a result of 1:8 were classified as negative, 1:16 and 1:32 as risk due to them having a low immune level, 1:64 to 1:512 as immunity caused by vaccination, and ≥1:2048 as field virus. This classification is based on previous bibliography references (2).

Results
Comparing vaccinated and non-vaccinated animals (Table 1), the percentage of negative animals is higher in those which are non-vaccinated. A high percentage of animals with titers compatible with field virus contact is observed in both groups.
With reference to the 3 vaccination plans (Table 2), the animals with a complete vaccination programme have the lowest percentage of negative and risk animals. As expected, the non-vaccinated have the worst results, with a high percentage of animals susceptible to suffering PPV-related clinical problems.

Table 1. Categories of immune response in vaccinated and no vaccinated animals.

Table 2. Categories of PPV immune response in animals with different vaccination plans

Discussion & Conclusion
The present study demonstrated the current situation of Porcine Parvovirus immune status based on different vaccination plans in China.
Based on these results, it is clear that PPV virus is circulating and the current vaccines and vaccine programmes are not offering enough protection level. This is due to there still being negative animals and a considerable percentage of animals at risk, independently of the vaccination plan used. When comparing partial and complete programmes, those animals under a complete one, present better immune protection against PPV. This is in accordance with previous studies where it was demonstrated that the farms with a complete vaccination

plan had a lower percentage of mummified fetuses and litters with >30% of mummies than those with a partial vaccination plan.

Acknowledgments

References
1. Christopher-Hennings J et al. 2019. In: Diseases of swine
2. Andre F. Streck et al., 2011. Berliner und Munchener Tierarztliche Wochenschrift 124, Heft5/6 (2011), Seiten 242-246
3. Tummaruk et al. IPVS 2022 Rio de Janeiro.

Table 1

		% Negative	% Risk	% Vaccine	% Field virus
Non-vaccinated	G	42	10	7	40
	P	13	4	12	72
	M	2	8	21	69
	Total	14%	8%	16%	61%
Vaccinated	G	10	13	22	56
	P	3	9	19	68
	M	0	5	21	74
	Total	4%	9%	21%	67%
Total		6%	9%	20%	66%

Table 2

		% Negative	% Risk	% Vaccine	% Field virus
Complete	G	11	13	26	51
	P	2	7	21	70
	M	0	5	20	74
	Total	3%	8%	22%	67%
Partial	G	10	13	13	64
	P	7	13	15	65
	M	2	8	22	68
	Total	6%	11%	17%	66%
Non-vaccinated	G	42	10	7	40
	P	100	0	0	0
	M	5	3	20	71
	Total	33%	8%	11%	48%

P-V-088

RETROSPECTIVE STUDY OF THE RELATION BETWEEN PCV2 BREEDER VACCINATION AND PCV2 VIREMIA IN BREEDERS AND PIGLETS TO FINISHER PIGS

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Introduction
Porcine Circovirus Type 2 (PCV2) caused major losses to the farm production in form of both clinical (PCVAD) and sub-clinical infection. The virus has been reported as eight genotypes (PCV2a-2h) availability but the main genotypes are PCV2a, PCV2b, and PCV2d (2, 3). PCV2 vaccination in piglets has been reported the advantages to performance, viremia, fecal shedding, and clinical signs of pigs from weaning to finishers. Furthermore, the breeder vaccination (gilt and sows) has been reported the improvement of sow immunity, reduction of virus in circulation, and vertical transmission to fetus (1, 4, 5). This retrospective study aimed to collect the field data of the farm serological monitoring from year 2015-2021 to evaluate the relation and effect of sow and boar PCV2 vaccination to the PCV2 viremia of 2-24 WOA fattening pigs in Thailand and some Southeast Asian (SEA) countries.

Materials and Methods
The serological data from year 2015-2021 of 30 pig farms in Thailand (24), Cambodia (2), Indonesia (1), and Malaysia (3) were selected and verified. The data of sows (P1-P≥5) and boars (AI) were investigated for the information including PCV2 vaccination (mixed program) and the incidence of viremia (PCR or qPCR) of adult breeders and piglets to finishers (2-24 WOA) in the period of data record, cross-sectionally. Statistical analysis aimed to test for the risk of no vaccination breeders to themselves and their progeny at 2-24 WOA, by ODD ratio (OR) and Fisher's Exact Test (Chi-square) P<0.05.

Results
Table 1 illustrates the breeder number (835 pigs) and the data distribution in term of countries and genders (sow or boar) in this retrospective study. The result of study is illustrated in table 2. The ODD ratio of no PCV2 breeder vaccination to the viremia incidence in breeder and piglets to finishers are 0.3168 (95% CI: 0.185-0.543) and 3.5741 (95% CI: 2.584-4.951), respectively. Both results are statistical significance, P<0.001.

Conclusions and discussion
According to the result of this study, PCV2 breeder vaccination provides the reduced incidence of viremia of fattening pigs at 2-24 WOA, in other words, no PCV2 vaccination in breeders causes more risk of pig viremia at 3.571 times than pigs from vaccinated breeders (OR=3.571). However, the viremia incidence of breeder themselves has not improved by the vaccination, only (OR=0.317). In conclusions, PCV2 vaccination in breeders (sows and boars) is important for the prevention of PCV2 viremia in fattening herds from this retrospective study.

References
1. Cybulski et al., 2020, Agriculture, 10, 639.
2. Guo et al., 2022, Viruses, 14, 2005.
3. Lopez-Rodriguez et al, 2016, Can Vet J, 57:619-628.

4. Opriessnig et al., 2009, Vaccine, 27 (7):1002-7.
5. Plequezuelos et al., 2021, Porcine Health Management, 7:35.

Table 1

Table 1. Distribution of breeders in the study

Breeder number	Boars (pigs)	Sows (pigs)	Total (pigs)
Thailand	104	638	742
Cambodia	5	45	50
Indonesia	5	25	30
Malaysia	1	12	13
Total (pigs)	115	720	835

Table 2

Table 2. Risk of viremia in breeder and piglets to finishers by ODD Ratio

Breeder Vaccine	Breeder (pigs)		Piglets to finisher (pigs)	
	Viremia	No viremia	Viremia	No viremia
No vaccination	21	458	404	75
Vaccination	45	311	214	142
ODD Ratio	0.317		3.571	
95% CI*	0.185-0.543		2.584-4.951	
Fisher's Exact Test**	*P* <0.001		*P* <0.001	

*Conficence interval; **Chi-square

P-V-089

SAFETY EVALUATION OF A MRNA PED VACCINE IN THAILAND

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Background
Porcine epidemic diarrhea virus (PEDV) was first recognized in 1977. The PEDV genome contains the gene for the spike (S) protein(180–220 kDa). The PEDV spike (S) protein is a viral glycoprotein responsible for receptor binding and fusion of host cell receptors, which plays a critical role in the early steps of infection. S protein is the primary immunogen due to its multiple neutralizing epitopes, the major target of neutralizing antibodies. PED outbreaks were first reported in Thailand in 2007 and this disease has since become endemic with production losses caused by piglet mortality and morbidity . Recently, an alphavirus replicon vaccine has become available in Thailand. The replicon forms the basis of the Sequivity®RNA Particle vaccine platform which is currently licensed in the US for multiple swine applications. In this system, the foreign gene of interest, in this case, PEDV S, is inserted in place of VEEV structural genes generating a self-amplifying RNA capable of expressing the gene of interest upon introduction into cells. The self-amplifying replicon RNA directs the translation of large amounts of protein in transfected cells, reaching levels as high as 15–20% of total cell protein. As the replicon RNA does not contain any of the VEEV structural genes, the RNA is propagation-defective. The replicon RNA can be packaged into replicon particles (RP) by supplying the VEEV structural genes in trans in the form of promoter-less capsid and glycoprotein helper RNAs and, when the helper and replicon RNAs are combined and co-transfected into cells, the replicon RNA is efficiently packaged into single-cycle, propagation-defective RP which are used in the vaccine formulation. RP vaccines induce both innate and adaptive immune responses including virus neutralizing antibodies and T cell responses.

Materials & Methods
50 sows in the 3rd trimester of gestation were vaccinated with Sequivity® PED Animals were vaccinated in the left neck and rectal temperatures of 20 individual animals were measured at 0h, 3h and 24h post vaccination. All animals were further observed for a period of 1 week post vaccination.

Results
In the vaccinated animals body temperature rise at 3h and 24h post vaccination was not significantly above the baseline (0h). Results were analysed using Student's t-test. Cut-off values of $p<0.05$ were deemed as statistically significant. All results are summarized in Table 1. No adverse clinical events such as feed refusal, discomfort or abortions were noted after vaccination during the 1 week observation period.

Conclusion
The data demonstrates that Sequivity® PED is a safe choice for prevention of PED related losses for swine operations in Thailand.

References
1. Langereis, M.A., Albulescu, I.C., Stammen-Vogelzangs, J. et al. An alphavirus replicon-based vaccine expressing a stabilized Spike antigen induces protective immunity

and prevents transmission of SARS-CoV-2 between cats. npj Vaccines 6, 122 (2021). https://doi.org/10.1038/s41541-021-00390-9

2. Mogler, M.A., Gander, J., Harris, D.L., 2014. Development of an alphavirus RNA particle-based vaccine against porcine epidemic diarhea virus. Proceedings of the Annual Meeting of the American Association of Swine Practitioners. Vol. 45, 63-64.

3. Mogler, M.A., Gander, J.F., Ray, D.D., Harris, D.L., 2015. Evaluation of PED RNA vaccine efficacy in dam vaccination, suckling piglet challenge model. Proceedings of the Annual Meeting of the American Association of Swine Practitioners. Vol. 46, 257-258

4. Sawattrakool K, Stott CJ, Bandalaria-Marca RD, Srijangwad A, Palabrica DJ, Nilubol D. Field trials evaluating the efficacy of porcine epidemic diarrhea vaccine, RNA (Harrisvaccine) in the Philippines. Trop Anim Health Prod. 2020 Sep;52(5):2743-2747. doi: 10.1007/s11250-020-02270-1. Epub 2020 Apr 11. PMID: 32279241.

Table 1

Table 1. Average body temperature post vaccination (n=20, ºC)

Time	Sequivity® PED
	Vaccinated with Sequivity® PED
0h	37.06^{a}
3h	37.57^{a}
24h	37.11^{a}

*a, b: values with different superscripts within a row are statistically significantly different ($p<0.05$).

P-V-090

SERO- AND VIRAL-DYNAMICS ANALYSIS OF PORCINE CIRCOVIRUS TYPE 2 IN THE FIELD

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Introduction:
Porcine circovirus type 2 (PCV-2) is associated with a number of diseases and syndromes, collectively called porcine circovirus associated disease (PCVAD). PCV-2 is considered to be one of the most important viral pathogens of pigs, and PCVAD is a major viral swine disease worldwide. Several commercial PCV2 vaccines have been used in controlling PCVAD in many countries that significantly increase the average daily weight gain, and antibody levels, reduce virus load and mortality, showing good protective efficacy. Several studies have indicated that vaccination in face of high maternally derived antibody (MDA) levels may affect such efficacy.

Materials and Methods:
The objective of this study is to analyze sero- and vral- dynamics of PCV2 after PCV2 immunization and to further understanding the situation of PCV2 infection pigs in the field. The second goal of this study is to investigate the correlation between the level of MDA and the efficacy of vaccination against PCV2. Serum samples from 7 PCV2-contaminated pig herds sent to the Animal Disease Diagnostic Center in National Pingtung University of Science and Technology from 2019 to 2021 were tested based on Enzyme-linked immunosorbent assay (ELISA) and Real-time polymerase chain reaction (Real-time PCR). In the second study, according to the antibody values of sows, their piglets were divided into three groups, low, medium, high maternally derived antibody. Antibody and viral load were compared using Wilcoxon signed-rank test and Kruskal-Wallis test.

Results:
In the first study, results showed that the ELISA S/P values started to decline since 3 weeks of age, and seroconversion during 7-11 weeks of age. PCV2 nucleic acids were also detected during that time Comparing the S/P values of piglets between two groups of low and high S/P values of sows, it can be found that the group of low maternal-derived antibody in the F farm has a trend that antibody level increase after 3 weeks of age when vaccinated at 2 weeks of age, which was different from other groups (figure 1).

Conclusions:
Pigs vaccinated with low MDA showed an earlier seroconversion than the others with high MDA.

Acknowledgement:
This work was supported by the Animal Disease Diagnostic Center, College of Veterinary Medicine, National Pingtung University of Science and Technology, Pingtung, Taiwan.

References:
[1] Dvorak CM, Yang Y, Haley C, Sharma N, Murtaugh MP. National reduction in porcine circovirus type 2 prevalence following introduction of vaccination. Veterinary Microbiology 189: 86-90, 2016.

[2] Kristensen CS, Baadsgaard NP, Toft N. A meta-analysis comparing the effect of PCV2 vaccines on average daily weight gain and mortality rate in pigs from weaning to slaughter. Preventive Veterinary Medicine 98: 250-258, 2011.
[3] Lin CN, Ke NJ, Chiou MT. Cross-Sectional Study on the Sero- and Viral Dynamics of Porcine Circovirus Type 2 in the Field. Vaccines 8: 339, 2020.

Graphs or Images 1

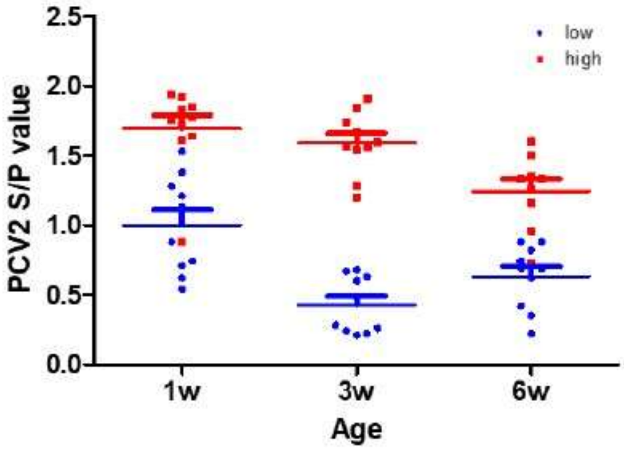

Figure 1. PCV2 S/P value between the groups of low and high maternally derived antibody at different ages in farm F.

P-V-091

SEROEPIDEMIOLOGICAL SURVEY OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS PREVALENCE PATTERN IN MALAYSIAN COMMERCIAL SWINE FARMS

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Introduction
Porcine reproductive and respiratory syndrome virus (PRRSV) infection, characterized by reproductive failure in sows and respiratory problems in growing pigs, is one of the most economically important disease in pig industry worldwide. In Malaysia, high farm seroprevalence of 81.25% to 89.2% had been reported over the years (1,2,3). Both PRRSV type I and type II genotypes had been reported in Malaysia (3).

Materials and Methods
Serum samples were collected from 36 Malaysian commercial pig farms in the year 2021 as part of routine herd health monitoring program. The 36 farms were 200 – 2,000 sows in size; adopting husbandry practices typical of pig production in Malaysia. For a comprehensive representation of the pig herds, five to ten serum samples from each age group (three, six, nine, 12, 16, 20, 24 weeks of age) were processed as pooled sample. Serum viremia titer and PRRSV genotype were determined by semi-quantitative qPCR (Intervet International B.V., Netherlands). Positive and negative samples were defined as a quantification cycle (Cq) value of ≤ 40 and > 40.

Results
In year 2021, Malaysian commercial swine farms showed the highest farm prevalence recorded: 97.2% (35/36 farms). Both PRRSV type I and type II genotypes were detected to be circulating in the local commercial pig population. Mapping the prevalence pattern across productive lifespan of pigs from suckling to finisher stage, we can see that PRRSV detection with concerning Cq values of <30 were clustered from age groups of three weeks to 16 weeks old (Figure 1).
Zooming into the production age groups, prevalence of PRRSV spiked drastically from 25% in suckling stage to 89.7% in nursery stage. It then decreased gradually from 80.6% to 41.7% in grower stage, and dropped sharply to 6.3% in finisher stage (Table 1).
Co-infection of PRRSV type I and type II occurred in age groups six to 16 weeks old, with the highest rate of 44.8% (13/29) recorded at weaners of nine weeks old.

Discussion & Conclusion
PRRS viral load in serum has been shown to be correlated with the severity of PRRSV disease manifestation (4,5). Seroepidemiological pattern of PRRS in Malaysia mainly concerns PRRS viremia detected in young pigs as early as three weeks old and continuing into nursery pigs of six and nine weeks with severe viremia of Cq value below 25. This disease pattern may be mitigated by practicing early PRRS vaccination for two weeks old piglets. PRRS viremia usually resolves by six weeks post infection (6,7). Hence, we postulate the PRRSV circulating in finisher herds of 20 and 24 weeks is not a continuity from nursery PRRS infection. This could reflect a breach of biosecurity, possibly by transport lorries collecting pigs for slaughter. Strict biosecurity steps currently in place for African Swine Fever prevention could be adapted for PRRS management.

References
1. Jasbir, S. et al., 2008. EMPRES Transboundary Animal Diseases Bulletin 30:38-39.
2. Vania, K. T. L. et al., 2012. 24th Vet Assoc Malaysia Congress 2012, Marriot Putrajaya.
3. Jaganathan, S. et al., 2013. In Ho Chi Minh: The 6th Asian Pig Veterinary Society Congress (pp. 23-25).
4. Murtaugh, M. P. et al., 2011. Vaccine 29(46), 8192-8204.
5. Han, K. et al, 2013. Vet 195(3), 313-318.
6. Wang, H. et al., 2021. Vaccines, 9(5), 480.
7. Wills, R. et al., 2003. J Clin Microbiol, 41(1), 58-62.

Figure 1

Figure 1. PRRSV prevalence pattern across age groups with regards to genotype and Cq value detected

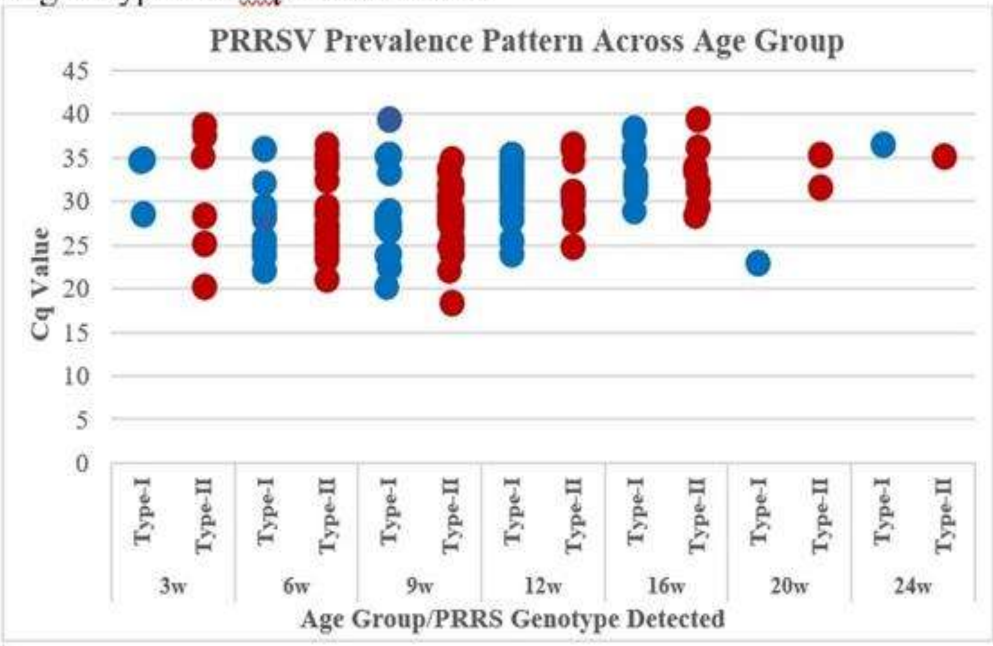

Table 1

Table 1. Farm prevalence and genotypic distribution of PRRSV over pig production lifetime.

Age Group	Farm Prevalence	PRRS Genotype		
		Type-I	Type-II	Co-infection
3 weeks	25.0% (9/36)	33.3% (3/9)	66.7% (6/9)	0%
6 weeks	89.7% (26/29)	7.7% (2/26)	65.4% (17/26)	26.9% (7/26)
9 weeks	80.6% (29/36)	13.8% (4/29)	41.4% (12/29)	44.8% (13/29)
12 weeks	77.8% (28/36)	57.1% (16/28)	21.4% (6/28)	21.4% (6/28)
16 weeks	41.7% (15/36)	40.0% (6/15)	26.7% (4/15)	33.3% (5/15)
20 weeks	8.3% (3/36)	33.3% (1/3)	66.7% (2/3)	0%
24 weeks	6.3% (2/32)	50.0% (1/2)	50.0% (1/2)	0%

P-V-092

SPATIO-TEMPORAL DYNAMICS OF PORCINE CIRCOVIRUS TYPE 2 IN GROW-FINISH PIG HERDS INOCULATED WITH PCV2 VACCINES IN CHINA

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Introduction:
Porcine circovirus associated disease (PCVAD) is initiated by porcine circovirus type 2 (PCV2). PCV2 has been damaging the pig industry worldwide, and was first reported in China in the late 1990's. Since the use of PCV2 vaccines, the clinical presentation of PCV2 have been reduced significantly in China [1-2]. Our study objective was to provide an update of the spatio-temporal dynamics of PCV2 following its existing vaccination programs in grow-finish pigs by evaluating PCV2 viremia based on quantitative polymerase chain reaction (qPCR) technique.

Materials and methods:
950 sera samples from 31 intensive grow-finish herds inoculated with PCV2 vaccines over 26 provinces in China were analyzed from May 2021 to July 2021. PCV2 vaccines used were international and local brands and applied at around 2-3 weeks of age. These samples were randomly collected from PCVAD suspected grow-finish pigs at 6, 8, 10, 12-14, 16-18, and above 20 weeks of age. All samples were tested for PCV2 viremia by qPCR, which were performed in the Zoetis swine disease diagnosis laboratory (Beijing). The qPCR positive rates were calculated according to samples collected in herds and production stages. Based on their PCV2 viral loads (log_{10} genome equivalent copies/mL), they were individually categorized into five groups: [i] PCV2 negative, [ii] Low (less than $2log_{10}$), [iii] Medium ($2\text{-}4.3log_{10}$); [iv] High ($4.3\text{-}5.4log_{10}$) and [v] Very High (more than $5.4log_{10}$). The percentages of each quantified PCV2 category by productions stage were also tabulated.

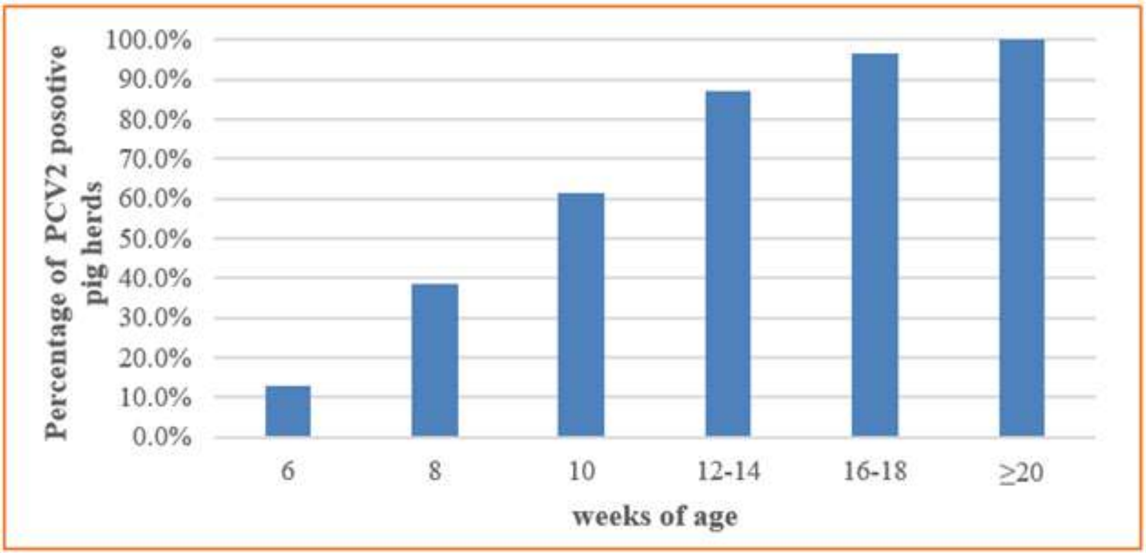
Results:
PCV2 positive rate for herds was low at 6 and 8 weeks of age (12.9% and 38.7% respectively), but steeply increased to 61.3%, 87.1% and 96.8% at 10, 12-14 and 16-18 weeks of age, respectively, and then reached 100% till the end of production cycle (Figure 1). The sera samples categorized by virus load were either free from and low PCV2 viral load predominantly at 6, 8 and 10 weeks of age with 94.6%, 99% and 78.6%, respectively, then dramatically dropped to 55.2% at 12-14 weeks of age, then kept steadily around this level afterwards (Figure 2). Interestingly, samples collected from pigs above 12 weeks of age were predominantly belonged to category 3 of PCV2 viral load (medium), and the proportion of category 4 and 5 of PCV2 viral loads were seen at 12-14, 16-18 and more than 20 weeks of age with 13.6%, 12.7% and 7.5%, respectively (Figure 2).

Conclusions and discussion:
This study showed that despite PCV2 vaccination in grow-finish pigs was widely implemented in China, all herds enrolled in this study were naturally infected with PCV2, but the most impactful PCV2 infection might appear on pigs older than 10 weeks of age, especially beyond 12-18 weeks of age. It is necessary to take actions to improve the effectiveness of vaccination in order to reduce the impact of PCV2 infection. Phylogenetic

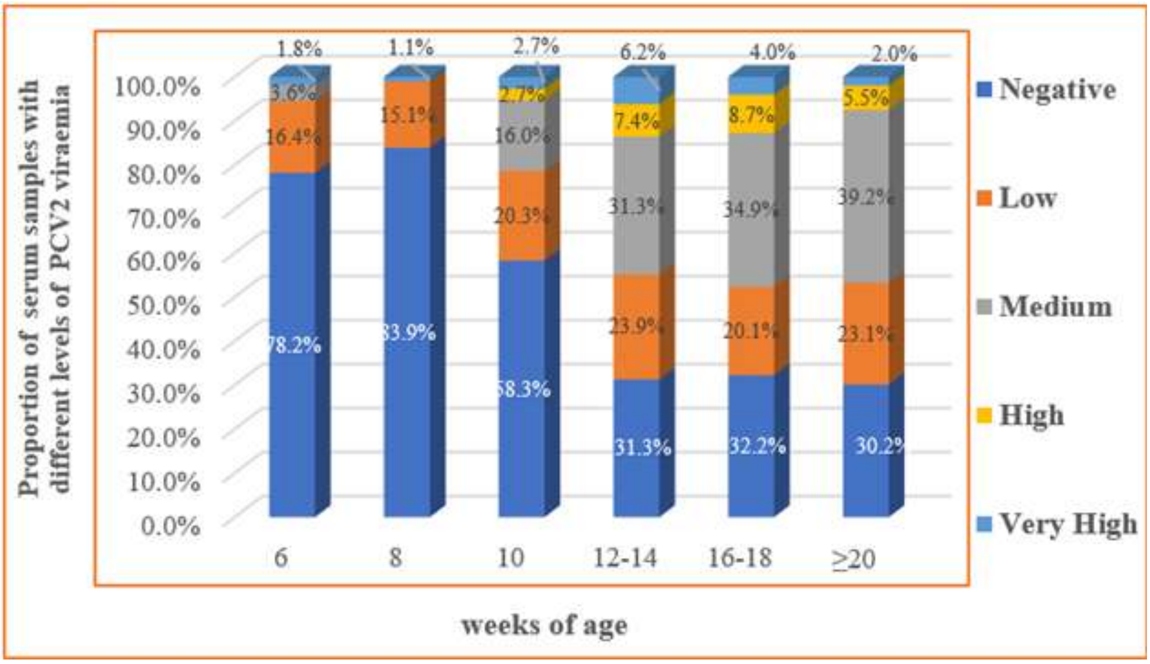
analysis of these PCV2 isolates based on the ORF2 sequence would be meaningful in understanding which are predominant circulating genotypes in pig herds.

References:
[1] Kewen Wang, et al., IPVS 2020 Proceedings, P749
[2] Yun-Hee Noh, et al., Vaccines. 2022, 10, 1469

Graphs or Images 1



Graphs or Images 2



P-V-093

STABILIZATION OF A SOW HERD IN A TAIWANESE FARM ENDEMICALLY INFECTED WITH PRRSV2 AFTER MASS VACCINATION WITH A PRRSV1 MLV

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Introduction
Currently, Porcine reproductive and respiratory syndrome type-1 (PRRSV1) and PRRS type-2 (PRRSV2) modified-live vaccine (MLV) are commercially available in Taiwan, where most of pig productions are infected with PRRSV2 belonged to lineages Ⅰ and Ⅵ to Ⅸ (1).
Minimization of the impact of the disease is a primary objective and vaccination with MLV vaccines has been reported to achieve farm stabilization more quickly than other methods (2), a farm with stable PRRSV status is able to achieve an increase of 1.28 weaned piglets per sow per year if PRRSV stability is maintained for a one-year period (3).
This study aimed to evaluate the efficacy of using PRRSV1 MLV to improve the stability of a sow herd in a Taiwanese pig farm endemically infected with PRRSV2.

Materials and methods
From December 2021 to January 2022, a 3000 sow-farrow-to-finish farm with over 2 years PRRSV1 MLV (UNISTRAIN® PRRS, HIPRA) vaccination history (60-day pregnant in cycle vaccination), suffered a new PRRS outbreak, this resulted in 20% abortion and a decrease in the number of weaned piglets.
Sequences of ORF5 gene were done to check the homology between the PRRS virus circulating in the farm during the outbreak and the current commercial vaccine strain.
Mass vaccination with the current PRRSV1 MLV in the sow herd was done in January 2022.
Vaccine efficacy in PRRS control was evaluated on the basis of these criteria: viremia-positive rate in weaned piglet and reproductive performance (average number of weaned piglets and farrowing rate).

Results
Sow herd PRRS stability improved at 68 days after PRRSV1 MLV mass vaccination, from positive unstable to positive stable status due to 100% of monthly samples from the herd tested negative (Fig.1).
The reproductive parameters were back to baseline performance in 3 months, the farrowing rate and the average number of weaned piglets improved from 73.8% to 81.2% and from 10.7 to 11.5 respectively (Table.1).
The results of complete ORF5 gene phylogeny (Fig.2) of PRRS virus isolates which were circulating during the epidemic in this production belonged to the PRRSV2, lineage Ⅲ. The strains divergence between these isolates ranged from 0.0 to 2.7%, whereas comparing with commercial MLV strains VR2332 and UNISTRAIN® PRRS were 21.0 to 21.7% and 49.7 to 51.6% respectively.

Fig.1 Positive rate of PRRS virus viremia in weaned piglets

Table 1. Reproductive performance

Fig 2. Sequencing and phylogenic analysis

Discussion and Conclusion

This study concluded that PRRSV1 MLV was effective against PRRSV2 field infection due to the achivement of sow herd stability and an improvement of the reproductive performance. For the sequencing and phylogenic analysis, the PRRSV2 which was circulating in this farm belongs to lineage Ⅲ, which is different from the results of the molecular epidemiology survey published in 2015 (1); however, PRRSV1 MLV conferred a solid heterologous protection with scientific evidence.

It was noteworthy that the time to stability (TTS) and the time to baseline production (TTBP) in this study was 68 days and less than 3 months, remarkably shorter than the known scientific publications either using PRRSV1 or PRRSV2 MLV in Taiwan, in which TTS was 3 and 9 months respectively (4,5).

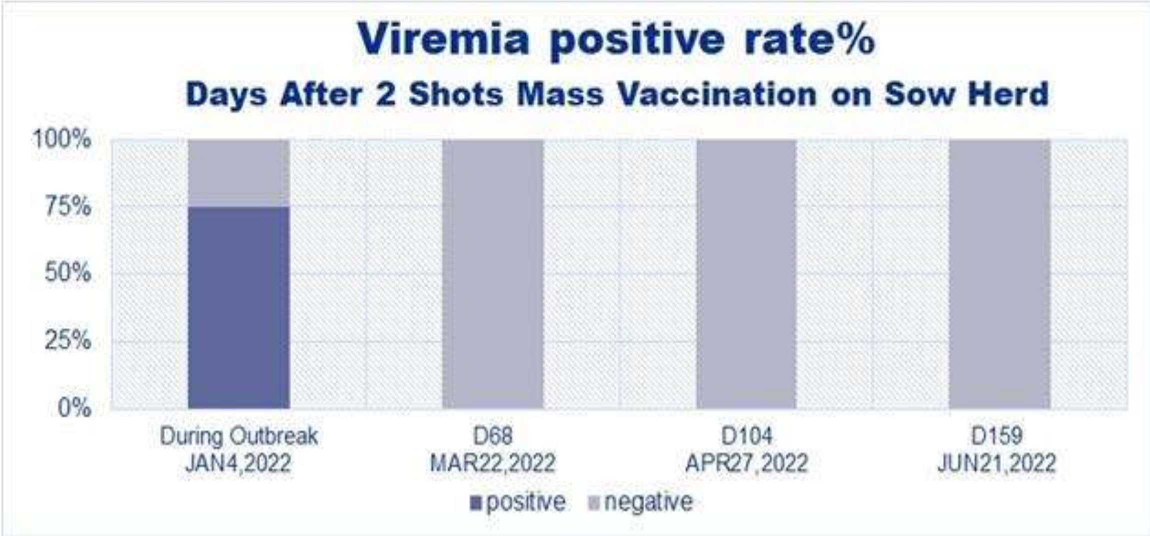
Reference

1. M.C. Deng, et al., 2015. Arch Virol 160: 2709-2718.
2. Linhares et al., 2014. Preventive veterinary medicine. 116.10.1016/j.prevetmed.2014.05.01
3. Torrents et al. 2021. Porcine Health Management, 7:21
4. Y.H. Yiu, et al., 2019. Comparison of the field efficacy of commercial porcine reproductive and respiratory syndrome virus (PRRSV)-1 and PRRSV-2 vaccines against prrsv-2 in Taiwan. APVS proceedings.
5. H.C. Kuo, et al., 2017. Evaluate the efficacy of PRRS MLV mass vaccination on sow herd in a PRRS unstable farm in Taiwan. APVS proceedings.

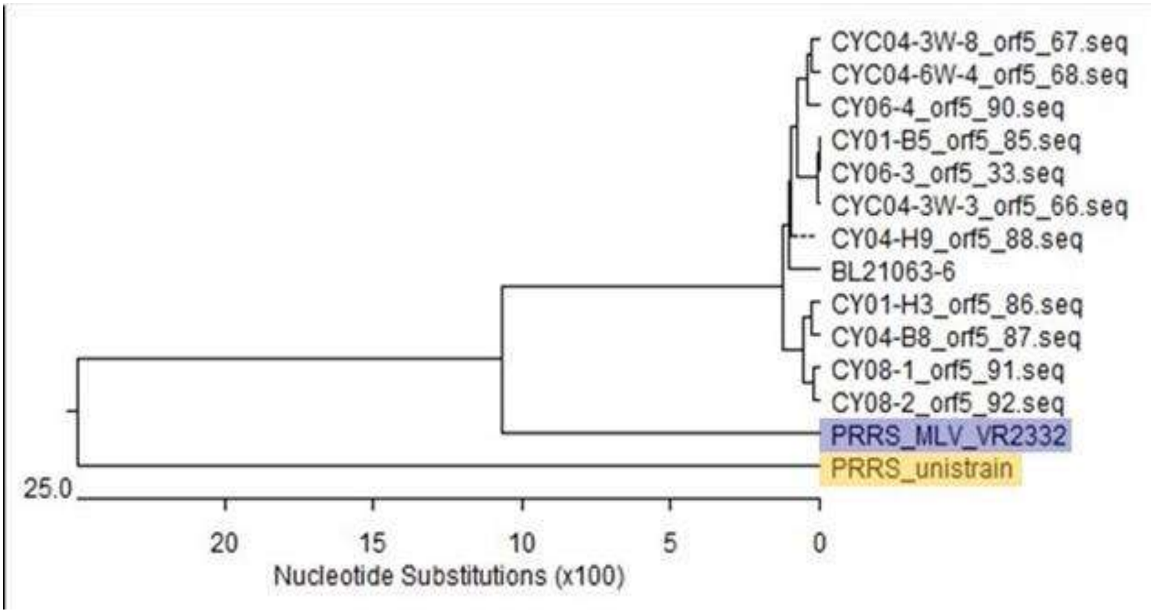
Table 1

	Before Epidemic (JAN-NOV 2021)	During outbreak (DEC 2021-FEB 2022)	After outbreak (MAR-JUL 2022)
Farrowing rate	84.8%	73.8%	81.2%
Avg. number of weaned piglet	12.2	10.7	11.5

Graphs or Images 1



Graphs or Images 2



P-V-094

SURVEILLANCE OF CLASSICAL SWINE FEVER VIRUS ACTIVITY IN A PIG FARM WHICH CONVERTS LPC VACCINE TO E2 SUBUNIT VACCINE THROUGH ERNS ANTIBODY DETECTION

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- Introduction

Classical swine fever (CSF) is an important infectious swine disease. In recent years, Taiwan has been planning to eradicate classical swine fever virus in order to become a CSF free country in the future. Lapinized Philippines Coronel (LPC) has been utilized as the major and efficacious vaccination strategy against CSF in Taiwan for a long period. For differentiating CSF infected from vaccinated animals, E2 subunit vaccine and Erns enzyme-linked immunosorbent assay (ELISA) were developed. Therefore, this experiment aimed to monitor the activity of classical swine fever virus in pig farm which vaccinated with E2 subunit vaccine through detecting Erns antibody.

- Materials and Methods

The selected pig farm was a three-week batch farrowing commercial pig farm, whose CSF immunization program was changed from LPC to E2 subunit vaccine. The serum samples of sows, 3, 6, 9, 12, 18 and 24 week-old pigs were collected for each batch, with a total of three batches. A ELISA test kit (pigtype CSFV Erns Ab, Qiagen, Leipzig, Germany) was used to detect Erns antibody of serum samples. The other ELISA test kit (IDEXX CSFV Ab Test, IDEXX Laboratories, Inc, Westbrook, Maire, United States) was used to detect E2 antibody of serum samples.

- Results

The results showed that the Erns antibody positive rate of three batch sows were 30 %, 20% and 10%, respectively. Twelve-week-old pigs in the second and third batch also showed 5 % Erns antibody positive rates. The results showed that the E2 antibody mean Blocking% value of each batch piglets all gradually decreased over time. The Blocking% value of some 18 and 24 week-old pigs of the third batch were above 80%. However, no clinical sign of classical swine fever was found in the pigs during the experiment.

- Conclusions

Detection of Erns antibody is available for CSF virus surveillance, but it may have a limit on early infected animals. The pig farm still has sporadic signs of suspected circulating LPC vaccine strain activity within three batches after vaccine conversion. Further research is needed for how long the LPC strain will keep circulating in field and whether it will pose a potential risk.

- Acknowledgement

We gratefully acknowledge the technical and veterinarian in ADDC of NPUST for their contributions to this study.

- References

[1] Aebischer A et al., 2013. Vet. Microbiol. 161, 274–285.

[2] de Smit AJ et al., 2000. Vet. Q.22, 182–188.
[3] Fan J et al., 2021. Microorganisms. 9, 761.

Table 1

Table 1. S/P ratio and positive rate of E^{rns} antibody in sows and piglets between different batches.

Age	Batch 1	Batch 2	Batch 3
Sow	0.43 ± 0.67 (30)	0.28 ± 0.44 (20)	0.26 ± 0.24 (10)
3w	0.06 ± 0.09 (0)	0.03 ± 0.06 (0)	0.02 ± 0.05 (0)
6w	0.02 ± 0.04 (0)	0.00 ± 0.01 (0)	0.00 (0)
9w	0.00 ± 0.01 (0)	0.02 ± 0.11 (0)	0.003 (0)
12w	0.00(0)	0.01 ± 0.39 (5)	0.09 ± 0.42 (5)
18w	-*	0.00 (0)	0.00 (0)
24w	-*	0.00 (0)	0.00 (0)

Data is presented as S/P ratio ± standard deviation (positive rate).
*Serum samples of 18-week-old and 24-week-old piglets in batch 1 were not collected because they were housed together with former batch.

P-V-095

SURVEILLANCE STUDY OF PRRS STATUS IN UNVACCINATED PORKER HERD IN SEVERAL FARMS THROUGHOUT MALAYSIA

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[1]Boehringer Ingelheim (Malaysia), [2]Agritech Enterprise Sdn. Bhd., [3]Universiti Putra Malaysia

Introduction:
Porcine reproductive and respiratory syndrome (PRRS) causes significant economic losses in the swine industry worldwide. The PRRS virus (PRRSV) can be divided into two strains, Type I (European) and Type II (North American) (1). PRRSV not only affects the reproductive performance of sows and boars, it also causes respiratory disease
in wean-to-finish pigs. As pigs are often raised in areas of high density, the spread of infection is difficult to control (2) The objective of this study is to evaluate the PRRS status of unvaccinated porker herds in several farms in Malaysia during the year 2021.

Material and Methods:
A total of 30 farms were selected, where the porkers are apparently healthy and unvaccinated. The farms involved were from different states in Malaysia which includes Penang, Perak, Selangor, Johor, Sarawak and Sabah. 21 out of 30 of these farms has PRRS vaccination program only in the breeder herd, whereas 9 other remaining farms vaccinate neither the porker nor breeder herd. A total of 1157 sera were collected from these farms for the seroprevalence study. The sera include ages from 4 weeks old, 8 weeks old, 12 weeks old, 16 weeks old and 20 weeks old porker. Each age group consist of 3-5 sera, pooled together and tested for PRRSV via nested-PCR that enabled the differentiation of Type I and Type II PRRSV.

Results:
Preliminary findings in Pie Chart 1 showed that all unvaccinated porkers in all 30 farms were tested seropositive. 70% of the farms had heterologous infection of both American and European strains and 30 % the farms had only US strain circulating the porker.

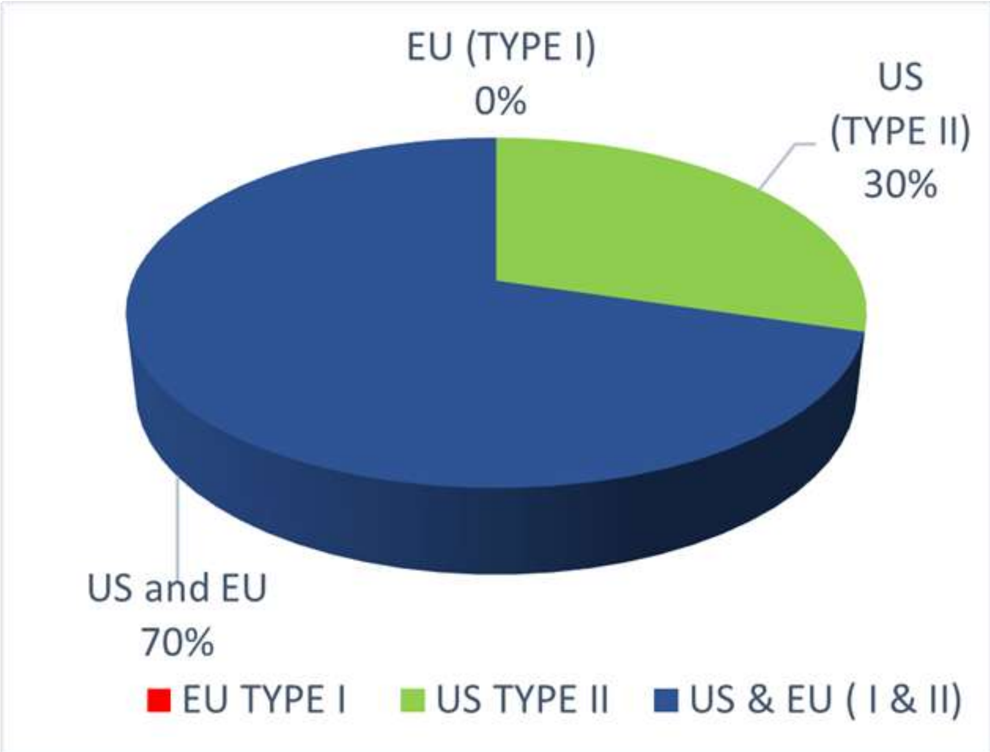
Image 1

A further look into the PRRS status of unvaccinated porker in a vaccinated breeder herd shows that 85.71% of the porker were infected with PRRSV (PCR positive) as early as 4 weeks old. At 8 weeks old, 100% of the farms were positive with PRRSV.

Discussion and Conclusion:
From this study, Type II PRRS is still predominant throughout most parts of Malaysia. The increase of genetic diversity of PRRSV threatens herd immunity which pose a challenge to the industry. Therefore, having vaccines that provide heterologous protection is essential (3,5) To date, there has been no report of highly pathogenic PRRSV in Malaysia. In addition, this study also shows that vaccination in breeder herd alone is insufficient to give a complete protection to the porker herd. In pig dense areas, breeder vaccination alone is insufficient to curb the disease. Therefore whole-herd vaccination against the virus' devastating effects is a sustainable way to control the infections and negative effects on production. Another study also shows that the financial benefits were highest if both sows and piglets (MSP) were vaccinated, with a median annual net benefits per working sow ranged from €170 to 340. (4)

References:
[1] Nelsen C. J. et al. 1999. J Virol 73:270-280.
[2] Kiu VTL et al., 2012. Characterisation of Porcine Reproductive and Respiratory Syndrome Virus
Strains in Selected Farms in Malaysia.
[3] Website:
https://www.prrs.com/disease-control/virus/prevalence-and-strains-prrs-disease
[4] Thomann, B., et al, 2020. Frontiers in Veterinary Science, 7.
doi:10.3389/fvets.2020.00500.
[5] Murtaugh M.P. et al Res. 2010;154: 18–30. 10.1016/j.virusres.2010.08.015

Graphs or Images 1



P-V-096

SWINE INFLUEZA VIRUS INFECTION IN KOREAN COMMERCIAL PIG FARMS BETWEEN 2021 AND 2022 AND SWINE INFLUENZA CONTROL VIA VACCINATION

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- Introduction

In Korea, the heat stress extremely increases in the summer, the hottest period of the year, resulting weight loss of weaning piglets caused by the insufficient breast milk and lactogenic immunity. It normally caused great damages to farm productivity in the second half of the year. In particular, pigs born between June and August had an average of 200 days of marketed age and the medication cost doubled due to continuous respiratory symptoms. The breeding rate for the finishing pigs also decreased by 3-7% compared to that of the first half. Cases of SIV (pandemic H1N1) infection and damages were confirmed at 7 customer farms of Smart Pig Clinic in 2021 and those status described above were observed in 6 out of the 7 farms above and then eased in February 2022. This study evaluated the efficacy of SIV vaccine (SuiShot® Flu-3) in order to control SIV infection and improve farm productivity.

- Materials and Methods

In one of the above farms, SuiShot® Flu-3 was applied to the breeding herds between May and June 2022 before the heat wave hit the farms. After 2-time mass vaccination, the antibody changes between the vaccinated farm and the unvaccinated farm were compared. Changes in clinical symptoms and mortality rate were also observed.

- Results

According to the monitoring of antibody positive rates against SIV in Farm A and Farm B since 2019, both farms were already infected with SIV. The positive rate at 30 days old was low between 0% and 50%, but it was rapidly increased up to 100% after 70 days old. The low positive rate at 30 days old could mean that the level of maternal antibodies was very diverse and had a large deviation. The rapid increase in 70 days old could be interpreted to mean that 50 to 70-day-old pigs were infected with SIV circulating in the farm. In Farm A, the antibody positive rate at 30 days old reached 100% after 2-time injection of SuiShot® Flu-3 on the breeding herds at intervals of 3 weeks in June 2022 and then it was gradually decreased after 70 days old. After that, there was a sharp increase at 130 days old after SIV infection. In Farm B, the antibody positive rate in October 2022 was similar to the past because the breeding herds were not vaccinated in June. A large difference in the positive rate was shown between Farm A and Farm B's 30-day-old pigs. It was speculated that a high level of maternal antibodies was transferred through SIV mass vaccination (Fig. 1).
In Farm A, the usual number of sows and the average monthly number of weaning piglets was 2,230 heads and 5,200 heads respectively, resulting in 27.9 pigs weaned per sow per year (PSY). The number of culled and dead pigs between October and December 2022 was 493 heads and was decreased by 98 heads compared to that of the same period last year. This was the result of the reduction of porcine respiratory disease complex (PRDC) involving SIV (Fig. 2).

- Conclusions and Discussion

This study confirmed that increasing the level of maternal antibodies through vaccination on breeding herds could be a way to protect pigs in the late stage of piglets and the early stage of grower from SIV infection and PRDC. As mentioned earlier, 2-time mass vaccination against SIV is essential to the breeding herds between May and June in order to prevent SIV infection after the heat wave. The booster vaccination every six month is highly recommendable.

- Acknowledgement and References

Kristien VR. et al. 2019. Diseases of Swine 11th ed.

Graphs or Images 1

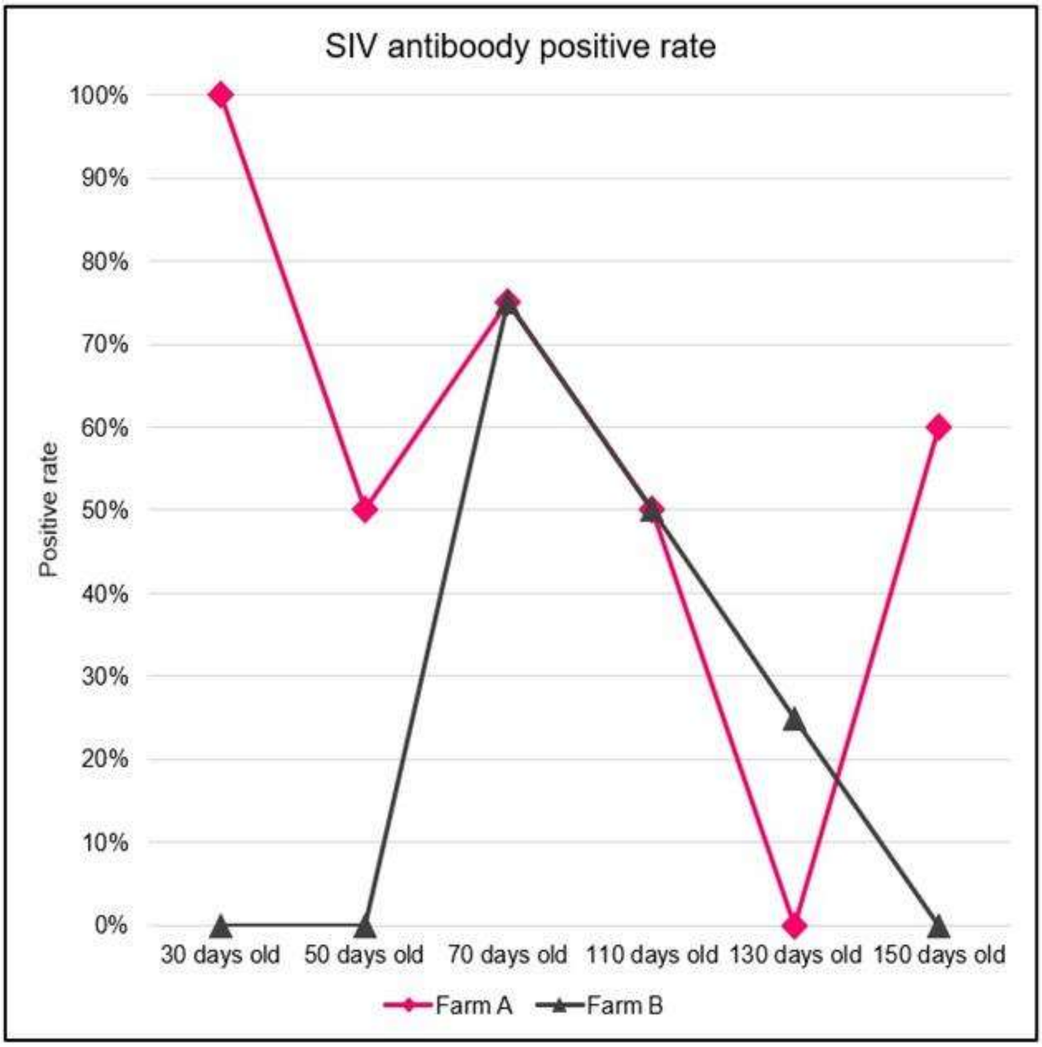

Fig. 1. SIV antibody positive rate change in the different age groups in Oct. 2022.

Graphs or Images 2

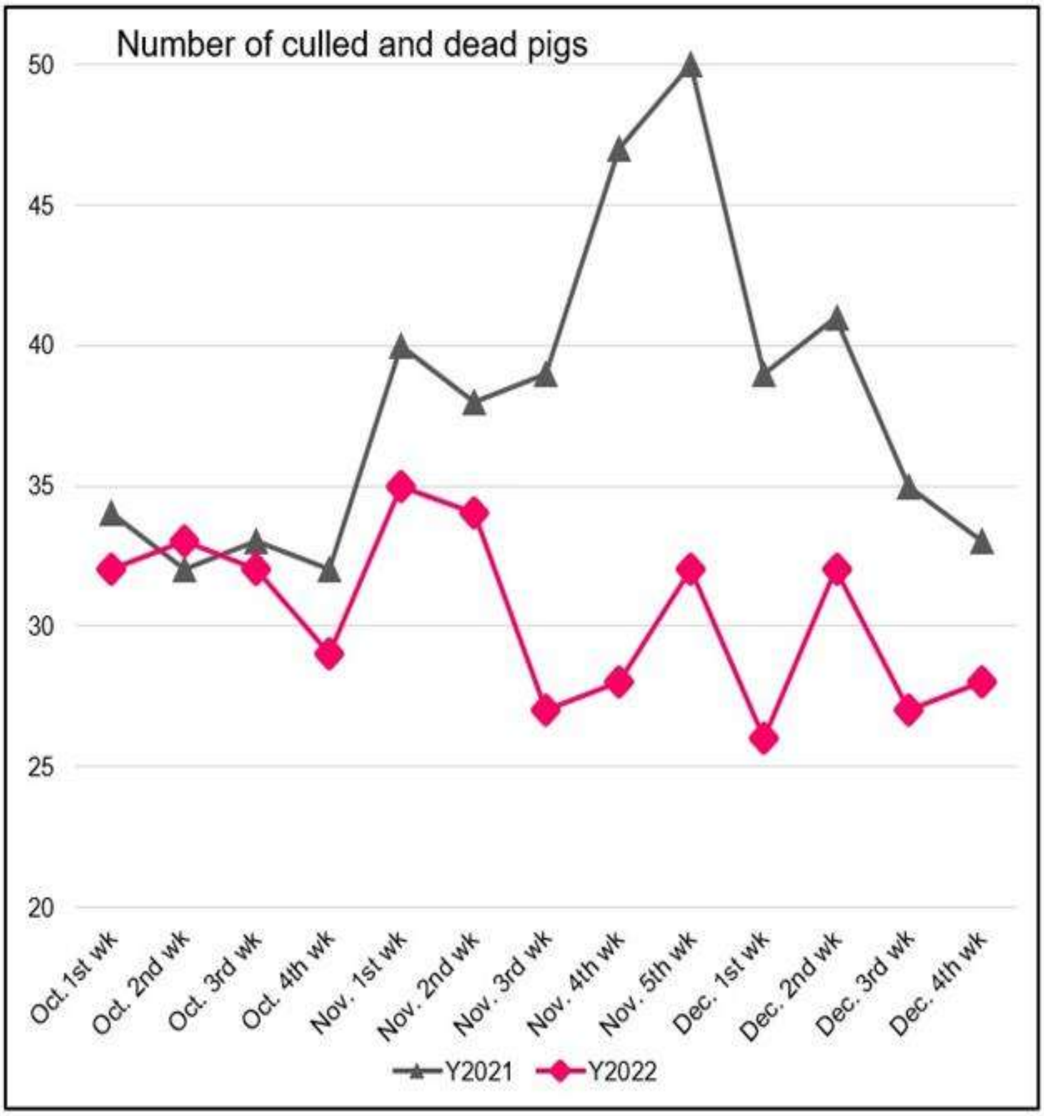

Fig. 2. Weakly number of culled and dead pigs in Farm A between Nov. and Dec. 2022.

P-V-097

T-CELL EPITOPE CONTENT COMPARISON BETWEEN COMMERCIAL VACCINES AND FIELD PORCINE CIRCOVIRUS TYPE 2 ISOLATES IN THAILAND

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Introduction

Porcine Circovirus type 2 (PCV2) may cause disease to both breeding and fattening pigs. Because of its high mutation rate and common recombination, PCV2 isolates have been clustered in 8 different genotypes, including PCV2a to PCV2h (1). Although, most studies in the last decade noted that PCV2d is becoming the predominant genotype in most global countries, the genetic variation of PCV2 should be investigated.

To protect animals from severe illness and production losses, the implementation of PCV2 vaccine in pig herds is the most common practice. However, there are different commercial PCV2 vaccine options in the market, not only monovalent PCV2 vaccines, but also a new bivalent PCV2 vaccine which contains PCV2a and PCV2b for broader protection (2).

The prediction of vaccine coverage using T-cell epitope content comparison (EpiCC) might be a useful in silico tool to assess the degree of homology between PCV2 field isolates and the PCV2 antigen in vaccines. Consequently, the most suitable vaccines would be selected for each individual farm to improve vaccine coverage and efficiency.

Materials and Methods

PCV2 suspected farm samples were sent from Central, Western and Eastern commercial herds in Thailand for PCR testing. All positive PCV2 PCR samples were submitted for whole genome sequencing. Then the similarity of PCV2 sequences were analyzed using the NCBI Basic Local Alignment Search Tool (BLAST).

Twenty-two different PCV2 sequences were evaluated for T-cell epitopes on ORF2 region using the PigMatrix epitope prediction tool (EpiVax Inc, Providence, Rhode Island, USA). Subsequently, the relatedness of T-cell epitopes between field isolates and commercial vaccines, including two monovalent PCV2a vaccines (VacAlt1 and VacAlt2) and bivalent PCV2a-PCV2b vaccine (VacAB; Fostera® Gold PCV, Zoetis, USA) was predicted using EpiCC analysis (EpiVax Inc, Providence, Rhode Island, USA).

Results

All PCV2 sequences were obtained from different regions in Thailand. Only 1 sample (4.5%) was classified as PCV2b genotype, while PCV2d genotype was the dominant, reported up to 95.4% of all sequences. The result of EpiCC indicated that bivalent PCV2a-PCV2b vaccine (VacAB) had highest EpiCC scores with % T-cell epitope coverage at 10.15 and 8.35 for PCV2b and PCV2d respectively. Meanwhile, monovalent PCV2a vaccines (VacAlt1 and VacAlt2) had lower scores ranging 6.21 – 6.91 (60.20 – 67.08%). Overall, the epitope conservation of the bivalent PCV2a-PCV2b vaccine and viral strains increased approximately 2.01 of EpiCC score and 20.57% of T-cell epitope coverage compared with monovalent PCV2a vaccines.

Conclusion and discussion

T-cell epitope content comparison (EpiCC) analyses resulted in the new bivalent PCV2a-2b vaccine having a higher score than the monovalent PCV2a vaccines. The EpiCC scores show the relatedness of T-cell epitopes between vaccine antigen (ORF2) and field isolates, therefore it could be hypothesized that the bivalent PCV2a-2b vaccine might result in higher field efficacy than monovalent PCV2a vaccines.

References
1. Link, E.K., Eddicks, M., Nan, L. et al. Discriminating the eight genotypes of the porcine circovirus type 2 with TaqMan-based real-time PCR. Virol J 18, 70 (2021).
2. Bandrick, M., Balasch, M., Heinz, A. et al. A bivalent porcine circovirus type 2 (PCV2), PCV2a-PCV2b, vaccine offers biologically superior protection compared to monovalent PCV2 vaccines. Vet Res 53, 12 (2022).

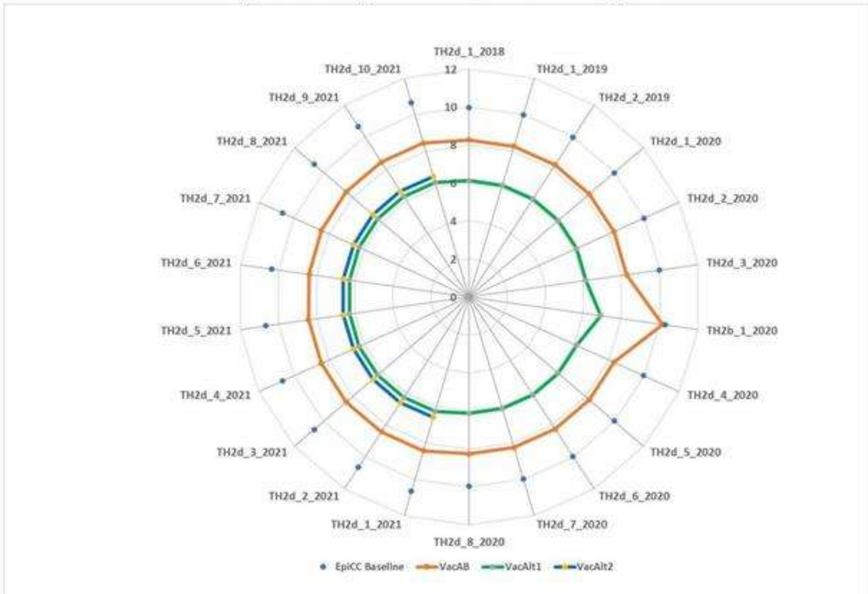
Table 1

Table 1. T cell epitope contents comparison (EpiCC) scores between commercial vaccines and field PCV2 isolates in Thailand.

PCV2 Genotype	EpiCC Baseline	EpiCC score (%T-cell epitope coverage)		
		VacAB (PCV2a-2b)	VacAlt1 (PCV2a)	VacAlt2 (PCV2a)
PCV2b	10.30	10.15 (98.56)	6.91 (67.08)	n/a
PCV2d	10.32	8.35 (81.03)	6.21 (60.20)	6.61 (62.21)
Total	**10.29**	**8.43 (81.99)**	**6.23 (60.62)**	**6.61 (62.21)**

Graphs or Images 1

Figure 1. EpiCC scores radar plot



P-V-099

THE EFFECT OF TILMICOSIN IN PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS INFECTED PIGS

Mr. Hsien-Jen Chiu[1,2], Mr. Shi-Meng Liew[1], Mr. Chao-Nan Lin[1,2], Mr. Ming-Tang Chiou[1,2]
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Introduction:
Porcine reproductive and respiratory syndrome virus (PRRSV) is a pathogen causing significant economic losses to the world swine industry. Tilmicosin is a semi-synthetic macrolide antibiotic developed from tylosin B. Previous studies related to Tilmicosin found that the drug could enter porcine alveolar macrophages (PAMs) and inhibit PRRSV replication in the cells. This study mainly evaluates the efficacy of Tilmicosin in PRRSV-infected pigs in vivo.

Materials and Methods:
In this study, 20 PRRSV-negative 28 days old pigs were selected and divided into 10 Tilmicosin group that were administered Tilmicosin (400 ppm) at the age of 32 days old until the end of the experiment, and 10 control group that were not administered Tilmicosin. Both groups were divided into 3 challenged subgroup that inoculated with the virus intranasally and 7 contact subgroup as contact infection. The efficacy of Tilmicosin administration was assessed by comparing viremia, temperature, clinical symptoms, gross lesions and histopathology. Pigs in the Tilmicosin group were given Tilmicosin, and the pigs were challenged intranasally at the age of 42 days old, and then the clinical symptoms were observed every day. Blood samples was collected for detecting viremia at 28, 42, 43, 45, 49, 52, and 57 days old.

Results:
The results showed that the viremia was no significant differences between the Tilmicosin group and the control group. The control group had more severe clinical symptoms than Tilmicosin group. The score of gross lesion and histopathology of lungs showed significant differences in Tilmicosin group compared to control group.

Conclusions:
The results of this study showed that the administration of Tilmicosin can effectively reduce the respiratory symptoms after PRRSV infection.

Acknowledgement:
We gratefully acknowledge the technician and veterinarian in ADDC of NPUST for their contributions to this study.

References:
[1] Cho JG et al., 2006. Theriogenology. 66(3), 655–662.
[2] Du Y et al., 2011. J. Antivir. Antiretrovir. 3(3), 28-33.
[3] Lin CN et al., 2015. J Agric Sci. 8(1), 154.

Graphs or Images 1

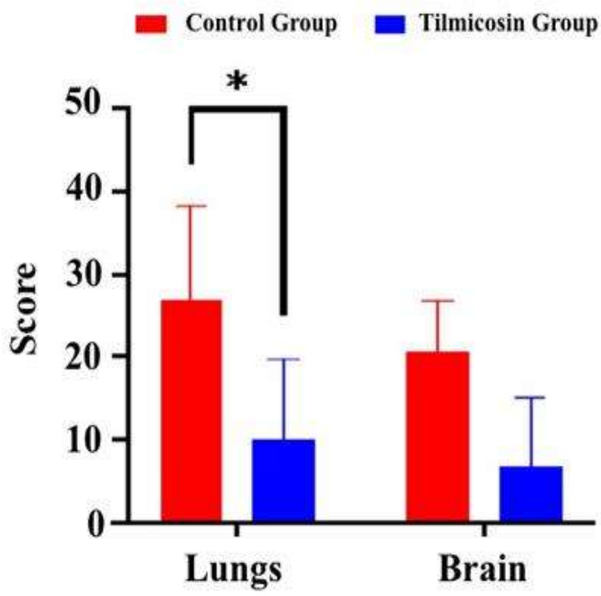


Figure 1. Histopathology score

Graphs or Images 2

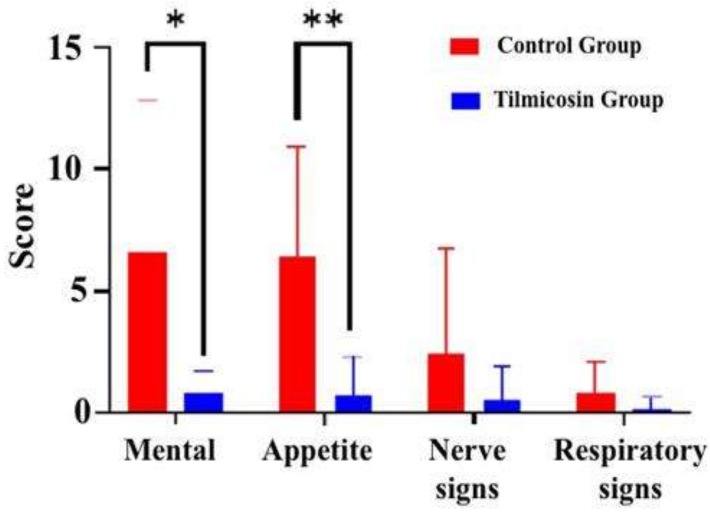


Figure 2. Clinical signs score

P-V-100

THE EVALUATION OF COMBINATION VACCINES HYOGEN® PLUS CIRCOVAC® VERSUS POSITIVE CONTROL ON GROWTH PERFORMANCES AND LUNG LESIONS UNDER FIELD CONDITIONS OF MYCOPLASMA HYOPNEUMONIAE AND PCV2 SUBCLINICAL INFECTIONS

Dr. Ye Xiong Wong, Dr. Ping Yee Saw[1], Dr. Suraphan Boonyawatana[2], Dr. Paiboon Sungnak[3], Dr. Chiou Yan Tee[4], Dr. Ye Xiong Wong[4]
[1]Ceva Animal Health Malaysia Sdn Bhd, [2]Ceva Animal Health Thailand, [3]Intercons 3P Co., Ltd, [4]Rhone Ma Malaysia Sdn Bhd

-Introduction
Porcine Circovirus type 2(PCV2) and Mycoplasma hyopneumoniae are the two major pathogens involved in porcine respiratory disease complex (PRDC), causing major economic loses. Vaccination is a major preventive measure against these diseases. This study evaluates differences in the growth performances and lung lesions between the vaccines Hyogen® and Circovac®(HC) and the ready-to-use vaccine against the PCV2 and M.hyo under field conditions.

-Materials and methods
This study was conducted in a farrow-to-finish farm with 300 sows. 700 piglets were equally divided into 2 groups. Group 1 was vaccinated with the PCV2 and Mhyo RTU vaccine, while group 2 was vaccinated with HC. Both groups were vaccinated at 3 weeks of age. Performance parameters were recorded from wean to finish period. Lung scoring was performed at slaughter using the Ceva Lung Program.
Results: From the zootechnical data, HC group showed lower mortality rate and higher total weight gains (Table 1). With the extra 540.77kg of selling weight at RM9.9/kg market price (April 2022), farmer got extra profit of RM15.30 per selling pig. The HC group had lower percentage of affected lung surface among the pneumonic lungs. EP index (calculated from the frequency and severity of EP-like lesions) was better in Group 2 compared to Group 1 (2.49 vs 2.90).

-Conclusion
The use of Hyogen® and Circovac® vaccines improved the overall performance results and lung health contributing a higher profitability to the farm compared to RTU vaccine.

-References
1. Sibila et al. Porcine Health Management (2020) 6:11.
2. Costa, W.M.T. et al. APVS 2019, IMM-020

Table 1

Table 1: Zootechnical data.

Parameters	Group 1	Hyogen® + Circovac®	Diff.
Pigs No.	350	350	
Avg. Weaning Weight, Kg	6.22	6.53	0.31
Avg. Body Weight at Sold, Kg	117.05	117.59	0.54
Mortality Rate, %	5.14%	4.00%	-1.14%
Total Weight Gains, Kg	36,682.14	37,222.91	+540.77
ADG, Kg/day	0.685	0.685	0.000

Table 2

Table 2: Enzootic pneumonia-like lesion.

Lung Lesion Score	Positive cotrol	Hyogen® + Circovac®	*P-value*
No. of Sample Lungs	49	49	
% Affected Surface/pneumonic Lung	11.19%	8.81%	*0.4845*
EP index	2.90	2.49	*0.6693*

P-V-101

THE IMPACT OF DIFFERENT COMBINATIONS OF SOW AND PIGLET VACCINATION AGAINST PCV2

Mr. Roman Krejci[1], Mateo del Pozo Vegas[2], Joaquin Morales[2], Juan Ramon Alba[3]
[1]Ceva, [2]PigCHAMP Pro Europa, [3]INIA

Introduction
Porcine circovirus remains one of major infection agents impacting pig performance in industrial farms. Vaccination has proven to reduce direct and indirect losses due to PCVD. The interference between high levels of maternal antibodies (MDA) and piglet vaccination was described previously1,2. The aim of this study was to assess the impact of different vaccination protocols in sows and piglets on PCV2 circulation in pigs and their performance

Materials and methods
The trial was performed at the experimental pig farm of PigCHAMP, Spain. In total 36 gilts were split into three groups and vaccinated with Circovac® 2ml/dose before first farrowing or at first weaning or not vaccinated (Groups A, B and C). Their offspring were vaccinated at 3(R1), or 4(R2) or 6(R3) weeks of age (WOA) or not vaccinated(R4) with Circovac® 1ml/dose. Antibody profiles in sows, PCV2 circulation patterns and performance of those pigs were measured in three consecutive reproduction cycles. To determine the protection against PCV2 infection, viremia was measured in the blood in pigs periodically until 20WOA. All animals with the ct values > 25 in all samplings until 20 WOA were considered negative. The percentage of negative pigs was calculated per treatment group.

Results
Effect of sow vaccination on sow PCV2 antibody profiles: IgG concentrations
All samples from sows were included in the statistical analysis using the R Project for Statistical Computing version 3.6.1 and SAS/STAT software. The Figure N°1 shows the profile of PCV2 IgG over time in different groups of sows.

Figure 1. PCV2 IgG levels over time in sows of groups A, B and C

Vaccination 3 weeks before farrow provides higher antibody titers against PCV2 than vaccination at weaning.

Effect of sow vaccination on piglet PCV2 antibody profiles: IgG concentrations
The evolution in time of IgG serum concentration in piglets by sow groups (piglets from sows of group A, of group B and of control sows) was measured.

Figure 2. PCV2 IgG concentration evolution in time (from week 1 to week 3 of life) in piglets of 3 different sow groups A, B and C.

Vaccination of sows 3 weeks before farrowing provides higher antibodies titers in their piglets.

Effect PCV2 viremia measured by PCR in pigs
When pigs born to sows A and B were considered, it was clearly observed that piglets born to sows A were more positive if they were vaccinated at 3 WOA compared to those vaccinated at 4WOA which were more positive than those vaccinated at 6 WOA. This

tendency was not observed in pigs born to sows B (especially not the highest positivity of pigs vaccinated at 3 WOA).

Tab 1. Percentage of negative pigs until 20 weeks of age.

No statistical difference was observed in the growth performance among groups R1-R4. Piglets of 2nd and 3rd parities born to vaccinated sows A, B had lower early mortality (7,35%b and 8,02%b) than those born to non-vaccinated sows C (15,72%a).

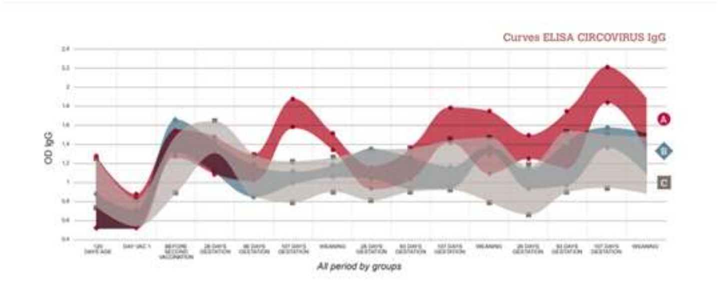
Conclusion
Sow vaccination at weaning avoided high peaks of antibodies during lactation. If sows are vaccinated pre-farrow, it is advisable to vaccinate their piglets not earlier than at 4WOA, better at 6 WOA, which is coherent with previously published reccomendations. Sow vaccination against PCV2 can reduce early piglet mortality.

References
Fraile L. Et al.,   Veterinary Microbiology 161 (2012) 229–234
Martelli P. et al. BMC Veterinary Research (2016) 12:77

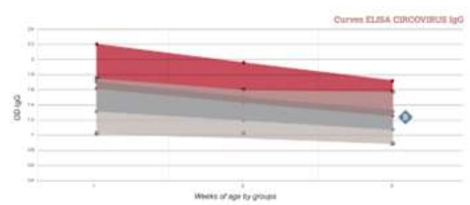
Table 1

Group Vaccine	A:R1	A:R2	A:R3	A:R4	B:R1	B:R2	B:R3	B:R4
TOTAL AVERAGE	64,1	77,5	83,35	68,1	82,7	75,25	87,5	73,05

Graphs or Images 1



Graphs or Images 2



P-V-102

THE POTENTIAL ADJUVANTICITY OF CAVANT®SOE FOR FOOT-AND-MOUTH DISEASE VACCINE

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[1]College Of Veterinary Medicine, Chungnam National University, [2]Choong Ang Vaccine Laboratory Co., Ltd, [3]Animal and Plant Quarantine Agency

Introduction:
In this study, we evaluated the efficacy of a novel water-in-oil (W/O) emulsion, called CAvant®SOE, as a novel vaccine adjuvant for use with inactivated FMD vaccines.

Materials and Methods:
To evaluate the efficacy of CAvant®SOE, the purified inactivated A22 Iraq virus antigen was emulsified with CAvant®SOE with the ratio 35:65 (volume: volume) of the aqueous antigen to adjuvant to form a water-in-oil emulsion. Similar antigen load emulsified with Montanide ISA 201, adjuvant known as efficacious in FMDV protection, was used as a positive control. Formulated vaccine preliminary assessed in mouse immunization model and finally assessed in swine as a novel vaccine adjuvant for use with inactivated FMD vaccines.

Results:
Inactivated A22 Iraq virus emulsified with CAvant®SOE (iA22 Iraq-CAvant®SOE) induced effective antigen-specific humoral (IgG, IgG1, and IgG2a) and cell-mediated immune responses (IFN-γ and IL-4) in mice. Immunization of pigs with a single dose of iA22 Iraq-CAvant®SOE also elicited effective protection, with no detectable clinical symptoms against challenge with heterologous A/SKR/GP/2018 FMDV. Levels of protection are strongly in line with vaccine-induced neutralizing antibody titers.

Conclusions:
These results indicate that CAvant®SOE-adjuvanted vaccine is a promising candidate for control of FMD in pigs.

Acknowledgement:
This work was supported by National Research Foundation of Korea (Grant no. 2021R1A6A1A03045495, 2018M3A9H4078703)

References:
1. Dar P. et al. 2013. Vaccine. 31: 3327-3332.
2. Cao Y. 2014. Expert Rev. Vaccines. 13: 1377-1385.

P-V-103

THE USE OF PORCINE CIRCOVIRUS (PCV) TYPE 2A-BASED VACCINE IN PCV2D PREDOMINATED FARM.

Mr. Tossapron Panya-ad[1], Ms. Naritsara Boonraungrod[2], Mr. Irawin Nimmansamai[2], Ms. Sasithorn Numkanisorn[2]
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Introduction
Porcine circovirus type 2 (PCV2) is circulating on almost all swine farms worldwide. PCV2 can be divided into eight different genotypes: a to h, based on sequence identity in ORF2(1,2). PCV2d genotype has become predominating particularly in farms with clinical forms of Porcine circovirus associated disease (PCVAD), including Thailand(3). Most of PCV2 vaccines are PCV2a based. However, there are PCV2b and PCV2d based vaccines are presenting in the market. The aim of this study is to compare the performance in finishing period of pigs vaccinated with PCV2a-based vaccine or PCV2d-based vaccine in PCV2d positive farm.

Materials and Methods
This study was conducted in 6,000 sow size farm from the Western part of Thailand with confirmed PCV2 infections in finishing period. Genome sequencing was performed and shown PCV2d predominant genotype in the farm. 19,000 piglets from 15 batches were included in this study and divided into 2 groups; group 1 (n=9,600) was vaccinated with PCV2a-based vaccine (Circovac®) 0.5 ml intramuscularly per pig and group 2 was vaccinated with PCV2d-based vaccine 1 ml intramuscularly per pig. Both groups were vaccinated twice at 4 and 7 weeks of age upon the request of the farmer, 132-142 days raised in finishing unit and performance data were recorded. The statistical analysis were performed using Two sample t-test.

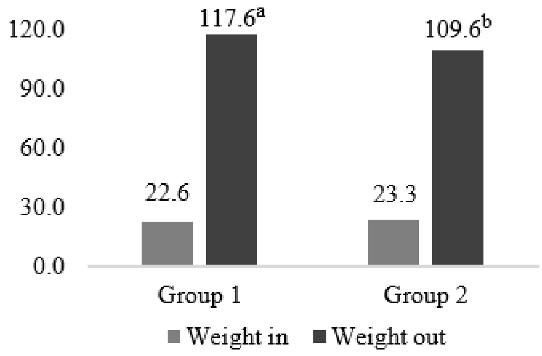
Results
Body weight when entered to finishing period was not significant difference between two groups but body weight at the end of finishing period was significant higher in group 1 as showed in the figure 1. Moreover, group 1 had significant lower mortality rate in finishing period when compared with group 2 (figure 2).

Conclusions and Discussion
According to the result from Opriessnig, 2017 that indicated cross-protection of Circovac® to PCV2d challenged in naïve pigs, PCV2a-based vaccine was effective in reducing PCV2d viremia, tissue loads, shedding and transmission(4). In this study, pigs that were vaccinated with Circovac® showed significant higher finishing weight and lower mortality rate than pigs that were vaccinated with PCV2d-based vaccine. This result indicated that PCV2a-based vaccine can improve the performance in farm with PCV2d infections.

Acknowledgement and References
1. Xiao et al. 2015. J. Gen. Virol. 96: 1830–41.
2. Davies et al. 2016. Virus Res. 2(217): 32–7.
3. Thangthamniyom et al. 2017. Vet. Mic. 208: 239-246.
4. Opriessnig et al. 2017. Vaccines. 35: 248-254.

Graphs or Images 1

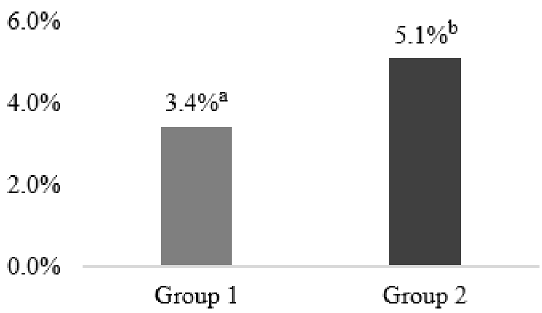
Figure 1. Body weight in and out from finishing unit.



a,b Two sample t-test $p<0.05$

Graphs or Images 2

Figure 2. Mortality rate in finishing period.



a, b Two sample t-test $p<0.05$

P-V-104

THE VARIATION AND FACTORS EFFECT OF THE NEUTRALIZING ANTIBODY AGAINST PRRSV BETWEEN SOWS AND SUCKLING PIGS

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Introduction
Porcine reproductive and respiratory syndrome virus (PRRSV) is responsible for respiratory disorders in nursery pigs and reproductive failures in sows. The maternal neutralizing antibody of maternally-derived antibodies (MDAs) can provide piglets for high protection [1]. There are many factors which may affect MDAs of piglets, including birth weight, birth order and parity of sows [2]. The aim of this study was to investigate the variation of PRRSV MDAs between sows and suckling pigs, and investigate factors associated with MDAs.

Materials and Methods
The sow parity, litter size, born alive, piglets birth weight, birth order, survival rate and teat order were recorded. On the 7 days post-parturition, blood samples of piglets and sows were collected. Sera were two-fold serial diluted in DMEM medium without FBS. 50 µl diluted sera were incubated with 4×10^3 $TCID_{50}$/ml of PRRSV at 37 °C and 5% CO_2 for 1 hour. Mixtures were transferred into MARC-YL cells prepared in 96-well plates and incubated, and the cytopathic effect (CPE) were observed at 5 days post-inoculation. The neutralizing antibody data was analyzed by the neutralizing antibody transfer ratio, which was divided the piglet neutralizing antibody titer (log_2) by the piglet neutralizing antibody titer (log_2). The simple regression analysis was analyzed by neutralizing antibody titer (log_2) of sows and piglets. Correlation analysis was performed by Pearson correlation coefficient for normally distributed data and Spearman correlation coefficient for non-normally distributed data.

Results
A total of 45 sows and 466 piglets were included in this study. The average coefficient of variation of piglet neutralizing antibody titer in litter was 22.7% (range, 8.8%-100%). Litter size of sows was significantly negatively associated with survival rate (P<0.05) and birth weight (P<0.01) of piglets. There is no significant correlation between parity, litter size of sows, birth weight, weight gain of piglets and survival rate of piglets. Neutralizing antibody transfer ratio of piglets was significantly positively associated with birth weight of piglets (P=0.001) and number of born alive in litter (P<0.001).

Conclusions and Discussion
The positive association between NA of sows and piglets can be considered and applied for PRRSV prevention and control strategies.

Acknowledgement
We gratefully acknowledge the technicians and veterinarian in ADDC of NPUST for their contributions to this study.

References
[1] Lopez OJ et al., 2007. Clin. Vaccine Immunol. 14: 269-275.

[2] Murtaugh PM et al., 2008. Vaccine. 46: 8192-8204.

Table 1

Table 1. Correlation analysis between neutralizing antibody transfer ratio and piglet's birth weight (BW), weight gain (WG), piglet's teat order, birth order and number of born alive in litter.

	Correlation coefficient	*P* value
BW (kg)	0.15	<0.01
WG (kg)	0.52	>0.05
Teat order	0.03	>0.05
Birth order	-0.02	>0.05
Born alive	-0.24	<0.001

BW, birth weight; WG, weight gain.

Graphs or Images 1

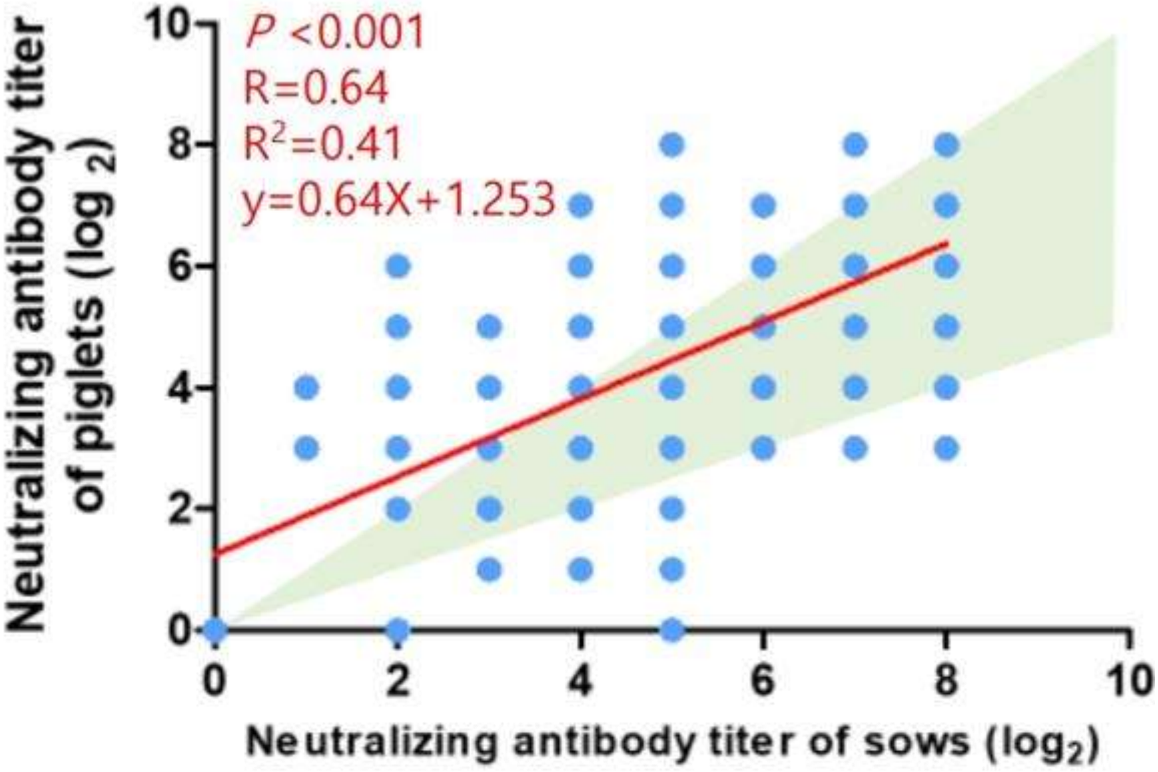

Figure 1. The relationship between the neutralizing antibody potency of piglets and sows, there is a significant positive correlation between piglets and sows. The green area indicates 0.5 to 1 times of the neutralizing antibody potency of sows (log2), 65.9% of the piglets were in this interval.

P-V-105

USAGE OF RT-QPCR AND SEQUENCING TO CHECK THE EFFICACY OF PIGLET VACCINATION WITH A PRRSV1 MLV IN A COINFECTED PRRSV1 AND PRRSV2 FARM IN KOREA

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Introduction
PRRS is a swine disease with a very important economic impact on the swine industry (1). Minimization of the impact of the disease is a primary objective and vaccination with MLV vaccines has been reported to achieve farm stabilization more quickly than other methods (2), a farm with stable PRRSV status is the key to achieving better productive parameters, being able to achieve an increase of 1.28 weaned piglets per sow per year if PRRSV stability is maintained for a one-year period (3). The purpose of this trial was to check if the stability of a site 2 in Korea coinfected with PRRSV1 and PRRSV2 was improved by switching from a PRRSV2 vaccine to a PRRSV1 vaccine.

Materials and Methods
The trial was conducted at a site 2 and 3 in South Korea where 70-days-old animals from a negative site 1 were introduced monthly and vaccinated with a PRRSV2 MLV (VR2332, 2 ml, IM). Site 3 was endemic to a field PRRSV2 (similarity with the PRRSV2 MLV VR2332 and with UNISTRAIN® PRRS in ORF5: 83.6% and 64% respectively). In April 2021 it suffered a severe outbreak of PRRSV1 (similarity with UNISTRAIN® PRRS in ORF5: 85%) causing high mortality in pigs (more than 20%) so in October the decision was to change the vaccine to UNISTRAIN® PRRS ID (PPRRSV1 vaccine, VP-046 BIS strain, 0.2 ml, ID, HIPRA) with an intradermal needle-free device (Hipradermic®, HIPRA). 15 blood samples were collected at 16 weeks of age (woa), 20 woa and 24 woa to perform PCR (pools of 5) and Sequencing (if the virus concentration was enough) before the use of UNISTRAIN® PRRS (UP), 1 month after UP, 2 months after UP and 4 months after UP.

Results
Before the use of UNISTRAIN® PRRS there was co-circulation of PRRS Type 1 and Type 2 field strains. 2 months after the use of UNISTRAIN® PRRS it was only detected vaccines strains (Table 1)

Table 1. PCR and Sequencing in site 3

Discussion and Conclusion
Based on the results of this trial, after vaccination with UNISTRAIN® PRRS in a site 2 and 3 coinfected with PRRSV1 and PRRSV2 field strains stabilization was achieved on the farm since it was not possible to detect Type 1 field virus and Type 2 field virus. This conclusion could be made thanks to differenciating vaccine strains from field strains. Not only PCR assay, but also sequencing should be performed to check the efficacy of the vaccine.

References
1. Holtkamp et al., 2013. J. Swine Heal. Prod. 21, 72–84.
2. Linhares et al., 2014. Preventive veterinary medicine. 116.10.1016/j.prevetmed.2014.05.01

3. Torrents et al. 2021. Porcine Health Management, 7:21

Table 1

Age of animals	Vaccine type & PCR result	Before Unistrain			1 month after Unistrain			2 months after Unistrain			4 months after Unistrain		
16 woa (right after entering)	Vaccine	VR2332			Unistrain(EU)			Unistrain(EU)			Unistrain(EU)		
	PCR result	NA	-	-	NA	NA	NA	EU+NA	EU+NA	EU	EU	EU	-
20 woa (4 wks after entering)	Vaccine	VR2332			Unistrain(EU)			Unistrain(EU)			Unistrain(EU)		
	PCR result	EU + NA	EU + NA	EU	-	-	-	NA	NA	-	EU	EU+NA	-
24 woa (8 wks after entering)	Vaccine	VR2332			VR2332			Unistrain(EU)			Unistrain(EU)		
	PCR result	EU	-	-	-	-	-	-	-	-	-	-	-
Similarity by comparing ORF5 (Detected wild strains vs Vaccine strains)	NA strain	VR2332		83.6%	Low abount of antigen (Sequencing was not possible)			*VR2332*		*99.2%*	Low abount of antigen (Sequencing was not possible)		
		Vaccine A		84.6%				Vaccine A		90.5%			
		Vaccine B		84.7%				Vaccine B		91.9%			
		Vaccine C		65.5%				Vaccine C		65.3%			
		VP046		64.0%				VP046		64.8%			
	EU strain	Lelystad		86.2%	-			Lelystad		94.9%	Lelystad		94.6%
		Vaccine A		63.3%				Vaccine A		63.2%	Vaccine A		63.1%
		Vaccine B		63.2%				Vaccine B		63.3%	Vaccine B		63.3%
		Vaccine C		85.2%				Vaccine C		93.6%	Vaccine C		93.1%
		VP046		85.0%				*VP046*		*99.2%*	*VP046*		*98.8%*

P-V-106

USE OF A KILLED PRRS VACCINE AS A COMPLEMENT TO A PRRS MODIFIED LIVE VACCINE TO ACHIEVE A PRRS STABLE STATUS ON A POSITIVE HIGHLY PATHOGENIC PRRSV1 FARM IN SPAIN

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[1]*HIPRA*

Introduction

On farms that are endemically infected with PRRS, modified live vaccines (MLV) are the first choice for immunological stimulation of animals, so any vaccination programme must include them. PRRS killed vaccines (KV) represent a booster of immunity against PRRS and are used as a complement to a previous vaccination with a MLV. The combination of MLV and KV has several benefits, namely an increase in neutralizing antibodies and cell-mediated immunity responses (1) and a remarkable improvement in the PRRSV status in breeding herds. A farm with stable PRRSv status is the key to achieve better reproductive parameters, and being able to get an increase of 1.28 weaned piglets per sow per year if PRRS stability is maintained for a one-year period (2). The objectives of this trial were to evaluate the efficacy of sow's revaccination with SUIPRAVAC® PRRS in reducing PRRS viraemia and its effect on reproductive parameters in a farm in Spain.

Materials and Methods

The study was conducted on a swine farm of 226 sows located in Spain (Tona, Barcelona) which was managed by a weekly farrowing system. Sows were vaccinated against PRRS with a MLV by blanket administration every 4 months. Piglets were not vaccinated against PRRS. The recruited farm was having reproductive disorders caused by a highly pathogenic Type 1 PRRS strain called Rosalia (3). Breeder sows at 45 days of gestation were recruited for this study. The latest vaccination against PRRS was administered 3 months before the study start (October 2021). The recruited sows were distributed in 2 groups. At 60 days of gestation, group 1 (n=14) was vaccinated with SUIPRAVAC® PRRS and group 2 (n=15) was vaccinated with PLACEBO. Once farrowed, the reproductive performances were recorded. The PRRS viraemia was monitored in the farrowed piglets during the first week after farrow (0 weeks) and at 3 weeks post-farrow with RT-qPCR. For this purpose, blood samples were collected from 58 piglets from sows in group 1 and 61 piglets from sows in group 2.

Results

Regarding reproductive performance, the percentage of stillborn piglets per litter was higher in group 2 (12,09%) versus those obtained in group 1 (6,28%), but the difference was not statistically significant (Figure 1). Despite this, the difference between both groups were 48,06% stillborn piglets less in sows from group 1 than in group 2. Regarding piglet's viraemia, between 8.20% (0 weeks) and 8.33% (3 weeks) of piglets were positive in group 2 while all piglets remained negative in group 1. The strain identified was a wild PRRS virus, specifically Rosalia strain. The difference between groups were statistically significant (Figure 2).

Figure 1. Reproductive performances of sows. Results are represented as average and standard deviation of the percentage of stillborn piglets per litter (Mann-Whitney Test, $p>0,05$).

Figure 2. Viraemia of PRRS in piglets. Results are represented as percentage of positive and negative piglets. Asterisks indicate a statistically significant difference between group (Fisher exact test 1 side; p>0.05).

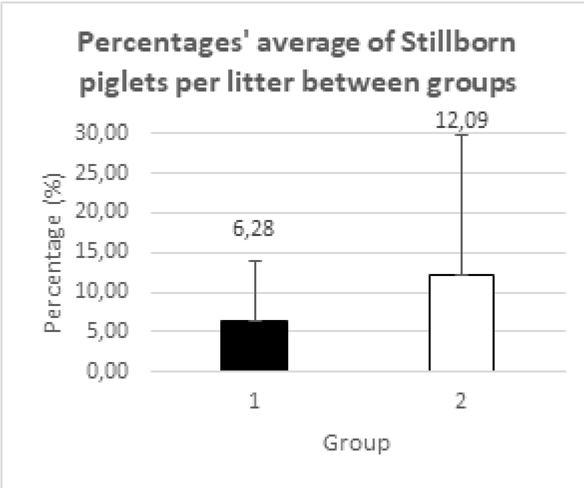
Discussion and Conclusion

The trial demonstrated that SUIPRAVAC® PRRS reduced significantly the viraemia in piglets during the lactation phase and produced 48.06% of stillborn piglets less than the control group in a positive Rosalia strain farm.

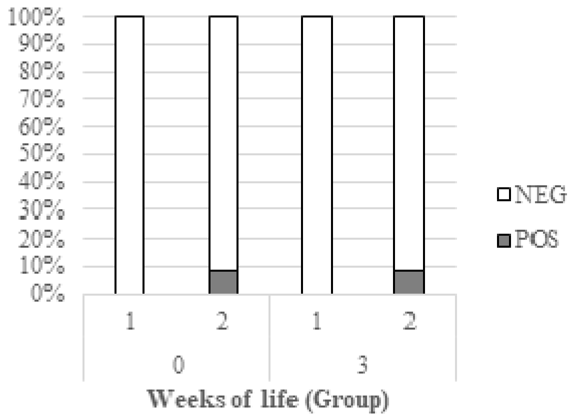
References

1. Diaz I et al. 2013. The Veterinary Journal 197: 438–444.
2. Torrents et al. 2021. Porcine Health Management, 7:21
3. Martín-Valls, G.E., Cortey, M., Allepuz, A. et al. Introduction of a PRRSV-1 strain of increased virulence in a pig production structure in Spain: virus evolution and impact on production. Porc Health Manag 9, 1 (2023). https://doi.org/10.1186/s40813-022-00298-3

Graphs or Images 1



Graphs or Images 2



P-V-107

USE OF A PORCINE ENDOGENOUS REFERENCE GENE (INTERNAL SAMPLE CONTROL) IN A PRRSV RT-QPCR

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[1]Iowa State University, [2]Pig Improvement Company Latinoamerica, [3]IDEXX Laboratories, Inc.

Introduction

Endogenous reference genes have been used extensively as qPCR internal sample controls (ISC) in gene expression research and, more recently in veterinary diagnostic. This study evaluated the detection of a porcine ISC from a commercial PRRSV RT-qPCR (IDEXX Laboratories, Inc.) using research samples of known PRRSV status.

Materials and Methods

Serum (n = 132), oral fluids (n = 130), and feces (n = 132) from 12 pigs individually housed under experimental conditions and vaccinated with a PRRSV MLV (Ingelvac® PRRS MLV) from -7 to 42 days post vaccination (DPV) were used herein. Samples were tested for PRRSV and ISC RNAs, and Cq values from the RT-qPCR were re-expressed as “Efficiency standardized Cqs (ECqs)” to account for PCR efficiency. Consistent expression of the ISC over time represented by DPVs was evaluated using a mixed-effects regression model (MRM) in R 4.2.1 (R core team, 2022).

Results

All pigs were negative for PRRSV RNA in all specimens at -7 and 0 DPV, with the first positive at 3 DPV. The ISC was consistently detected in all specimens (n = 394). The mean ISC ECqs was 1.8 in serum, 2.1 in oral fluid, and 1.3 in feces. DPVs and PRRSV did not affect the ISC expression in any specimen ($p > 0.05$; MRM), with a slope value of 0.00 ECqs (95%CI: -0.00, 0.01) in serum, 0.01 ECqs (95%CI: 0.00, 0.01) in oral fluids, and -0.00 ECqs (95%CI: -0.01, 0.00) in feces.

Conclusions and Discussion

In this study, the ISC response in samples collected under the “best-case scenario” (i.e., research conditions) was uniform over time and unaffected by PRRSV infection. By extension, these data suggest that not detecting the ISC would indicate a significant irregularity either with the sample itself or with the testing process. The use of an ISC could be a useful addition to quality management in routine PCR testing.

Acknowledgements

PRRSV RT-qPCR testing was partially supported by donations from IDEXX Laboratories, Inc.

P-V-108

VIRUS NEUTRALIZATION AND SHEDDING COMPARING THREE DIFFERENT PSEUDORABIES MLV VACCINES AGAINST A VIRULENT NIA-3 CHALLENGE

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Background and objectives
Pseudorabies virus (PRV), originally Aujeszky's disease virus, causes potentially devastating clinical problems, including reproductive failure, piglet and grower mortality, reduced growth and productivity, and compromised immunity against secondary infection and vaccine responses. Once infected the animal will be life-long infected with possible reactivation following stress and hormonal changes. The virus is shed in high levels for 3-4 weeks after infection and reactivation. Serum virus neutralising antibody (VN) titers are commonly used to estimate PRV-vaccine protective capacity. The aim of this study was to compare the VN and post-challenge shedding titers of three PRV-MLV marker vaccines, two based on the Ceva-China produced Bartha strain, and an international Bartha-K61 strain-based vaccine in a controlled virulent PRV challenge study.

Materials and methods
In a trial design according to the European Pharmacopeia forty-four eight-week-old pigs were randomly allocated to one of four groups, vaccinated (D0) at 8 weeks and boosted at eleven weeks of age (woa) (D21). With A1 (O/W adjuvated Auphyl® plus; Ceva, China), A2 (non-adjuvated Auphyl® plus; Ceva, China), IA (non-adjuvated international PRV-Bartha-K61 based), and non-vaccinated controls (ctrl) injected with phosphate-buffered saline (PBS). PBS is used as diluent in both non-adjuvated PRV-vaccines (A2 and IA). At 14 woa, three weeks after second vaccination (D42), all animals were challenged intranasally with 4 ml (2 ml/nostril) of the virulent NIA-3 PRV strain in a 7.7 log10 TCID50/ml solution. Seven days later, D49, all surviving animals were euthanised and investigated.
All pigs were serum sampled at D42, prior to challenge, and nasal swabs were taken at days 2, 5, 7 post-challenge. Serum virus neutralising antibody (VN) titers in response to vaccination were measured by virus neutralization assay using standard microneutralization method on MDBK cells against 100-300 TCID50 ADV-MNC+10a standard assay strain. Swabs were washed into MDBK cell culture solution for determination of PRV titer. For each group, the average area-under-the-curve (AUC) was computed applying the Trapezoidal rule and used to provide an accurate estimate of post-challenge PRV shedding. The AUCs were calculated into residual rate of shedding for each vaccine group relative to the ctrl-group, and into the relative shedding to the best vaccine group (A1), set as 1, for easier comparison of relative efficacy. Data were analysed in R/Python. VN and post-challenge shedding were analysed using the non-parametric Mann-Whitney U test. Results with p-values ≤0.05 are considered statistically significant.

Results
VN titers were 7.6, 6.4, 5.1, and 2.0 log2 in A1a, A2b, IAc, and ctrld group respectively (Fig 1); all statistically different.
Post-challenge (NIA-3-only) PRV AUC-shedding was 22.3, 23.6, 29.6, and 33.7 log10 TCID50/ml in A1, A2, IA, and ctrl group respectively, providing shedding rates relative to the best of 20-, 19,952,623-, and 251,188,643,151-times A1a shedding in A2a, IAb, and ctrlc

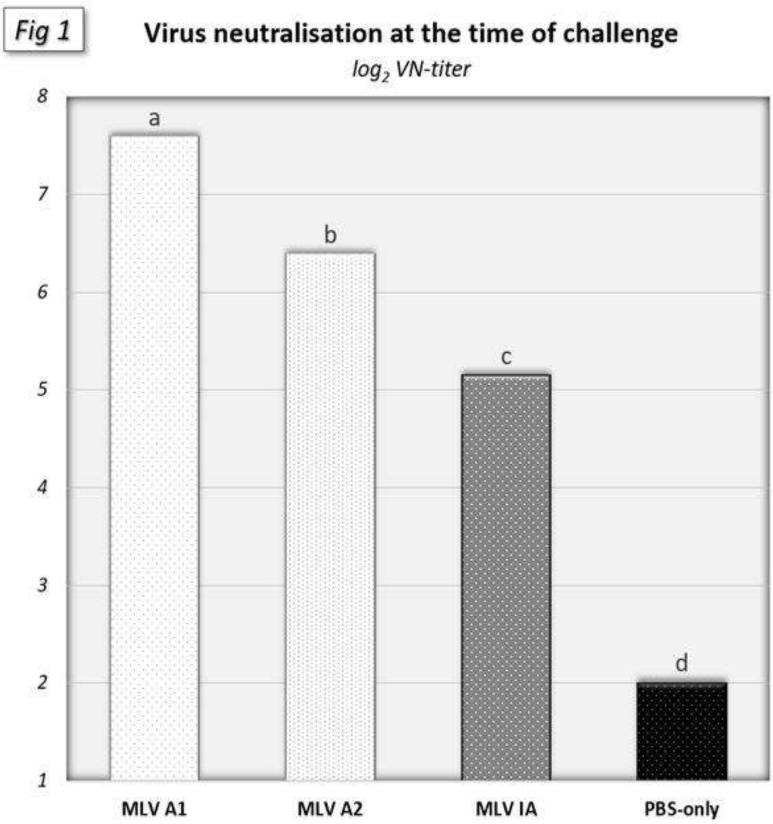
groups respectively (Table 1). Statistically significantly different groups are indicated by letters: a, b, and c.

Conclusion
The VN-titers indicated superior efficacy of A1 (O/W) vaccine, closely followed by the A2 (PBS) vaccine. This was confirmed by the post-challenge viral control (significant reduced shedding). The superior PRV viral control, with a potential of eradication, of the A1 vaccine makes it a strong candidate for all breeding-stock PRV-vaccination. In most farms the A2 vaccine will likely be sufficient for pig(let) PRV-vaccination and protection.

Table 1

Table 1: **Post-challenge PRV AUC-shedding**	**A1 (O/W)**[a]	**A2 (PBS)**[a]	**IA (PBS)**[b]	**Ctrl (PBS-only)**[c]
Residual rate	0.000,000,000,4%	0.000,000,007,9%	0.007,9%	100%
Relative to best	**1**	*20* x A1	*≈20 million* x A1	*> ¼ trillion* x A1

Graphs or Images 1



P-V-109

VULNERABILITY OF REAL-TIME PCR IN EVALUATING PORCINE CIRCOVIRUS TYPE 2 INFECTION AND ESTABLISHMENT OF A NOVEL DETECTION METHOD USING INVERSE PCR COMBINED WITH REAL-TIME PCR

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Introduction
Porcine circovirus type 2 (PCV2) is a tiny virus with a diameter of approximately 17 nm. PCV2 is nonenveloped, with a single-strand circular DNA genome of approximately 1.8 kb. PCV2 causes porcine circovirus-associated disease (PCVAD or PCVD), such as post-weaning multisystemic syndrome (PMWS), by weakening the immune function of piglets.
In addition, it was reported that the virus is superinfected with PRRSV, the responsible virus for porcine reproductive and respiratory failure syndrome (PRRS), affecting its proliferation and clinical presentation. These diseases cause significant detriments to the pig industry. Vaccination is particularly effective in preventing viral infections [1]. Post-vaccination PCV2 testing typically uses pooled sera from multiple pigs, with PCV2 generally being detected by real-time PCR, targeting approximately 100 bp of open reading frame 2 (ORF2). The detection sensitivity is high. However, detection of fragmented viral genomic DNA degraded by the immune system is possible (Fig. 1). In addition, although the use of pooled sera for PCV2 detection has some advantages in testing, false-negative results may occur because of dilution. These facts suggest that the copy numbers calculated using real-time PCR may not be accurate. Our study assessed whether the results of real-time PCR can be reflected as the accurate copy number of a potentially infectious virus. We also established a novel PCV2 detection method combining both real-time PCR and inverse PCR.

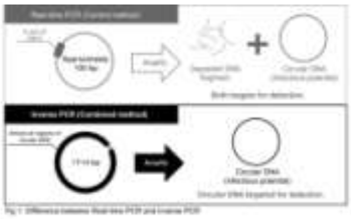
Materials & Methods
An inverse PCR system for detecting the circular DNA of potentially infectious viruses was validated and compared with a real-time PCR detection system. A total of 44 piglets used for this study were divided into two groups, the vaccinated and non-vaccinated (22 piglets per group). Piglets in the vaccinated group were vaccinated with Ingelvac CircoFLEX® at 21 days of age. All piglets from each group were kept under the same management conditions on the farm. Sera from PCV2-uninfected pigs in vaccinated and non-vaccinated groups were examined. The virus copy number was calculated using real-time PCR. Based on this copy number, inverse PCR was performed to check for the presence of viral DNA and fragment detection PCR was performed to amplify part of ORF2.

Results
Inverse PCR detects the circular DNA of PCV2. The detection sensitivity was 10^2 to 10^3 copies/ml. In the control test, the non-vaccinated group had a 100% real-time PCR positivity rate, of which 86% was inverse PCR positive. The vaccinated group had a 52% real-time PCR positivity rate, when this was considered 100%, the inverse PCR positivity rate was 42% (Fig. 2). In the general farm test, circular DNA was detected in only 25.9% of pooled samples with high copy numbers. The detection sensitivity of inverse PCR was also reduced to approximately 10^5 to 10^6 copies/mL in most pooled samples.

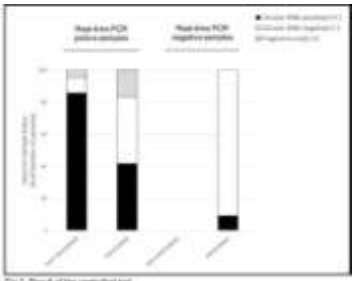
Conclusions and Discussion
Real-time PCR had a 58% possibility of detecting fragmented PCV2 genomic DNA, which did not accurately reflect the number of copies of viral DNA remaining in serum. Dilution of the sample in the pooled test may have reduced the detection sensitivity of inverse PCR. For accurate detection of PCV2, we recommend not only detection by real-time PCR, but also combined use of inverse PCR and individual testing.

Acknowledgement and References
1. Yi J., Liu C. Molecular characterization of porcine circovirus 2 isolated from diseased pigs co-infected with porcine reproductive and respiratory syndrome virus. Virol. J. 2010;7:286.
2. Vlasakova M, Leskova V, Sliz I, Jackova A, Vilcek S. The presence of six potentially pathogenic viruses in pigs suffering from post-weaning multisystemic wasting syndrome. BMC Vet Res. 2014;10:221.

Graphs or Images 1



Graphs or Images 2



P-V-110

GENETIC CHARACTERIZATION OF PORCINE CIRCOVIRUS 3 IN TAIWAN

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Introduction
Porcine circovirus type 3 (PCV3) is a non-enveloped, circular single-stranded DNA virus belonging to the genus Circovirus of the family Circoviridae. PCV3 have two major open reading frames (ORF), ORF1-2. The ORF1 encodes the replication-associated protein (Rep). ORF2 encodes the capsid protein (Cap), which contains the immunodominant antigenic epitopes. Phylogenetic studies based on open reading frame 2 (ORF2) region of PCV3 isolates reveal that the virus has been divided different subgroups (1). PCV3 PCV2 infection has been correlated as porcine dermatitis nephropathy syndrome (PDNS), reproductive failure and diarrhea in pigs. The aim of this study was to investigate the genetic diversity of PCV3 in Taiwanese pig farms.

Materials and Methods
A total of 5 Taiwanese isolates were collected in 2022. Complete genome of each isolate was amplified by PCR (1).The PCR-amplified DNA fragments were sequenced by the direct sequencing method as previous study (2). The complete genomes of PCV3 obtained in this study were aligned against those sequences available from GenBank (NCBI) (Fig. 1) using the MegAlign program within the DNASTAR package. Phylogenetic analysis was performed on the aligned data set and an unrooted tree was constructed using the distance-based neighbor-joining method as determined by the DNASTAR. The 5 Taiwanese PCV3 isolates were classified into subgroup as Franzo definition (2).

Results
In an effort to understand the interrelationships of the viral isolates, the complete genome sequences of the 5 Taiwanese PCV3 isolates from this study and 17 PCV3 isolates obtained from the GenBank (Fig. 1) database were aligned and analyzed. Phylogenetic tree analysis classified these isolates into 1 large group containing three minor subgroups (Fig. 1). The first subgroup contained 1 Canadian isolates (ON418890/Canada), 1 American isolates (KT869077/US), 2 Korean isolates (MF063071/Korea and MW168693/korea), 2 Chinese isolate (MW883350/China and MH184540 /China), 1 Japanese isolate (LC383840/Japan), 1 Hungary isolate (ON015890/ Hungary), 2 Spanish isolates (MT350525/Spain and MT350553/Spain) and 9 Taiwanese isolate (MK343155/Taiwan, MN510466/Taiwan, MN510467/Taiwan, MW803046/Taiwan, TS1/Taiwan, TS2/Taiwan, TS3/Taiwan, TS4/Taiwan, TS5/Taiwan). The second subgroup contained 2 Chinese isolates (KY924474/China and KY924475/China), and the third subgroup contained 1 Chinese isolates (MG372488/China).

The genome similarity between the PCV3 isolates of Taiwan was between 96.4% and 99.5%, and the similarity with the PCV3 genome registered in GenBank from all over the world is about 94.1% to 99.7%. Only a small number of nucleic acid mutations occur in PCV3 virus strains.

Conclusion and Discussion
Our study revealed that the 22 PCV3 isolates were divided into three subgroups, subgroup1, 2 and 3. Subgroup1 was the most commonly genotype isolated over world. Previous study

indicated that greater variations in ORF2 (2), which might influence viral virulence. Further study will be important for identifying the varied genetic and infectious factors associated with the pathogenesis of PCV3 in pig population. In addition, previous studies have shown that co-infection of PCV3 with other pathogens might increase the severity of clinical syndromes (3). Therefore, further studies are needed to define the role that dual infection of PCV3 with other viral pathogens plays in pigs.

Acknowledgement
This work was supported by a grant 111AS-8.1.1-HI-HD to Chun Wang from the Council of Agriculture (COA)

References

1. Franzo G, Legnardi M, Hjulsager C, Klaumann F, Larsen L,Segales J, Drigo M (2018). Full-genome sequencing of porcine circovirus 3 field strains from Denmark, Italy and Spain demonstratesa high within-Europe genetic heterogeneity. Transbound Emerg Dis 65(3):602–606.
2. Chen N, Li S, Ye M, Huang Y, Huang Y, Xiao Y, Yu X, Dong J, Tian K, Zhu J. (2019)A novel NADC30-like porcine reproductive and respiratory syndrome virus (PRRSV) plays a limited role in the pathogenicity of porcine circoviruses (PCV2 and PCV3) and PRRSV co-infection.Transbound Emerg Dis. 66(1):28-34.

Graphs or Images 1

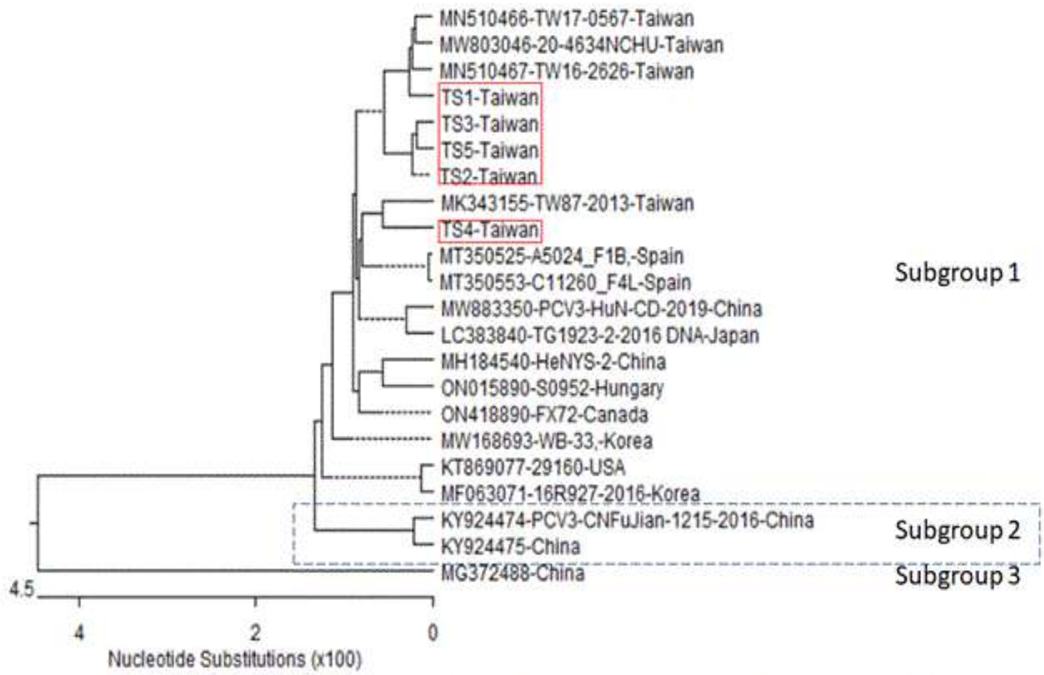

Fig. 1. Phylogenetic tree analysis of PCV3 isolates. An unrooted neighbor-joining tree was derived from aligned nucleic acid sequences representing the complete genomes of 22 PCV3 isolates. The PCV3 isolated in this study are denoted in red box.

P-V-111

EVALUATION OF THE EFFICACY OF VACCINATION AGAINST PORCINE EPIDEMIC DIARRHEA VIRUS

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Abstract
Porcine epidemic diarrhea virus (PEDV), a member of genus Alphacoronavirus in the family Coronaviridae of the order Nidovirales, is the causative agent of Porcine epidemic diarrhea (PED) that causes a devastating viral enteritis and high mortality in neonatal piglets. Since 2010, Highly pathogenic variant strains of PEDV were identified and caused a high morbidity of up to 100% in piglets, and since then, these strains become dominant, leading to most of the acute outbreaks of PED worldwide.[1] For the prevention of PEDV infection, several vaccines have been developed and a number of studies are actively underway to develop effective and safe vaccines against PEDV.[2] In this study, we evaluated immunogenicity and the protective efficacy of mutant strain which has two base pair(TT) deletion (4136-4137bp) in the S2 gene. We vaccinated sows via intramuscular (IM) route and virulent virus challenge in 5-day-old piglets, and confirmed the validity of vaccine for the current endemic strain SGP-M1.

Materials and Methods
The sows in this study(n=5) were healthy and were confirmed negative for PEDV antigens and antibodies. Three of five sows were intramuscularly inoculated with the 1st vaccine 4-6 weeks before delivery and 2nd vaccine 2 weeks after delivery. The remaining two sows were not vaccinated and served as the control group. For serologic analysis, blood was collected three times: before vaccination, 2 weeks after the first vaccination and again immediately after delivery. Colostrum was also collected after delivery. After delivery, Four-day-old piglets were randomly selected from farrowing sow. These piglets were challenged orally with 1ml ($10^{4.0}$TCID$_{50}$/ml) per piglet of virulent PEDV genotype 2b(SGP-M1 strain). Rectal swabs were collected and body weight was checked after challenge(dpc). Clinical symptoms and mortality were monitored daily up to 5 dpc. RT-PCR was performed to detect the amount of viral excretion in fecal swab samples. For the sow immunogenicity verification experiment, collected serum and colostrum was tested by neutralizing antibody titer test and ELISA.

Results
In colostrum and milk, the vaccine was able to induce significantly higher virus neutralization response. The survival rate of piglets after challenge with PEDV was 75% in inoculated group compared to , 25% in the groups of Traditional control vaccine strain which is far from the latest trend , 25% in the positive control group. Though all piglets showed watery diarrhea through certain period, the clinical symptoms of inoculated group were relatively compared to the positive control group. As a result, the novel vaccine strain using the current vaccine strain showed the effective and significant defense rate compared to the control vaccine and positive control group.

Discussion
The plant-derived PED vaccine developed from currently endemic strain SGP-M1 used in this

study showed efficacy in terms of immune protection in sow, reduction of death rate in piglets and mild clinical signs. However, compared to the positive control group which was unvaccinated, there was no significant difference in the amount of the virus shed. Therefore, it is suggested that future research should focus on improving viral shedding and the development of more effective vaccines, a disease that has significant impacts on the swine industry.

Acknowledgements
This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea Government (MEST)(No. NRF-2021M3E5E3083401)

References

1. Zhang et al. (2022) Viruses 14, 112434.
2. Li et al. (2020) Microb pathog 149, 104553.

P-V-112

ARTIFICIAL VIRAL-LIKE PARTICLE INHIBIT PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS INFECTION IN PRIMARILY PULMONARY ALVEOLAR MACROPHAGES

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Introduction:
Porcine reproductive and respiratory syndrome virus (PRRSV) is a highly contagious viral disease that has a significant economic impact on the porcine industry worldwide. Several studies indicated that PRRSV replicated primarily in pulmonary alveolar macrophages (PAMs) and resulted lung damage influenced by strain virulence. Here, we first demonstrated an artificial chelated-mineral particles, Artificial viral-like particle (AVLP), synthesized through innovative chelating platform (Cinteral®).

Materials and Methods:
Primary pulmonary alveolar macrophages was isolated from 4 to 5-week-old, crossbred, male and female, specific pathogen free (SPF) pigs. A stock of PRRSV 108-929 was prepared and titrated by cytopathic effect (CPE). For the pre-treatment group, cells were g/ml of AVLP ingredient before virus infection. treated with 100 After 24 h, the cells were either mock or co-treated with AVLP infected with 0.1 or 1 multiplicity of infection, respectively. Macrophage morphology was assessed by microscopy. Total RNA was isolated from PAMs using automated nucleic acid purification and then reverse transcribed into complementary DNA with MMLV Reverse Transcriptase. The viral gene copy numbers were calculated.

Results:
In this study, PRRSV 108-929 strain, the North American genotype lineage isolated from Taiwan field, was used for PAMs infection test. PAMs g/ml of AVLP and inoculated with PRRSV were treated with or without 100 108-929 strain at 0.1 or 1 multiplicity of infection (MOI), respectively. The data shown that PAMs infected by PRRSV developed cytopathic effect (CPE), which suggested the replication of PRRSV. However, the amount of CPE obviously reduced in AVLP-treated PAMs when compared to PRRSV-infected cells. Furthermore, the copy number of PRRSV were analyzed using quantitative RT-PCR. Interestingly, the results indicated that PRRSV copies were significantly decreased in AVLP-treated PAMs when compare with untreated group, especially by approximately one to two log step in AVLP-pretreated PAMs.

Conclusions and Discussion:
Taken together, AVLP particles effectively reduce the amount of virus copy numbers and prevent the cytopahtic effect of PRRSV in macrophages. In conclusion, AVLP might be a novel potentially protective strategy against PRRSV infection in porcine.

References:
[1] Lin TY, Chen MK, Wang KT, and Jan HJ. Sintered nanoparticles and use of the same against a virus. (US 2020/0138726 A1)

[2] Lin CN, Lin WH, Hung LN, Wang SY, and Chiou MT. Comparison of viremia of type II porcine reproductive and respiratory syndrome virus in naturally infected pigs by zip nucleic acid probe-based real-time PCR. BMC Vet Res 2013.

P-B-001

A FIELD COMPARISON OF INTRADERMAL PCV2 AND MYCOPLASMA HYOPNEUMONIAE VACCINES

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Introduction:
Intradermal vaccination of swine brings numerous benefits, such as improved welfare and reduced iatrogenic transmission of pathogens. Porcine Circovirus 2 (PCV2) viremia is correlated with reduced Average Daily Gain (ADG). One common metric to measure vaccination efficacy of PCV2 vaccines is thus reduction in viremia load. Mycoplasma hyopneumoniae (M.hyopneumoniae) is the primary pathogen of enzootic pneumoniae and results in increased cranioventral consolidation of lungs at slaughter. The extent of damage can be assessed through slaughter inspections of lungs. The aim of this study was thus to compare and observe the field efficacy of Porcilis® PCV ID and Porcilis® M Hyo ID Once against another intradermal ready to use (RTU) vaccine, in field conditions in Thailand.

Materials and Methods:
This study was performed in two separate production flows of a commercial multi-site swine production system in Thailand. 14 day old pigs were randomly allocated within litter to one of 2 treatments.
Group A - 513 piglets vaccinated concurrently at 4 weeks of age with Porcilis® PCV ID and Porcilis® M Hyo ID Once, housed in Production Flow A to 12 weeks of age.
Group B - 551 piglets vaccinated at 4 weeks of age with a single RTU intradermal vaccine, housed in Production Flow B to 12 weeks of age.
At 12 weeks of age, both Group A and Group B were comingled in Production Flow B and raised to sale age.
Efficacy parameters observed were lung lesion score at slaughter (LLS), rt-PCR for PCV2.
Results were analyzed using Microsoft Excel Data Analysis Toolkit. Lung Lesion Score was analysed using Chi-Square method and rt-PCR was analysed using two tailed t-test.

Results:
PCV2 qPCR mean was numerically different (A = not detected, B = 33.4; P=0.15). LLS was statistically different (A = 10.83 vs. B =25.1; P = 0.001).

Conclusions:
In a commercial farm with PCV2 and M.hyopneumoniae field challenge, pigs vaccinated with Porcilis® PCV ID and Porcilis® M Hyo ID Once showed both numerical and significant reduction in 1) lung lesions associated with M.hyo, 2) mean PCV2 viremia titres compared to pigs vaccinated with a competitor intradermal RTU vaccine.

References:
López-Soria S, Sibila M, Nofrarías M, Calsamiglia M, Manzanilla EG, Ramírez-Mendoza H, Mínguez A, Serrano JM, Marín O, Joisel F, Charreyre C, Segalés J. Effect of porcine circovirus type 2 (PCV2) load in serum on average daily weight gain during the postweaning period. Vet Microbiol. 2014 Dec 5;174(3-4):296-301. doi: 10.1016/j.vetmic.2014.09.016. Epub 2014 Sep 30. PMID: 25448444.

Madapong A, Saeng-Chuto K, Tantituvanont A, Nilubol D. Safety of PRRSV-2 MLV vaccines administrated via the intramuscular or intradermal route and evaluation of PRRSV transmission upon needle-free and needle delivery. Sci Rep. 2021 Nov 29;11(1):23107. doi: 10.1038/s41598-021-02444-3. PMID: 34845289; PMCID: PMC8629989.
Maes D, Segales J, Meyns T, Sibila M, Pieters M, Haesebrouck F. Control of Mycoplasma hyopneumoniae infections in pigs. Vet Microbiol. 2008 Jan 25;126(4):297-309. doi: 10.1016/j.vetmic.2007.09.008. Epub 2007 Sep 22. PMID: 17964089; PMCID: PMC7130725.
Temple D, Jiménez M, Escribano D, Martín-Valls G, Díaz I, Manteca X. Welfare Benefits of Intradermal Vaccination of Piglets. Animals (Basel). 2020 Oct 16;10(10):1898. doi: 10.3390/ani10101898. PMID: 33081216; PMCID: PMC7602853.

P-B-002

A NEW TOOL TO EVALUATE THE SEVERITY OF TURBINATE LESIONS IN PIGS

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Introduction
Atrophic rhinitis(AR) is a costly pig disease. After infection, the turbinates of pigs atrophy, causing other respiratory diseases. The diagnosis of the disease is carried out mainly by observing the nose and scoring the turbinate lesions (1). This method can lead to errors due to experience or subjective judgment. In order to avoid such subjectivity, HIPRA has developed an artificial intelligence diagnostic system, AI DIAGNOS, to objectively evaluate turbinate lesions using big data analysis (2).
The aim of this study was to evaluate the efficacy of AI DIAGNOS on Chinese farms comparing the results of its evaluation with the ones done by different vets.

Materials and methods
From July to August 2022, 60 pig noses from 5 farms were dissected, and turbinate lesions were evaluated by a veterinarian (Vet), Hipra technical service manager (TS) and AI DIAGNOS (AI) and compared.
The correlation of scorings between evaluators was measured through a coefficient of correlation of Pearson.
The difference in the proportion of severe lesions was compared between evaluators through a test of proportions.

Results
Figure 1 shows that there is a strong correlation between scorings between the three Evaluators. The coefficient of correlation between AI and TS is 0,98, between AI and Vet 0,94, and between TS and Vet 0,95. All of them are significant (P-val <0,001).
In Figure 2 it can be seen that the percentage of turbinates with severe lesions varies mainly between the Veterinarian (21,7%) and TS (40%) and AI (40%) being this difference significant (P-val < 0,05)

Discussion
The results show that there are subjective differences in the results of evaluation between people. The AI DIAGNOS evaluates turbinate lesions more objectively and faster. In addition, the situation of AR in Chinese farms is serious and needs to be prevented as the high prevalence of severe and moderate lesions is pointed out.

References
1. De Jong MF. 2006. Progressive and Non-Progressive Atrophic Rhinitis. Diseases of swine, 10th Edition, 34:577-602. Reference Donko.
2. Artificial intelligence as a new method of assessing enzootic pneumonia and atrophic rhinitis lesions. Jordà R. et al .IPVS 2022.

Graphs or Images 1

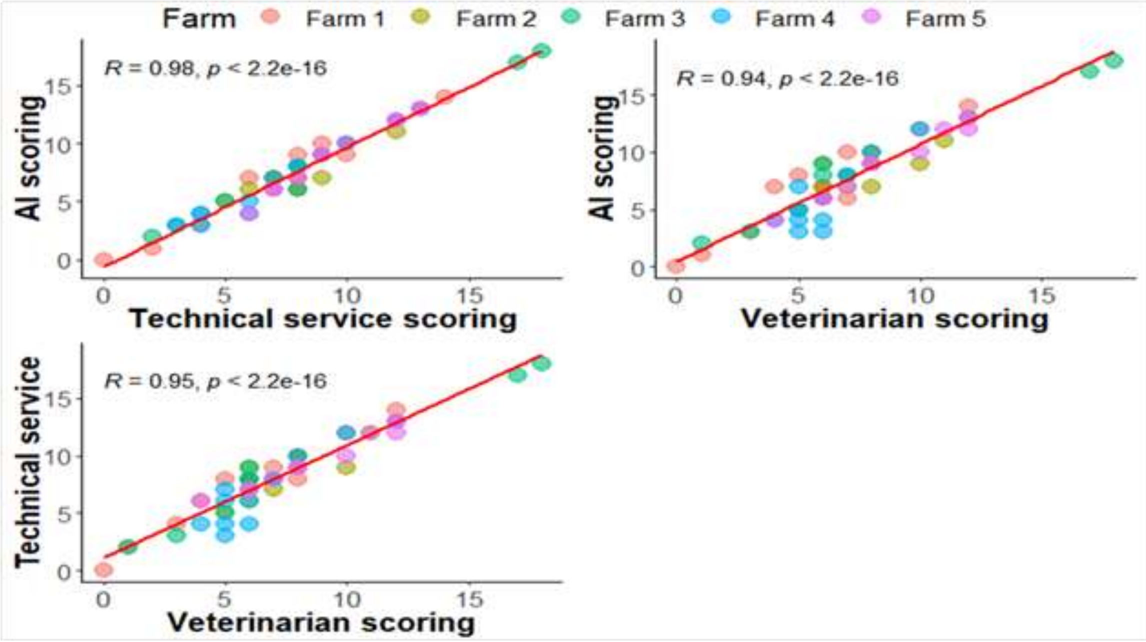

Figure 1 Correlation between different Evaluators

Graphs or Images 2

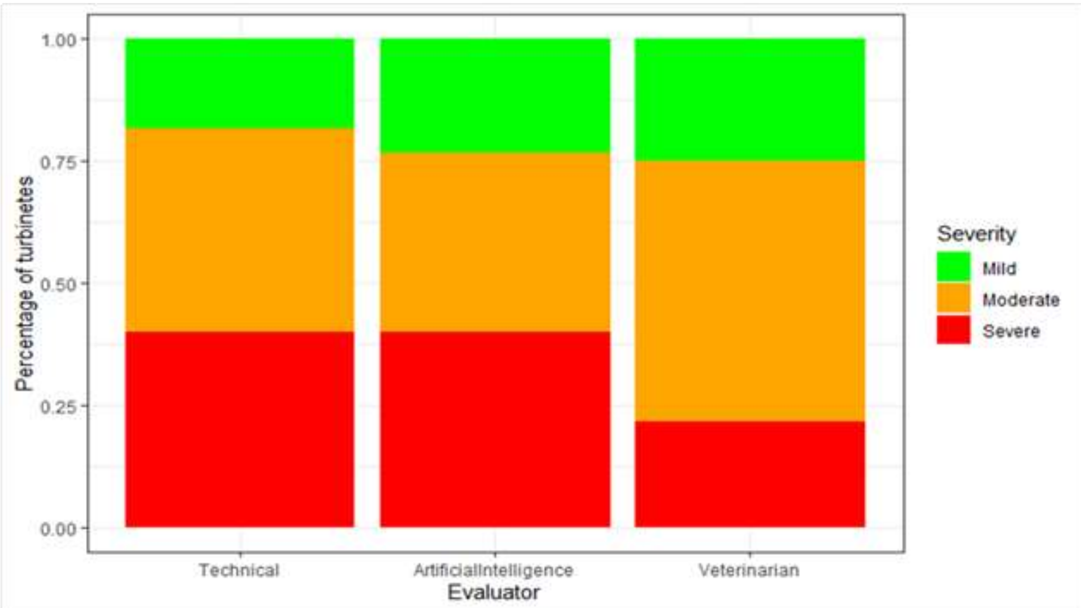

Figure 2 The proportion (%) of turbinate lesions assessed by different groups

P-B-003

A RETROSPECTIVE STUDY OF ENTEROTOXIGENIC AND ENTEROPATHOGENIC ESCHERICHIA COLI FROM DISEASED PIGS IN THE CENTRAL AND SOUTHERN AREA OF TAIWAN.

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Introduction
Enterotoxigenic Escherichia coli (ETEC) and enteropathogenic E. coli (EPEC) are common pathogens that cause diarrhea in newborn and weaned pigs. Infection of ETEC and EPEC leads to poor growth performance and the higher risk of respiratory infections in weaned pigs, which cause significant economic losses to the swine industry. In this study, we aim to explore E. coli isolates collected from Yunlin-Chiayi-Tainan of Animal Disease Diagnosis Center from 2018 to 2021 for the presence of the virulence factors and analyze the correlation between virulence factors and the intestinal lesions of the disease pigs.

Materials and Methods
Isolates were collected from liver, lungs, brain, synovial fluid, small intestine contents, mesenteric lymph nodes, diarrhea feces or anal swabs. Bacterial identification was carried out by PCR using primer pair designed for the E. coli tuf gene. The strains isolated and identified as E. coli were detected by PCR and multiplex PCR for virulence factors STb, EAE, fimbriae F4, F5, F6, F18; STa, LTI, and verotoxin. Antimicrobial drug susceptibility test was carried out by broth microdilution.
Sections from EAE-positive samples were stained with hematoxylin and eosin staining and histological damage scores were determined by quantifying the intensity of epithelial tissue damage. Immunohistochemical staining was performed on the slides with rabbit anti-EPEC commercial antibody to observe the colonization of EPEC in the small intestine and the severity of lesions.

Results
The strains isolated in this study included 145 strains of ETEC, 78 strains of EPEC, 9 strains of shiga toxin-producing E. coli (STEC), 6 strains of ETEC/EPEC, and 17 strains of ETEC/STEC. The enterotoxins of ETEC were mainly STb alone, STa alone and STa+LTI, and the adhesin is mainly F18 (Table 1). Antimicrobial agent susceptibility testing revealed that EPEC had a higher resistance rate to doxycycline, ciprofloxacin, nalidixic acid, flumequine, and enrofloxacin than ETEC (Table 2). Microscopic lesions were observed in the duodenum, jejunum, and ileum in the cases of EPEC (n =15) and ETEC/EPEC (n =4). Anti-EPEC staining showed that the positive rate was 84.2% (16/19) in ileum, which was higher than in duodenum and jejunum ($p < 0.05$) (Figure 1).

Conclusions and Discussion
The pathogenic E. coli are mainly ETEC and EPEC in diseased pigs in Taiwan, and all pathogenic E. coli are multi-drug resistant. A comparison of the microscopic lesions of the small intestine tissues of pigs infected with EPEC and ETEC/EPEC showed that E. coli carrying multiple virulence genes may cause more serious pathological changes. E. coli is often co-infected with other gastrointestinal and respiratory pathogens, making the disease difficult to control, and the severity of lesions may not be directly comparable to the results of IHC.

Acknowledgments
Many thanks to the supervisors, Mr. Kuo Hung-Chih and Ms. Wu Ching-Fen, for supervising the experiments. I would also like to express my special thanks to all the lab members who have given me guidance for the study.

References

1. Fairbrother JM, Nadeau É. Colibacillosis. In: Zimmerman JJ, Karriker LA, Ramirez A, Schwartz KJ, Stevenson GW, Zhang J, ed. Diseases of swine. 11th ed. John Wiley & Sons Ltd, USA, 807-832, 2019.
2. Dubreuil JD, Isaacson RE, Schifferli DM. Animal enterotoxigenic Escherichia coli. EcoSal Plus 7: 10, 2016.
3. Ledwaba SE, Costa DVS, Bolick DT, et al. Enteropathogenic Escherichia coli infection induces diarrhea, intestinal damage, metabolic alterations, and increased intestinal permeability in a murine model. Front Cell Infect Microbiol 10: 595266, 2020.

Table 1

Table 1 Distribution of ETEC virulence factors in 2018-2021.

Year（ETEC）	Enterotoxin（%）						
	STa alone	STb alone	LTI alone	STa+STb	STa+LTI	STb+LTI	STa+STb+LTI
2018 （*n* =67）	13 （19.4）	22 （32.8）	3 （4.5）	7 （10.5）	5 （7.5）	10 （14.9）	7 （10.5）
2019 （*n* =39）	6 （15.4）	13 （33.3）	2 （5.1）	2 （5.1）	11 （28.2）	3 （7.7）	2 （5.1）
2020 （*n* =16）	3 （18.8）	5 （31.3）	4 （25.0）	1 （6.3）	2 （12.5）	1 （6.3）	0
2021 （*n* =23）	5 （21.7）	11 （47.8）	3 （13.0）	3 （13.0）	0	1 （4.4）	0
Total （*n* =145）	27 （18.6）	51 （35.2）	12 （8.3）	13 （9.0）	18 （12.4）	15 （10.3）	9 （6.2）

Year（ETEC）	Adhesin（%）			
	F4	F5	F18	Unknown
2018 （*n* =67）	3 （4.5）	2 （3.0）	20 （29.9）	42 （62.7）
2019 （*n* =39）	1 （2.6）	0	8 （20.5）	30 （76.9）
2020 （*n* =16）	0	0	2 （12.5）	14 （87.5）
2021 （*n* =23）	0	0	7 （30.4）	16 （69.6）
Total （*n* =145）	4 （2.8）	2 （1.4）	37 （25.5）	102 （70.3）

Table 2

Table 2 Antimicobial agents susceptibility testing of ETEC and EPEC.

	ETEC（n =142）			EPEC（n =74）			p-value[b]
	MIC_{50}	MIC_{90}	R[a]	MIC_{50}	MIC_{90}	R[a]	
CFZ	>1024	>1024	69%	512	>1024	73%	0.54
CEF	8	>256	57%	8	64	64.9%	0.27
GEN	32	>256	60.6%	64	>256	70.3%	0.16
OTC	1024	>1024	100%	512	>1024	98.6%	0.17
DOX	16	32	64.1%	16	64	79.7%	<0.05*
FFC	512	1024	100%	1024	>1024	100%	-
NAL	64	512	71.1%	256	512	94.6%	<0.01*
UB	8	1024	54.9%	16	1024	82.4%	<0.01*
ENR	0.5	32	31.7%	2	64	60.8%	<0.01*
CIP	0.25	32	32.4%	1	16	55.4%	<0.01*
TS	>1024	>1024	88.7%	>1024	>1024	95.9%	0.08
COL	4	16	62.7%	4	8	68.9%	0.36

CFZ, Cefazolin; CEF, Ceftiofur; GEN, Gentamicin; OTC, Oxytetracycline; DOX, Doxycycline; FFC, Florfenicol; NAL, Nalidixic acid;

UB, Flumequine; ENR, Enrofloxacin; CIP, Ciprofloxacin; TS, Trimethoprim-sulfamethoxazole; COL, Colistin;

MIC, Minimum inhibitory concentrations.

[a]: Resistance percentages, [b]: *Chi*-square test, *: Significant（p <0.05）.

Reference of cut off: CLSI, 2022

Graphs or Images 1

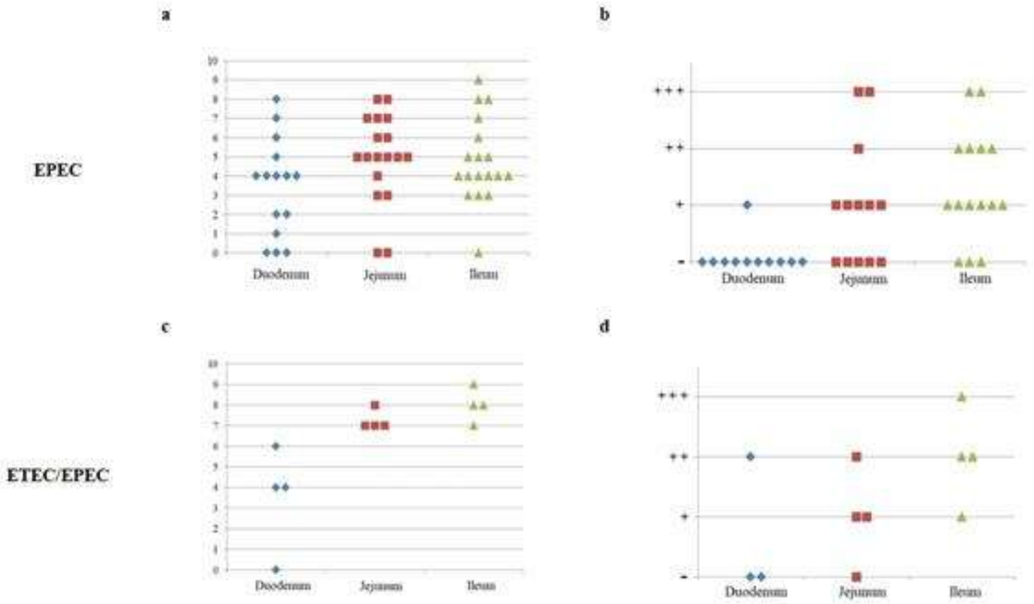

Fig. 1 Histopathologic scoring of intestines tissues examined.（a, c）Total scores of histopathology lesions, H&E stain.（b, d）Scores of anti-EPEC immunohistochemistry.

P-B-004

A SURVEY OF RESPIRATORY HEALTH IN FINISHING PIGS AT SLAUGHTER AGE IN TAIWAN

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Introduction
Lung scoring at the slaughterhouse is a valuable tool for assessment of the farm's respiratory health status covering a large group of animals and avoiding risks of contamination due to farm visits. It provides more information to the farm, as more samples could be evaluated at a single lung scoring session, the data are stored electronically for detailed evaluation, and could be collected from all finishers, compared to necropsies on the farm. Moreover, a clear relationship between lung lesions present at slaughterhouses and the economic impact of respiratory disease has been reported[1], making lung scoring an attractive tool for decision-making and effective monitoring of veterinary interventions.
Ceva updated the lung scoring results among 7 Asian countries back in 2017 [2]. That report shows that a median of 72.5% of lungs had bronchopneumonia, and a median of 14% of lungs had dorsocaudal pleurisy. Here we present data from Taiwan to show the high prevalence of lung lesions in recent years.

Material and Methods
Between January 2019 and Dec 2022, a total of 177 batches of pigs (6,155animals) were scored at the time of slaughter, using CLP.
Lungs were scored following the CLP method [3], with the presence, type, and extension of lung lesions described as:
• Enzootic pneumonia (EP)-like lesions following a modified Madec methodology.
• Cranio-ventral pleurisy, to describe EP-associated secondary pleurisy.
• Scarring, describing the prevalence of fissures associated with older EP-like lesions.
• Dorsocaudal pleurisy (DCP), to describe Actinobacillus pleuropneumoniae (APP)-like lesions
- Actinobacillus pleuropneumoniae Index (APPI), using prevalence and grade of DCP (scale 0-4).

Results
Results for the Asian region are presented in Table 1 and 2 using percentiles (P_{25} - median - P_{75}).

Conclusion
Compared to the publications in other countries, both EP- and APP-like lesions are more prevalent and severe in Taiwan, as demonstrated in this study. More research would be warranted on the reasons and the solutions to these problems.

References
[1] Hill et al., 1994. Res Vet Sci. 56: 240-4.
[2] Boonyawatana et al., 2019. Proceedings 7th Asian Pig Veterinary Society Congress.

[3] Krejci et al., 2013. Proceedings 6th Asian Pig Veterinary Society Congress, OR6.

Table 1

Table 1: EP-like lesions

	P_{25}	Median	P_{75}
Prevalence of bronchopneumonia	80.0%	90.6%	95.0%
% lung surface with bronchopneumonia	5.5%	8.6%	11.6%
% Cranio-ventral pleurisy	10.1%	23.4%	40.7%
% Scars	6.7%	9.2%	12.4%

Table 2

Table 2: AP-like lesions

	P_{25}	Median	P_{75}
DCP prevalence	10.0%	26.7%	47.8%
APP index	0.30	0.80	1.33

P-B-005

A SURVEY ON PURULENT VERTEBRAL OSTEOMYELITIS OF SWINE CARCASSES IN KOREA

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Background
Vertebral osteomyelitis is a type of osteomyelitis (infection and inflammation of the bone and bone marrow) that affects the vertebrae. Purulent vertebral osteomyelitis is most often secondary bacterial infections by skin trauma or septicemia, such as wounds from castration, tail biting, and tail docking. Trueperella pyogenes and other pyogenic bacteria, like Streptococcus spp. and Staphylococcus spp., are common causes of purulent osteomyelitis in pigs and other farm animals.

Purulent vertebral osteomyelitis is the highly portion of carcass disposal in slaughter house. In national regulation, Purulent vertebral osteomyelitis carcass must be remove as unfit for meat hygiene, because of affected by a generalized disease, such as septicemia, pyemia, toxemia, or viremia. For this reason, It has a significant impact on farm income.

The purpose of this study was to investigate the classification of Purulent vertebral osteomyelitis using macroscopic findings on slaughter house and to find the cause of occurrence.

Materials and Methods
This survey was conducted on 1,086,200 pigs in one slaughter house from 2021 to 2022. During this period, 487 cases were investigated as purulent vertebral osteomyelitis. The incidence of purulent vertebral osteomyelitis were compared by month of occurrence or season. Total 317 pigs in 2022 which have purulent vertebral osteomyelitis were divided into region of affected vertebrae by macroscopic characteristics.

Results
From the 1,086,200 finisher pigs slaughtered during 2021 and 2022, 487 carcasses (0.04%) were totally condemned. Compared to 2021, the number of slaughtered finisher pigs in 2022 is the same, but the incidence of purulent vertebral osteomyelitis increased by 53%. Both years showed the highest occurrence of purulent vertebral osteomyelitis in November and December. As a result of the macroscopic inspection, there were no lesions in the cervical region, and 30.4% in the thoracic spine, 52.1% in the lumbar spine, 26.0% in the sacrum, and 4.3% in the coccygeal were classified.

Discussion
Based on the Korea Meat Inspection legislation, meat from purulent vertebral osteomyelitis presenting signs of pyemia should be declared unfit for human consumption which causes great economic loss. Further survey is needed to investigate the cause of the purulent vertebral osteomyelitis to reduce the economic loss on farms.

References
1. Madalena et al. 2020 Oct 20;9(10):1502.

P-B-006

ACTINOBACILLUS PLEUROPNEUMONIAE SURVEY IN VIETNAM

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Introduction
Porcine pleuropneumonia is a common disease in Vietnam1. It is an economically devastating disease usually affecting heavy finishers and even replacement animals. This study aims to update previous prevalence studies on Actinobacillus pleuropneumoniae (APP) in Vietnam.

Materials and Methods
The study involved nationwide testing sampling using serum samples from farms in North (n=8), Central (n=4) and South Vietnam (n=11), totaling 1700+ samples. Sampling was done in 2022. A total of 10 samples fromon ages 6, 10, 12, 16, and 20 weeks, including Gilts and Sows of variusvarious parities were sampled.Thecollected. The APP determination was done using the App APX IV Test kit (IDEXX) which detects all known serotypes. Analysis of trends (effect of age, geography, temporal use of vaccine) was also carried out.
Results
Out of the more than 1700 samples, 774 samples (45.34%) were positive for the App antibodies, with the rest either being negative or suspect. Overall, 83% of farms were positive for APP, with some regional variation (Figure 1). The highest positivity (at 47,82%) was found in samples from South of Vietnam while the lowest was in Central Vietnam (41.80%) (table 1).

Table 1.

Declining maternal derived antibodies were detected until 12 weeks of age. Seroconversion was detected in some herds around 8 weeks or after 16 weeks. There were also some regional differences in Parity positivity. In the North, except for replacement gilts, the positivity rate was above 50% from Parity 1-5 while in the South, the positivity rate was increasing as the Parity number increases. Vaccination status of the herd did not appear to influence sow serological status.

Figure1.

Conclusion
In this study, the prevalence of Actinobacillus pleuropneumoniae was updated and was high in all regions of Vietnam. While most of the positives were in finishing pigs and Sows, there were some regional, herd and parity differences in the samples and farms tested. It is important to note that some farms were negative or had a very low number of positive samples in each Region. Typically, as previously described colostral antibodies may persist for about 5-12 weeks2. Control programs should consider these epidemiological features. Further studies should look further into these differences
References
1. Phu et al (2005) Determination the causes of respiratory disease in pigs rearing at some different provinces in North Vietnam. Agris.fao.org
2. Zimmermann et al (2015) Diseases of Swine (10th ed) pp. 653-665.

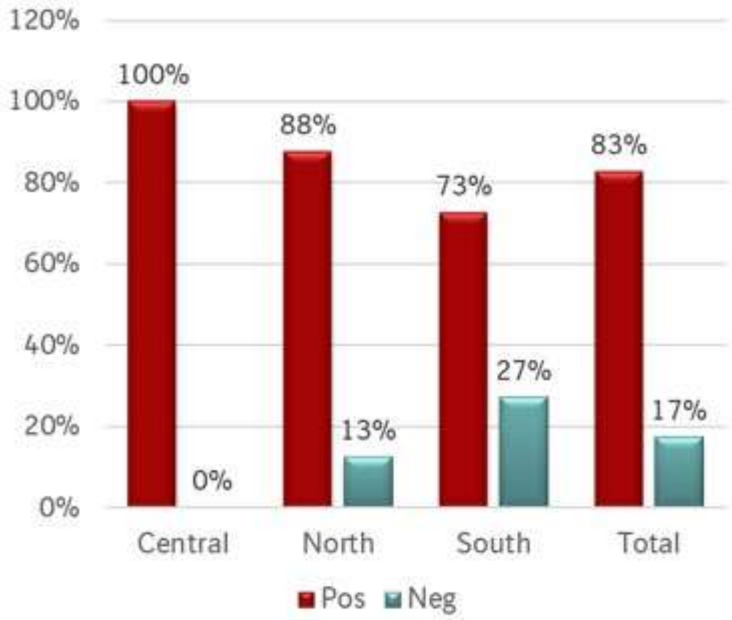
Table 1

Table 1. App Positivity Rate of Samples from 3 regions in Vietnam

Area	Pos	Neg	Sus	Total	% Positive
North	288	363	9	660	43.64%
Central	102	135	7	244	41.80%
South	384	397	22	803	47.82%
Total	774	895	38	1707	45.34%

Graphs or Images 1

Figure1. Prevalence of APP positive farms (%)



P-B-007

ANALYSIS OF 4 TRIALS IN KOREA OF 2 COMBINATION PORCINE CIRCOVIRUS 2 (PCV2) AND MYCOPLASMA HYOPNEUMONIAE (MHYO) VACCINES

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Introduction
Porcine Circovirus 2 and Enzootic Pneumonia are economically damaging diseases1, affecting mortality and growth rates in pigs. Vaccination is one major way to prevent or lessen the impact of these 2 diseases. Pigs in Asia are exposed to higher challenges related to different pathogens, so an effective prevention of PCV2 and M. hyopneumoniae becomes even more critical. . This review compares the efficacy results observed in four studies evaluating two different vaccine treatments in farms in South Korea.

Materials and Methods
The studies were performed in four different PRRS negative commercial farrow-to-finish single site farms, ranging from 180 to 650 sows in various provinces of South Korea (Table 1). The efficacy parameters measured as mortality %, body weight gain and average daily weight gain (ADG), as well as group homogeneity (reduced weight variation within the group) were evaluated. Two PCV2 and M. hyopneumoniae vaccine treatments were applied side by side: FLEXcombo (Boehringer Ingelheim) and Porcilis PCV Mhyo (MSD), randomly and equally assigned as T1 and T2 respectively.
Table 1. Farms included in the analyses.
Treatments were administered at weaning (21-28 days) according to label recommendation. Pigs were individually weighed at weaning (D0), nursery (D70) and three days before shipment to slaughter. In total, 1042 animals within the four trials were included in this analysis.
Analysis of mortality was done using Chi-Square analysis. Weights were analyzed using One Way Analysis of Variance (ANOVA) and Histogram distribution curves (Minitab 18) for uniformity. An economic analysis was performed considering the cost/benefit of the different treatments and the return on investment (ROI) was calculated using BECALTM.

Results
Individual trial results are shown in Table 2. In all farms, T1 group was statistically superior in average Ending Weight (Farm A: +3.2 kg; Farm B: +2.7 kg; Farm C: +4.6 kg; Farm D: +5.9 kg) and ADG (Farm A: +19g; Farm B: +16g; Farm C: +35g; Farm D: +42g). In 3 out of 4 farms T1 group had better uniformity, demonstrating lower weight variation within the group. Final weights were all statistically significant in favor of T1 group. Mortality differences were not statistically significant.
Table 2. Individual Trial Results.
The Return on Investment of implementing T1 over T2 was 2.47:1 for Farm A, 8.7:1 for Farm B 12.89:1 for Farm C. 13.9:1 for Farm D.

Discussion
Analyzing the results of the 4 studies together, there was a 4.2 kg difference of the final weight in favor of T1 group (FLEXcombo) with a lower Standard Deviation, confirming a

better group homogeneity. The differences on Final Weight and ADG were statistically significant (P <0.0001) between groups (Figure 1 and 2). The favorable results for FLEXcombo are consistent with previous studies in Asia2, reinforcing the impact of making the right PCV2/Mhyo vaccine selection on optimal animal performance.

References

1. Holtkamp and Rotto (2008) Proc ISU Swine Disease Conf, 91-97
2. Oh et al (2021). Proc of the Leman Conference. Poster Proc Page 1.

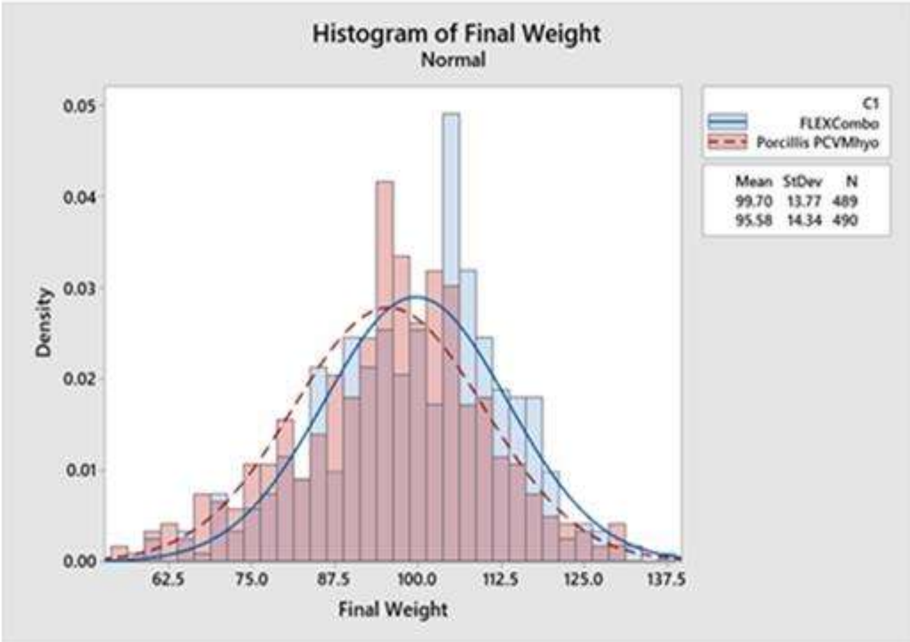
Table 1

Farm	Farm Size	Number of pigs
Farm A	250 sows	258
Farm B	300 sows	240
Farm C	650 sows	290
Farm D	180 sows	254

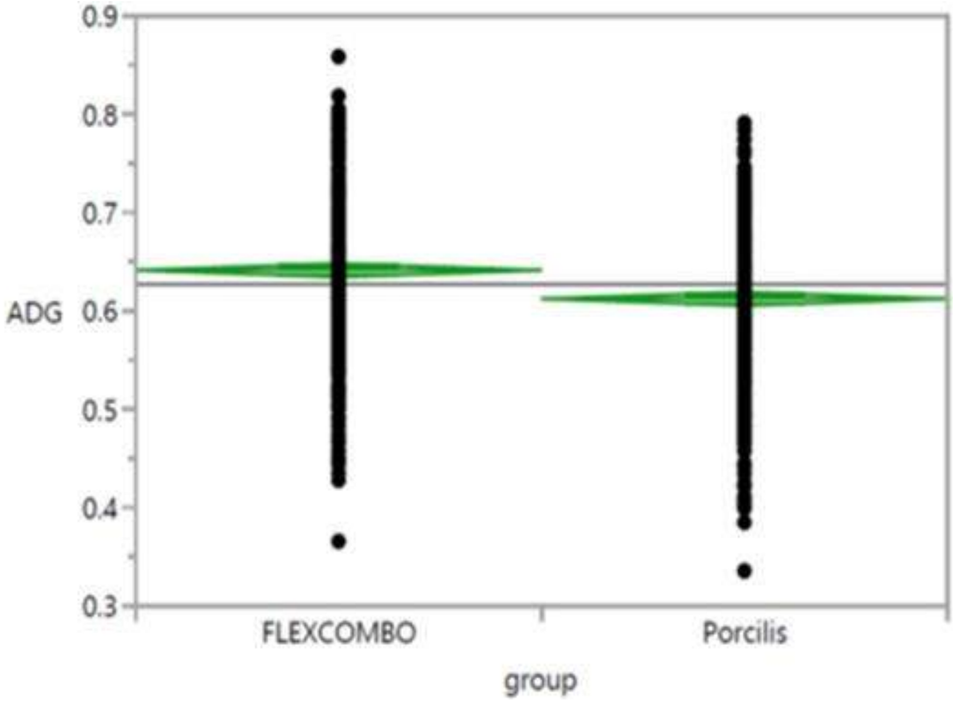
Table 2

Farm	Group	Mortality	Average Body Weight (kg)	Weight Variation (Stdev)	P value of Weights ($P \le 0.05$.)	ADG (kg/day)	P Value of ADG
Farm A	T1	15%	105.5	11.43	0.0186	0.586	0.0186
	T2	12%	102.2	12.13		0.568	
Farm B	T1	3.3%	107.7	9.91	0.0253	0.645	0.0253
	T2	4.2%	105.0	10.69		0.629	
Farm C	T1	6.21%	84.7	10.38	0.0002	0.621	0.0002
	T2	6.90%	80.1	10.84		0.586	
Farm D	T1	2.31%	103.5	8.475	<0.0001	0.707	<0.0001
	T2	2.31%	97.61	7.719		0.665	

Graphs or Images 1



Graphs or Images 2



P-B-008

ANTIMICROBIAL SUSCEPTIBILITY OF ACTINOBACILLUS PLEUROPNEUMONIAE IN TAIWAN

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Introduction:
Actinobacillus pleuropneumoniae (App) is the etiologic agent of porcine pleuropneumonia that causes great economic losses in the swine industry, this pathogen has high morbidity and mortality rates which affects pigs of all ages, and induces fibrinohemorrhagic and necrotizing pleuropneumonia. The clinical course can be divided into three different stages, peracute, acute and chronic [1]. Sudden death and outbreak of peracute and acute cases grab attention but chronic cases also impair profits of pig farms due to poor feed conversion rates. Vaccination can basically reduce clinical signs and lung lesions, but therapy based on accurate antimicrobial advice is still needed to decrease mortality and case numbers [2]. Therefore, the aim of this study was to evaluate the antimicrobial susceptibility of Actinobacillus pleuropneumoniae in Taiwan in 2021.

Materials and Methods:
A total of 35 Actinobacillus pleuropneumoniae isolates were collected in 2021 in Taiwan. The 19 examined antimicrobial agents included trimethoprim/sulfamethoxazole (1.25/23.75 μg), lincomycin/spectinomycin (109 μg), amoxicillin (25 μg), ampicillin (10 μg), enrofloxacin (5 μg), flumequine (30 μg), cephalexin (30 μg), cephalothin (30 μg), ceftiofur (30 μg), doxycycline (30 μg), oxytetracycline (30 μg), florfenicol (30 μg), gentamicin (10 μg), spiramycin (100 μg), tilmicosin (15 μg), streptomycin (10 μg), kanamycin (30 μg), apramycin (15 μg) and colistin (10 μg). The agar disk diffusion method was employed in antimicrobial susceptibility testing according to the procedures outlined in the Clinical and laboratory standards institute document M31-A3 [3].

Results:
Our results showed that more than 70% of Taiwanese Actinobacillus pleuropneumoniae isolates were sensitive to trimethoprim/sulfamethoxazole, lincomycin/spectinomycin, enrofloxacin, cephalexin, cephalothin, ceftiofur, tilmicosin and colistin. Besides, most Taiwanese Actinobacillus pleuropneumoniae isolates were resistant to ampicillin, doxycycline, oxytetracycline, spiramycin, streptomycin and apramycin.

Conclusions:
For oral group medication in pigs, trimethoprim/sulfamethoxazole, lincospectin, and tilmicosin can be added to feed or drinking water. Cephalexin, cephalothin, ceftiofur and enrofloxacin can be used for individual treatment of pigs with clinical signs of porcine pleuropneumonia.

Acknowledgement:
We gratefully acknowledge the technicians and veterinarians in ADDC of NPUST for their contributions to this study.

References:
[1] Zimmerman, J. J., 2019. Diseases of swine (11th ed.)
[2] Vanni M. et al., 2012. Antimicrobial resistance of Actinobacillus pleuropneumoniae isolated from swine.
[3] Clinical and laboratory standards institute (CLSI) Performance standards for antimicrobial disk and dilution susceptibility test for bacteria isolated from animals; Approved Standard, 3rd ed. CLSI document M31-A3. Wayne, USA: CLSI; 2008

Graphs or Images 1

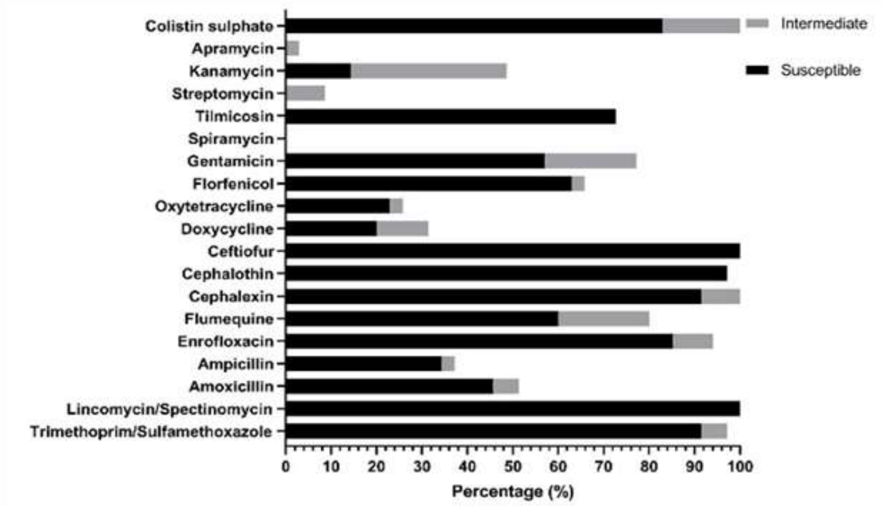

Figure 1. Antimicrobial disk susceptibility of *Actinobacillus pleuropneumoniae*

Data were shown as percent of sensitive and intermediate isolate (number of sensitive and intermediate isolate/examined isolate).

P-B-009

ANTIMICROBIAL SUSCEPTIBILITY TESTING OF BRACHYSPIRA HYODYSENTERIAE IN TAIWAN

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Introduction
Brachyspira hyodysenteriae are the most important pathogen of swine dysentery (SD). This disease mostly occurs in growing to finishing pigs. In recent years, it has also been seen in suckling pigs and led to severe economic losses due to decreased feed intake and increased feed conversion ratio. Antimicrobial agents, such as tylosin, lincomycin and tiamulin, are widely used to control the infection of Brachyspira hyodysenteriae in Taiwan. However, some antimicrobial agents with low clinical efficacy are used for treating Brachyspira hyodysenteriae and resistance of antimicrobial agents has been reported in many countries. In those studies, it is suggested that reduced susceptibility to pleuromutilins is correlated with tva(A) in combination with single point mutations, which leads to higher-level resistance in Brachyspira hyodysenteriae strains [1].The aim of this study is to analyze the antimicrobial susceptibility of Brachyspira hyodysenteriae isolated from Taiwan and to investigate the mechanism of decreased susceptibility to pleuromutilins.

Materials and Methods
A total of 37 Brachyspira hyodysenteriae isolates obtained from diseased pigs were evaluated in this study. Antimicrobial susceptibility testing was performed to examine selected antimicrobial agents. All antimicrobial agents were evaluated using the agar dilution method. Detection of tva(A) gene was performed by using polymerase chain reaction (PCR).

Results
The results showed that 50% and 90% minimum inhibitory concentration (MIC50 and MIC90) of pleuromutilins were both higher than 32 μg/mL. The MIC50 of lincomycin, tylosin, tylvalosin, carbadox, doxycycline and enrofloxacin were 16, 512, 2, 0.625, 0.25 and 0.25 g/mL. Additionally, results obtained from PCR were confirmed that pleuromutilins resistance strains carry tva(A) gene.

Conclusions and Discussion
Our results are consistent with the study from other country, in which MIC value of the strains carrying tva(A) gene is higher than the strains without tva(A) gene [2]. In conclusion, the results of this study indicate that Brachyspira hyodysenteriae may be developing resistance to antimicrobial agents commonly used for the treatment of SD in Taiwan.

Acknowledgement and References
I would like to express our thanks to all the members of the Bacteriology Laboratory in the department of Veterinary Medicine, National Chiayi University, Taiwan.
[1] Card RM et al., 2018. Front Microbiol 9: 1183.
[2] Stubberfield E et al., 2020. Vet. Microbiol. 244: 108645

Table 1

Table 1. MIC_{50}, MIC_{90} of *Brachyspira hyodysenteriae* isolated from diseased swine.

Antibiotics	MIC_{50} (μ g/mL)	MIC_{90} (μ g/mL)
pleuromutilins	>32	>32
lincomycin	16	256
tylosin	512	>512
tylvalosin	2	32
carbadox	0.0625	0.5
doxycycline	0.25	2
enrofloxacin	0.25	4

P-B-010

ANTIMICROBIAL SUSCEPTIBILITY OF GLAESSERELLA PARASUIS IN TAIWAN

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Introduction:
Glaesserella (Haemophilus) parasuis (G. parasuis), is the causative agent of Glässer's disease, which is characterized by sudden death, polyserositis, polyarthritis, meningitis, pneumonia and poor production performance and cause severe economic losses in the swine industry [1, 2]. One of the main strategies to control and prevent Glässer's disease is using antimicrobials. If Glässer's disease doesn't be treated with effective antimicrobials in time, the lesions process from acute fibrinous serositis to chronic fibrous serositis which impairs swine growth rate and feed conversion rate. It usually takes longer time to perform G. parasuis antimicrobial susceptibility testing than other common porcine bacterial pathogens due to its slow growth rate. Therefore, the aim of this study is to screen out effective antimicrobials for initial Glässer's disease treatment and to monitor the antimicrobial resistance of G. parasuis.

Materials and Methods:
A total of 200 G. parasuis isolates were collected from Glässer's disease cases in 2021 in Taiwan. The 19 examined antimicrobial agents included trimethoprim/sulfamethoxazole (1.25/23.75 µg), lincomycin/spectinomycin (109 µg), amoxicillin (25 µg), ampicillin (10 µg), enrofloxacin (5 µg), flumequine (30 µg), cephalexin (30 µg), cephalothin (30 µg), ceftiofur (30 µg), doxycycline (30 µg), oxytetracycline (30 µg), florfenicol (30 µg), gentamicin (10 µg), spiramycin (100 µg), tilmicosin (15 µg), streptomycin (10 µg), kanamycin (30 µg), apramycin (15 µg) and colistin (10 µg). The agar disk diffusion method was employed in antimicrobial susceptibility testing according to the procedures outlined in the Clinical and laboratory standards institute document M31-A3 [3].

Results:
Our results showed that more than 80% of Taiwanese G. parasuis isolates were sensitive to amoxicillin, cephalexin, cephalothin, ceftiofur, doxycycline, florfenicol and colistin. Besides, most Taiwanese G. parasuis isolates were resistant to trimethoprim/sulfamethoxazole, flumequine oxytetracycline, spiramycin, tilmicosin, streptomycin and apramycin.

Conclusions:
For initial treatment, amoxicillin, florfenicol and doxycycline can be considered to use in feed or drinking water to prevent and control Glässer's disease in pig herds. Cephalexin, cephalothin and ceftiofur can be used for individual treatment of acute-phase diseased pigs.

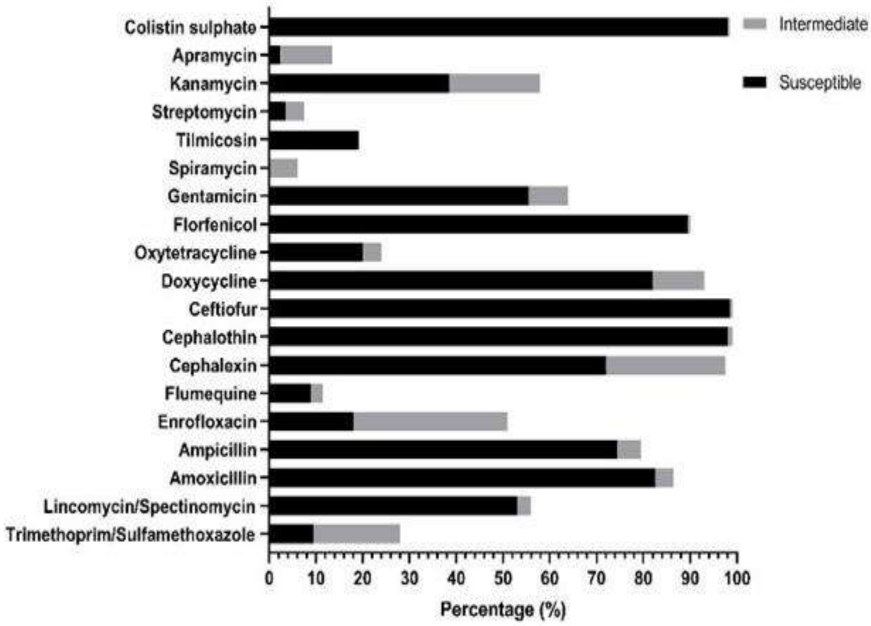
Acknowledgement:
We gratefully acknowledge the technicians and veterinarians in ADDC of NPUST for their contributions to this study.

References:

[1] Gatto I.R.H. et al., 2019. Front. Vet. Sci. 6: 35
[2] Hause B.M. et al., 2015. J. Gen. Virol. 96(10): 2994-2998.
[3] de Groof A. et al., 2016. Viruses. 8(10): 271

Figure 1. Antimicrobial susceptibility of G. parasuis
Data were shown as percent of sensitive and intermediate isolate (number of sensitive and intermediate isolate/examined isolate).

Graphs or Images 1



P-B-011

ANTIMICROBIAL SUSCEPTIBILITY OF PASTEURELLA MULTOCIDA IN TAIWAN

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Introduction:
Pasteurella multocida is an important swine respiratory pathogen causing progressive atrophic rhinitis and pneumonia. It is regarded as a common component agent of the porcine respiratory disease complex (PRDC) [1]. The usage of antimicrobial agents, such as β-lactams, sulphonamide/trimethoprim, florfenicol, macrolides and tetracyclines remains to be the common treatment to control PRDC. Antibiotics have been widely used for the treatment of Pasteurella multocida, and the incorrect long-term antibiotic exposure will cause antimicrobial resistance strain issues [2, 3]. The objective of this study is to report antimicrobial susceptibility for Pasteurella multocida in Taiwan.

Materials and Methods:
A total of 66 Pasteurella multocida isolates were collected from diseased pigs in 2021 in Taiwan. The 19 examined antimicrobial agents included trimethoprim/sulfamethoxazole (1.25/23.75 µg), lincospectin (109 µg), amoxicillin (25 µg), ampicillin (10 µg), enrofloxacin (5 µg), flumequine (30 µg), cephalexin (30 µg), cephalothin (30 µg), ceftiofur (30 µg), doxycycline (30 µg), oxytetracycline (30 µg), florfenicol (30 µg), gentamicin (10 µg), spiramycin (100 µg), tilmicosin (15 µg), streptomycin (10 µg), kanamycin (30 µg), apramycin (15 µg) and colistin (10 µg). The agar disk diffusion method was employed in antimicrobial susceptibility testing according to the procedures outlined in the Clinical and laboratory standards institute document M31-A3 [4].

Results:
Our results indicated that more than 80% of Taiwanese Pasteurella multocida isolates were still sensitive to lincomycin/spectinomycin, enrofloxacin, cephalexin, cephalothin, ceftiofur, tilmicosin and colistin. Besides, most Taiwanese Pasteurella multocida isolates were resistant to spiramycin, streptomycin and apramycin. injection in Taiwan.

Conclusions:
For Pasteurella multocida pneumonia control, lincospectin, enrofloxacin and tilmicosin can still be used in feed as herd treatment in the nursery stage. Cephalexin, cephalothin and ceftiofur can be used for individual treatment of PRDC pigs with Pasteurella multocida infection to decrease the mortality rate. The antimicrobial resistance of Pasteurella multocida should be further concerned in the future due to zoonotic public health issues.

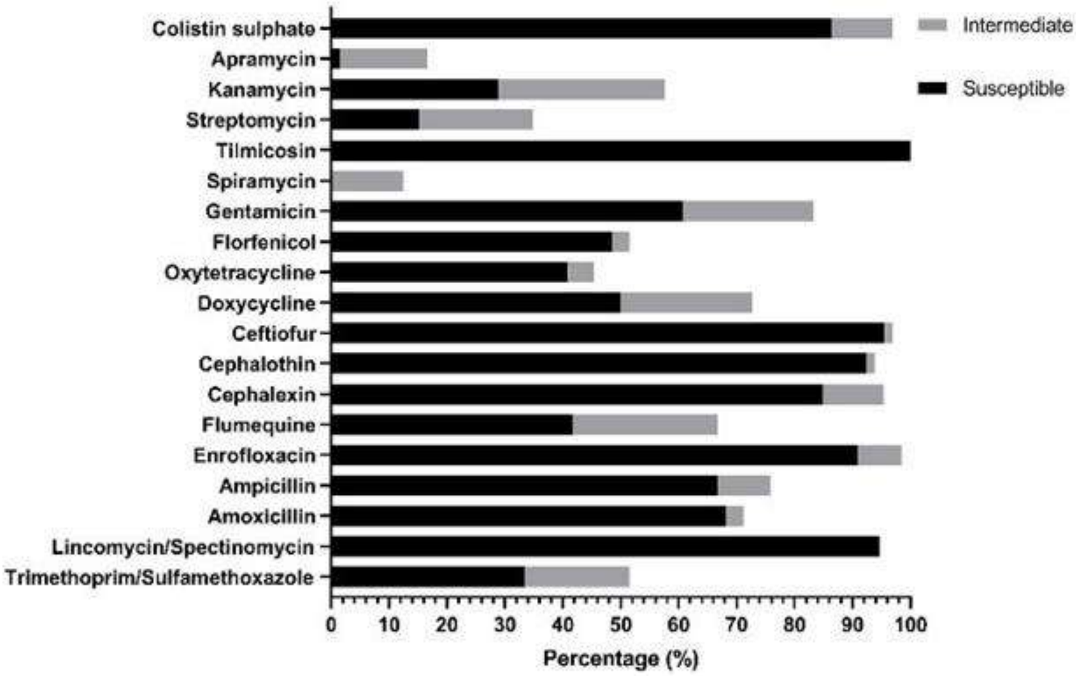
Acknowledgement:
We gratefully acknowledge the technicians and veterinarians in ADDC of NPUST for their contributions to this study.

References:
[1] Zimmerman, J. J., 2019. Diseases of swine (11th ed.)

[2] Vu-Khac H. et al., 2020. Prevalence of virulence factor, antibiotic resistance, and serotype genes of Pasteurella multocida strains isolated from pigs in Vietnam.
[3] Petrocchi-Rilo M. et al., 2020. Antimicrobial resistance of Pasteurella multocida isolates recovered from swine pneumonia in Spain throughout 2017 and 2018.
[4] Clinical and laboratory standards institute (CLSI) Performance standards for antimicrobial disk and dilution susceptibility test for bacteria isolated from animals; Approved Standard, 3rd ed. CLSI document M31-A3. Wayne, USA: CLSI; 2008

Figure 1. Antimicrobial susceptibility of Pasteurella multocida
Data were shown as percent of sensitive and intermediate isolate (number of sensitive and intermediate isolate/examined isolate).

Graphs or Images 1



P-B-012

ASSESSMENT OF ENZOOTIC PNEUMONIA-LIKE LESIONS IN SLAUGHTERED PIGS IN CHINA IN 2022

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[1]Ceva Sante Animale, [2]Ceva Sante Animale

Introduction
Lung scoring at the slaughterhouse is a valuable tool for assessment of the respiratory health status including Enzootic pneumonia (EP) causing economic losses in swine production (1). Different from post-mortem investigation, the scoring method assesses lung health across the whole batch of animals. Moreover, a clear relation between lung lesions present at slaughterhouse and economic impact of respiratory disease has been reported (2), making lung scoring an attractive tool for decision making and efficient monitoring of veterinary interventions.
Ceva Lung Program (CLP) has been widely used, facilitating lung scoring more efficient and hygienic at slaughterhouses (3). Wechat and iOS versions of CLP are all available in China.

Materials and Methods
From 1st January 2022 to 31st December 2022, a total of 698 batches of slaughtered pigs' lungs (35,494pigs) were scored, using CLP methodology (Fig 1). It was performed in more than 10 different Provinces in China, with presence, type and extension of lung lesions described by:
-% of lungs with EP-like lesions following a modified Madec methodology.
-% of affected lung parenchyma- to describe average infected area in lungs with EP-like lesions.
-Enzootic pneumonia Index (EP index), to describe average score of pneumonia-like lesions in all lungs.
-% of lungs with scars, describing prevalence of fissures associated with older EP-like lesions.

Results
Results of lung lesion scoring in slaughtered pigs were presented in Table 1 using percentiles (P25 -median - P75). The results of the lung lesion scoring indicated high prevalence of EP-like lesions in China under African swine fever epidemic environment. The median prevalence of lungs with EP-like lesions and affected lung parenchyma was 54.50% and 4.56% respectively.

Conclusions and Discussion
The results of lung lesion scoring clearly indicated there was high room for improvement in China. Ceva Lung Program (CLP) could be a very useful tool for lung lesion scoring, as well as a benchmark for farms to improve enzootic pneumonia status.

References
1. Ostanello F. et al. Journal of Veterinary Medicine Series A. 20.
2. Hill etal, 1994. Res Vet Sci. 56: 240-4.
3. Krejci et al, 2013. Proceedings 6th Asian Pig Veterinary Society Congress, OR6.

Table 1

Table 1 EP-like lesions results

	P25	Median	P75
% of lungs with EP-like lesions	38.71	54.50	71.43
% of affected lung parenchyma	3.03	4.56	6.56
EPI	1.00	1.52	2.46
% of lungs with scars	5.88	14.29	26.54

Graphs or Images 1

Fig.1 Lung lesion scoring at the slaughterhouse

P-B-013

ASSESSMENT OF PORCINE LUNG LESIONS AT SLAUGHTERHOUSE FORM BATCHES VACCINATED WITH DIFFERENT COMMERCIAL MYCOPLASMA HYOPNEUMONIAE & PCV2 VACCINES

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Introduction
Enzootic pneumonia, which is mainly caused by Mycoplasma hyopneumoniae (M. hyo), can be effectively controlled by vaccination. And one of the ways to evaluate vaccination processes is through lung lesions in the slaughterhouse scoring methods (1). Among the available vaccination protocols, single-dose ready-to-mix (RTM) formulations stand out. The aim of this study was to examine the possible association of lung lesions at slaughter with two different commercial single-dose vaccines against M. hyo.

Material & Methods
From January to November 2022, a total of 23,865 lungs were evaluated at slaughterhouse according to the Ceva Lung Program methodology, in Ecuador and Venezuela. The animals involved in this evaluation underwent two different protocols at weaning: Animals in Group 1: vaccination with a single dose of Hyogen and Circova (Vaccine A), with 131 batches of 16,586 lungs in total and the Group 2: Single dose vaccination of Mhyo + PCV2 (RTM) of 2 ml IM (Vaccine B), with 75 batches of 7,289 lungs in total. Lung lesion scores were analyzed at the batch level. The frequency of bronchopneumonic lungs (BP) and the percentage of lung surface affected in both vaccination groups were statistically compared.

Results
The evaluated lungs of the animals vaccinated with vaccine A showed a frequency of BP of 40.4% while that of vaccine B was 78%. Regarding the severity of bronchopneumonic lesions, the percentage of affected area was 4.4% and 10% for vaccines A and B, respectively. Animals vaccinated with vaccine A had an average of 19.4% of scar lesions, and animals vaccinated with vaccine B had 26.3%.

Tab 1. CLP results of Vaccine A or Vaccine B vaccinated batches of pigs

Discussion and conclusion
The results showed that Hyogen and Circovac was able to reduce the prevalence and severity of bronchopneumonia lesions when compared to the protocol with the B vaccine. This is reflecting better respiratory health of pig batches.

Table 1

CLP results of Vaccine A or Vaccine B	Vaccine A	Vaccine B
# CLP	131	75
# Lungs	16586	7289
% Bronchopneumonic Lungs	40,4%	77,8%
% Affected Area of BP	4,4%	10%
% Scars	19%	26%

P-B-014

ASSESSMENT OF THE FIELD EFFICACY OF A READY-TO-USE TRIVALENT VACCINE ADJUVANTED WITH HIPRAMUNE®G ON A COMMERCIAL PIG FARM IN THAILAND

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[1]*HIPRA*, [2]*HIPRA Thailand*

Introduction
Swine Erysipelas (SE), parvovirosis, and leptospirosis are the most frequent infectious diseases that cause reproductive disorders in breeder pigs worldwide, caused by Erysipelotrix rhusiopathiae, Porcine Parvovirus (PPV) and Leptospira spp. (1). For their prevention, inactivated trivalent vaccines containing antigens of Erysipelothrix rhusiopathiae, PPV and 6 serovars of Leptospira spp. are commonly applied in each productive cycle (2). The objective of this study was to evaluate the efficacy of a ready-to-use trivalent vaccine adjuvanted with HIPRAMUNE®G on a commercial pig farm in Thailand.

Material and Methods
The study was conducted in a PRRSv, PPV, and SE endemic area in a commercial 3,200-sow farrow to fattening pig production unit located in Lopburi province, Thailand from January 2020 to December 2020. During 2019, reproductive parameters such as the percentage of mummified fetuses (6.41%) and stillbirth piglets (8.54%) were indicating disorders related with infectious diseases. Different reproductive indicators were compared during the period of 2019 using an inactivated trivalent vaccine, containing only 5 serovars of Leptospira interrogans adjuvanted with aluminum hydroxide (Group 1: Vaccine B) (5 mL, IM). And after switching during 2020 to an inactivated trivalent vaccine containing 6 serovars of Leptospira spp. and adjuvanted with HIPRAMUNE®G (2 mL, IM; ERYSENG® PARVO/LEPTO, SPAIN) (Group 2: EPL. Different parameters related to sow performance in group 1 and group 2 were compared using the SPSS statistical program (version 22.0).

Results
Statistically significant lower percentages of mummified foetuses and stillbirth piglets were observed during the period using ERYSENG® PARVO/LEPTO (Table 1).

Discussion & Conclusion
Based on this study's results, after implementing the vaccination with ERYSENG® PARVO/LEPTO reproductive parameters related to infectious diseases were significantly improved compared with the period using Vaccine B. Field data provides key general insights on the evaluation of the impact of the HIPRA vaccine must be considered to use for controlling the swine reproductive failure in Thailand.

References
1. Wrathall AE. 1975. Reproductive disorders in Pigs, rev. series no. 11. Farnhem Royal, England: Commonwealth Agricultural bureau: 1960.
2. Kitchodok et al., 2020. Proceedings IPVS BRAZIL 2020.

Table 1

Table 1. Reproductive parameters comparison between vaccines

Reproductive parameters	EPL	Vaccine B	Difference
Nº vaccinated sows	1,465	1,648	-183
Litter size (pig)	14.08[a]	13.91[a]	+0.17
Weight at birth (kg)	1.46[a]	1.35[a]	+0.11
Total born alive/litter	13.11[a]	12.74[a]	+0.37
Stillbirth piglets (%)	3.48[a]	5.03[b]	-1.55
Mummified foetus (%)	2.21[a]	4.15[b]	-1.94

[a, b] indicates significant differences within main effect ($p \leq 0.05$)

P-B-015

ASSESSMENT OF THE SITUATION OF THE PORCINE ENZOOTIC PNEUMONIA AND PORCINE PLEUROPNEUMONIA IN THAILAND USING LUNG LESION SCORING AT SLAUGHTERHOUSE

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[1]*Ceva Animal Health (Thailand)*

Introduction
Porcine Enzootic Pneumonia (EP) caused primarily by Mycoplasma hyopneumoniae (M. hyo) and Porcine Pleuropneumonia caused by Actinobacillus pleuropneumoniae (A. p) are two of main respiratory problems in pigs and cause a major economic loss in pig's industry. (1,2) Both diseases can cause the lung lesion that is commonly found when finishing pigs enter to slaughterhouse. Lung lesion scoring is an important tool to monitor the prevalence and severity of both diseases. The aim of this study is to describe the EP-like lesion and Ap-like lesion situation in difference regions of Thailand.

Materials and Methods
This study was conducted throughout the year 2022, 96,120 lungs from 2,040 pig batches from both integrated and commercial farms in 6 regions of Thailand were included. Lung lesions were evaluated for EP-like lesion and Ap-like lesion using Ceva Lung Program scoring methodology (CLP): a standardized method assessing the presence and extension of EP and Ap-like lesion described previously. (3)

Results
The lung lesion score results of Thailand and each region of Thailand are shown in table 1 and 2 respectively.

Conclusions and Discussion
The lung lesion score results of 96,120 lungs from 2,040 pig batches at slaughterhouse in 6 regions of Thailand indicated a high rate and severity of EP- in all country, especially in Eastern, Western and Northeastern region that had high density of pig population. Likewise, the severity of Ap-like lesion was higher in Eastern and Northeastern region. Control of M. hyo and A. p remain an important practice in Thailand's farm and should be monitor regularly. The CLP methodology is an effective and convenient tool to monitor the prevalence and severity of EP and Ap-like lesions at slaughterhouse.

Acknowledgement and References
1. Maes et al. 2018. Transboundary and Emerging diseases. 65: 110-124.
2. Sassu et al. 2018. Transboundary and Emerging diseases. 65: 72-90.
3. Mombark et al. 2019. APVS 2019 abstract book. 50.

Table 1

Table 1. EP and Ap-like lesion score results of Thailand.

	P25	Median	P75
Bronchopneumonic lungs (%)	35.9%	46.0%	80.8%
Affected surface/pneumonic lungs (%)	2.1%	6.2%	10.1%
Affected surface/all lungs (%)	5.0%	12.2%	22.3%
Scar scoring (%)	2.0%	10.0%	36.7%
Cranial pleurisy scoring (%)	0.0%	6.0%	11.8%
EP index	1.22	2.90	4.26
Dorsocaudal pleurisy (%)	0.0%	4.0%	10.0%
AP index	0.00	0.12	0.30

Table 2

Table 2. EP and Ap-like lesion score results in each region of Thailand, show as the median, P25 and P75.

	C	E	NE	N	S	W
Broncho-pneumonic lungs (%)	49.0 (49.0, 78.5)	76.7 (53.0, 93.3)	45.0 (38.0, 77.2)	30.0 (10.0, 75.0)	32.0 (21.0, 44.0)	75.2 (44.9, 88.5)
Affected surface/ pneumonic lungs (%)	4.7 (4.7, 9.7)	11.5 (7.3, 17.3)	14.7 (7.3, 22.5)	14.8 (3.4, 33.4)	5.8 (2.1, 12.2)	6.9 (3.3, 10.6)
Affected surface/ all lungs (%)	2.2 (2.2, 5.8)	8.6 (4.2, 13.8)	7.6 (4.4, 10.1)	2.8 (1.0, 8.1)	1.5 (0.5, 4.4)	5.2 (1.6, 10.1)
Scar scoring (%)	6.0 (6.0, 14.0)	23.3 (2.9, 50.0)	12.0 (7.4, 70.0)	0.0 (0.0, 15.2)	10.0 (4.0, 21.0)	16.0 (4.0, 23.5)
Cranial pleurisy scoring (%)	1.0 (1.0, 12.0)	3.3 (0.0, 16.7)	10.0 (6.0, 12.0)	0.0 (0.0, 2.4)	0.0 (0.0, 0.0)	11.0 (2.8, 16.5)
EP index	1.50 (1.50, 3.09)	4.51 (0.00, 6.83)	3.18 (2.10, 3.91)	1.25 (0.56, 3.35)	0.78 (0.39, 2.05)	2.91 (1.25, 5.46)
Dorsocaudal pleurisy (%)	0.0 (0.0, 4.0)	10.0 (3.3, 20.0)	6.0 (0.0, 10.0)	3.4 (0.0, 7.6)	0.0 (0.0, 2.0)	2.0 (0.0, 8.3)
AP index	0.00 (0.00, 0.12)	0.30 (0.07, 0.60)	0.18 (0.00, 0.32)	0.08 (0.00, 0.20)	0.00 (0.00, 0.04)	0.04 (0.00, 0.29)

C = Central, E = Eastern, NE = Northeastern, N = Northern, S = Southern, W = Western.

P-B-016

ATROPHIC RHINITIS AS A CAUSATIVE FACTOR FOR POLYSEROSITIS PROBLEMS ON KOREAN SWINE FARMS

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[1]*HIPRA*

Introduction
The nasal turbinate is known to act as a filter that removes pathogens in the air that is inhaled into pigs' respiratory tract (1). One of the causative agents of atrophic rhinitis (AR), Bordetella bronchiseptica is considered not only as destroyer of healthy nasal turbinates but also as predisposing factor to infection of other respiratory pathogens, especially the ones causing polyserositis such as Streptococcus suis and Glaesserella parasuis (2, 3). However, the agent which gets high virulence under the presence of AR problem in the situation of Korean swine farms has not been studied well. The aim of this study was to find out the main secondary infective agent following AR situation in the environment of Korean swine farms.

Materials and Methods
Necropsy was conducted for 20 dead pigs from 7 commercial swine farms distributed in different provinces of South Korea. Lung and snout samples were collected from all animals. Nasal lesions of a transection of snouts at the level of 1st upper premolar and lung lesions of each animal were assessed following the guidelines of European Pharmacopoeia(having a maximum score of 18) and the scoring method described by Madec and Kobisch (having a maximum score of 28), respectively. Information of the existence of polyserositis was also collected from each animal. The PCR assay was performed for each lung sample to detect causative agents for polyserositis such as Glaeserella parasuis, Streptococcus suis and Mycoplasma hyorhinis, which are known as secondary infectious pathogens that follow atrophic rhinitis (AR). In order to check the relationship between nasal lesion score and lung lesion score, Pearson correlation was analyzed. To see the correlation of polyserositis and nasal lesion score and the correlation of polyserositis and lung lesion score, ANOVA was performed. The correlation between polyserositis and PCR positivity of each pathogen was analyzed by binominal regression with Tukey as a posthoc test.

Results
Nasal lesion score had a positive correlation with lung lesion score ($r=0.77$). In case the animals had polyserositis (7 out of 20 animals), the average nasal lesion score and lung lesion score were significantly higher (53.9% and 92.1%, respectively) than the animals without polyserositis ($p<0.05$). The animals with polyserositis showed higher positivity of Glaesserella parasuis by PCR (57.1%) than animals without polyserositis (7.7%). All samples were negative for Streptococcus suis by PCR. The positivity of Mycoplasma hyorhinis was higher in animals with polyserositis (100%) than animals without polyserositis (53.8%), but there was no significant correlation ($p>0.05$).

[Figure 1]. Correlation of polyserositis and nasal lesion score(upper), and correlation of polyserositis and lung lesion score(lower)

[Figure 2]. Correlation of polyserositis and positivity of Glaeserella parasuis

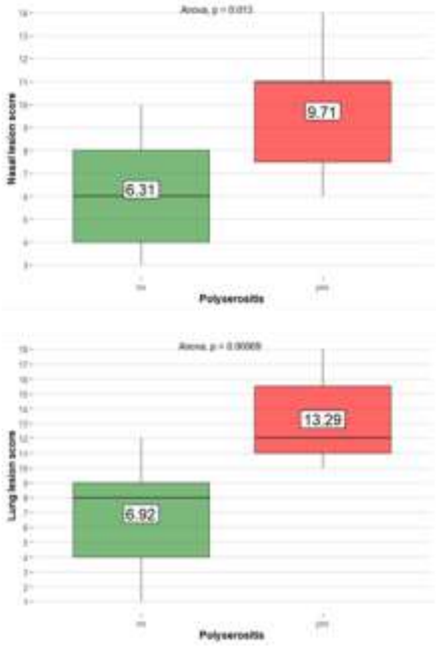
Discussion and Conclusion
As atrophic rhinitis (AR) is highly relevant to lung lesions, AR should be controlled to prevent respiratory problems caused by secondary infectious agents following it (4). If a farm has an

AR problem, it can lead to an infection of Glaeserella parasuis, causing Glasser's disease in the situation of Korean commercial swine farms. To demonstrate the correlation between AR and the infection of Streptococcus suis and Mycoplasma hyorhinis, further study is needed with a higher number of samples.

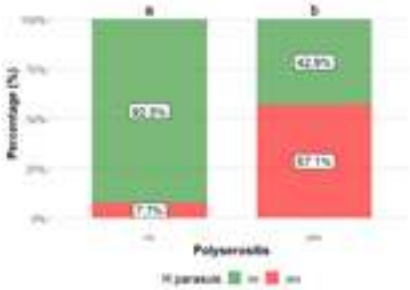
Acknowledgments
The authors would like to acknowledge all the veterinarians and farmers who supported them in obtaining the samples.

References
1. Brockmeir S. Et al. 2019 Diseases of Swine 11th Edition, p. 767-775
2. Brockmeier S. 2003. Veterinary Microbiology 99 (2004) 75-78
3. Vötsch D. Et al. 2020. Virulence 2020, Vol 12, NO. 1, 84-95
4. Hau, S.J., Brockmeier. S. The Pig Site. Review Article. p.1-21.

Graphs or Images 1



Graphs or Images 2



P-B-017

BENEFITS OBSERVED IN THE FIELD AFTER USING AN ORAL LIVE VACCINATION AGAINST LAWSONIA INTRACELLULARIS IN MALAYSIA

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Introduction
Porcine proliferative enteropathy (PPE) also known as ileitis, is caused by Lawsonia intracellularis (LI). It is a common disease worldwide affecting 57% to 100% of herds, including Malaysia (1, 2). Clinically, ileitis manifest itself in acute and chronic forms. It can reduce the average daily gain (ADG) by 32% (3). In another study, with the use of oral LI vaccination, the total economic benefit is €8.30 per vaccinated pig (4). Together with biosecurity and proper husbandry, the use of oral vaccination can have a positive outcome for the composition of the gut microbiome, pig performance (ADG, FCR) and transmission of the pathogen (5). The objective of this trial was to evaluate the efficacy of oral vaccination against LI on the zootechnical performance in a Malaysian pig farm.

Materials and Methods
This study was conducted in a 1200 sows farrow-to-finish-site located in the northern part of Malaysia. Although no clinical signs associated with ileitis were observed, infections with LI starting at the age of 14 weeks and older was confirmed by ELISA test. A total of 450 finishers from the same farrowing batch were included in the study (225 controls and 225 finishers vaccinated with an oral live ileitis vaccine (Enterisol® Ileitis Boehringer Ingelheim Animal Health USA Inc.). A 2-ml dose was administered by drench for the vaccinated pigs at the age of 18-days. Pigs were evaluated for side effects due to vaccination by measuring the rectal temperature 12 hours before and 12 hours after vaccina nation.

The vaccination efficacy was assessed by comparing productive parameters among groups (28 days old body weight (kg), 42 days old body weight (kg), final live weight (kg), days to slaughter and average daily gain (ADG, g/day). Statistical analysis of the data was performed using a T-test for normal distributed data using Kolmogorov-Smirnov and a Mann Whitney test for non-normal distributed data.

Results
There was no significant rise in rectal temperature after vaccination. There was no significant difference between the bodyweight of vaccinates and control on the age of 28 days. ADG of the vaccinated animals was significantly higher during the finishing period (641 vs 665 gram/day; $p<0,01$) resulting in 3.73 kg heavier pigs at slaughter (107.73 kg CON vs 111.46 kg VAC; $p<0.01$)). The return on investment for the oral vaccination was 5.67.

The results are summarized in Table 1.

Discussion and Conclusion
This study demonstrated that the use of oral live ileitis vaccine significantly improved the final live weight at 185 days of age and increased the average daily gain. In addition, there is no significant difference in the body temperature before and after vaccination, indicating

absence of side effects which is in line with the Summary of Product Characteristics. The significant improvement in the final live weight for the vaccinated group, resulted in a ROI of 5.67.

References

1. Ng Z.Y. et al, 2020 Proc 26th IPVS, 329
2. McOrist S et al, Pig J. 2003; 51: 26-35
3. Helm, E.T., et al. 2022 IPVS Brazil Oral vaccination mitigates weight and feed efficiency loss during Lowsonia intracellularis experimental challenge
4. Rutger et al, Leman conference, St Paul September 2022 A meta-analysis of ileitis oral vaccination field studies highlights its zootechnical effect and their economic impact under current market circumstances
5. Paradis MA, et al. J Swine Health Pro. 2012;20 (3):137-141

Table 1

	Mean		
Vaccination	**No**	**Yes**	**Significance**
Body temperature °C (before vaccination)	36.6	36.6	NS
Body temperature °C (after vaccination)	36.5	36.8	NS
28 days old body weight (kg)	7.11	7.12	NS
Final live weight at 185 days (kg)	107.73	111.46	**p<0.01**
Average daily gain (ADG, gram/day)	641	665	**p<0.01**
ROI	5.67		

P-B-018

BRONCHOPNEUMONIA COST CALCULATION BY LUNG LESION SCORING IN A COMMERCIAL SWINE FARM IN THE PHILIPPINES

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INTRODUCTION
Bronchopneumonia is chronic lung lesions located at the cranio-ventral lobes often associated with Mycoplasma hyopneumoniae.1 These lesions can be assessed by lung lesion scoring (LLS) which provide valuable information on the incidence, severity of swine respiratory pathogens on the farm; these can be correlated to farm data and can complete the animal health program assessment. LLS gave information when monitoring changes in following the implementation of changes in the medication program. Calculation of disease cost is an important guide for veterinarians in disease management.

MATERIALS AND METHODS
A commercial farm in the Philippines decided on treatment and control using in-feed Pulmotil® to address respiratory diseases in the herd. LLS was done across several months for a period of three years. Score system used for bronchopneumonia was the Straw method. 2 Lungs were further classified as normal (green), with moderate pneumonia (yellow, orange) or with severe pneumonia (pink). The cost of disease calculations were based on farm production data in relation to severity of lesions.3

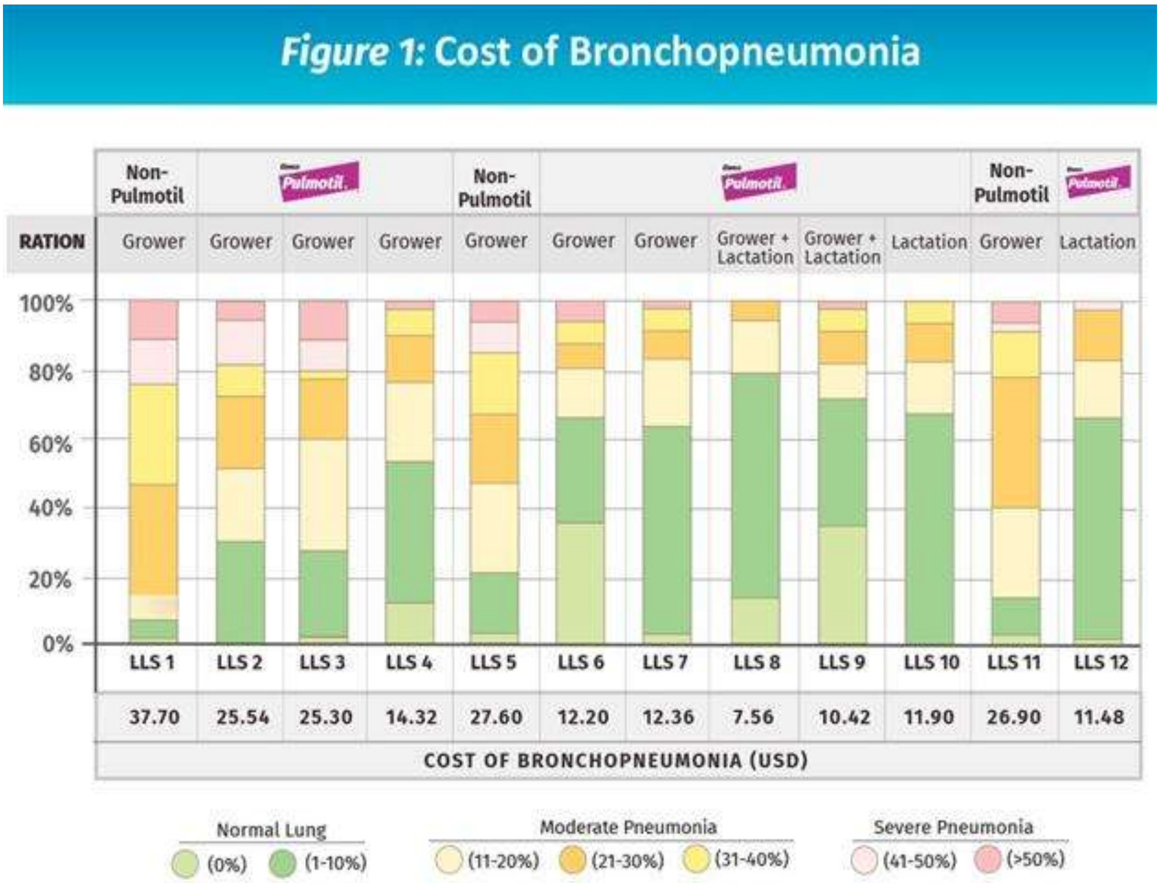
RESULTS AND DISCUSSION
Twelve LLS activities were done to monitor the treatment with Pulmotil® in the grower feeds at 2kg/ton for 21 days as well as control in the lactation feed. A baseline LLS1 was done before adding Pulmotil® (tilmicosin) and a gradual increase in the number of normal lungs were observed during LLS2-4. The farm decided to use a generic tilmicosin and LLS5 showed a resurgence of moderate to severe pneumonia lesions. Pulmotil® was used again in grower with LLS6-10 showed more normal lungs and which prompted the farm to consider the transition from grower to lactation diet as monitored in LLS8-9 with further improvements, with high numbers of normal lungs. However, the farm decided to use a generic tilmicosin in the grower feed during a respiratory disease break and the lungs had more pneumonia lesions by LLS11. Pulmotil® was returned and used in grower feeds, which was eventually discontinued but remained in the lactating feed and more normal lungs were seen by LLS12.

Figure 1 shows the cost of bronchopneumonia. The LLS batch was used to extrapolate the % of lung severity. An ideal ADG was assumed and the % ADG reduction per lung classification was calculated in addition to the feed cost and extra days to consume to reach the ideal weight of harvest. We noted that in LLS 8 (using Pulmotil in both grower and lactation diets) with 80% normal lungs, we had the lowest at USD 7.56, while the higher ones included LLS1 at USD 37.70 and at times when the farm used generic tilmicosin evaluated at LLS5 and LLS11 (USD27.60 and 26.90).

CONCLUSION
LLS is an effective tool for monitoring and classifying bronchopneumonia as a consequence of swine respiratory diseases. It alerted the farm veterinarians about the efficacy of their medication program and the cost of disease incursions.

REFERENCES:
1.VanAlstine, WG. Chapter 21. Respiratory System. Diseases of Swine 10th ed.
2.Straw, B. E., L. Backstrom, and A. D. Leman, 1986b: Examination of swine slaughter. Part II. Findings at slaughter and their significance. Compend. Contin. Educ. Vet. Pract. 8, 106–110.
3.Muirhead, M.R. Alexander, T. J. L., 2013: Managing Pig Health A reference for the farm 2nd Ed.(Carr, J., Ed.) 5M Publishing Benchmark House. UK. p.309

Table 1



P-B-019

CLINICAL CASE REPORT: DIFFERENTIAL DIAGNOSIS TO CONFIRM SUSPECTED SALMONELLA OR PCV2 INFECTION ON A COLOMBIA'S FARM

Mr. Roman Krejci[1], E Vargas[1], J Calveyra[1]
[1]*Ceva Animal Health*

Introduction
Gastrointestinal and systemic disorders in nursery and fattening pigs can occur due to several factors and the correct diagnosis is the key to success in the treatment and control of infectious pathogens. The aim of this study is to report a clinical case with initial suspicion of clinical signs and macroscopic lesions compatible with PCV2 and/or Salmonella confirming through laboratory techniques.

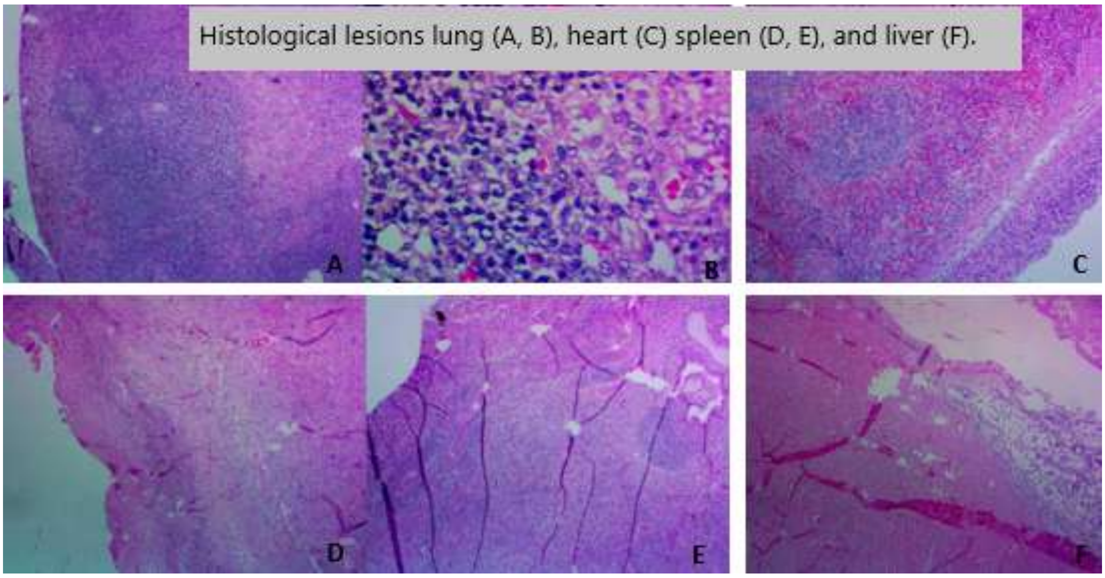
Description
The study was conducted on a farrow to finish farm, located in the Antioquia region. The region is endemic to PCV2 and Mycoplasma hyopneumoniae (Mhyo), with the animals in the study farm being vaccinated for both etiological agents at weaning (0.5 mL and 2 mL respectively). After 18 months with this protocol, the animals began to show clinical symptoms PCVD and / or Salmonellosis.
Tissue fragments (liver, spleen, kidney, lymph node and intestine) were collected from dead animals in nursery, for histopathology and bacterial isolation in order to obtain a confirmatory information for the correct diagnosis.

Results and discussion
The clinical signs and macroscopic lesions found in necropsied animals could have lead to a presumptive diagnosis of PCVD or Salmonellosis. The findings described moderate diffuse lymphohistiocytic enteritis, mild diffuse mixed peritonitis, with evidence of an acute systemic process in the liver. The samples were cultured for bacterial growth, with the result of isolation of Salmonella Typhimurium. From the isolated colonies, antibiogram was performed with sensitivity to Ciprofloxacin, Trimetoprim sulfamethoxazole, Neomycin and Fosfomycin.
Cases like this are being reported more frequently in commercial farms in which false diagnosis of PCVD is sometimes set. These results confirm previous studies, pointing out the importance and need to use specific laboratory techniques to conclude the presumptive diagnosis performed on the farm.

Conclusion
The association of different diagnostic tools is essential to clarify the disease and the etiological agent present in the herd.

Graphs or Images 1

Graphs or Images 2



P-B-020

CLINICAL CASE WITH DIFFERENTIAL DIAGNOSIS OF PCV2 AND SALMONELLA IN A BRAZILIAN FARM

Mr. Roman Krejci[1], M Souza[2], J Calveyra[2]
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Introduction
Infectious diseases have a great economic impact on pig farming. To reduce these losses, proper treatment and prevention, based on correct diagnosis, are essential. Therefore, the aim of this study is to report a case of suspected circovirus infection that was opposed in the laboratory.

Description
The clinical case was reported in a closed system farm with 800 sows, located in Minas Gerais - Brazil. The farm had a history of culling and severe diarrhea in animals between 90 and 110 days of age. The initial suspicion, based on clinical signs, was PCV2. The animals were vaccinated against Mycoplasma - 2 ml and Circovirus - 0.5 ml at weaning. Necropsy and collection of organs from 8 animals were performed for histopathology and bacterial isolation. Blood was collected from animals of 4 different ages for PCRq analysis for PCV2: 50, 75, 95 and 112 days of age, 15 samples, with 3 pools per age.

Results and discussion
Macroscopic findings showed non-pathognomonic lesions, requiring other tools to complete the diagnosis. Two samples of ileum, cecum and colon from animals with 107 DOA were positive in the isolation of Salmonella Typhimurium. These results are correlated with prevalence studies that show the growing number of clinical cases of Salmonellosis in the country, pointing to it as an endemic disease.
The qPCR results found show titers of up to 1+04 viral copies. Some studies, which used real-time PCR to detect PCV2 in serum, have shown differentiation of subclinical and clinical circovirus, proposing a genome-wide limit of > 1+07 PCV2 / mL of serum as indicative of clinically significant PCV2 infection.
Figure 1. Macroscopic lesions:presence of colitis, enlarged mesenteric lymph nodes and mesenteric edema.

Fig 2. Presence of grey scours in the pen

Conclusion
In the reported clinical case, the conclusive and final diagnosis was Salmonella Typhimurium. The association of different diagnostic tools is essential for the correct diagnosis, which helps to apply the most appropriate prevention and control measures.

Graphs or Images 1

Graphs or Images 2

P-B-021

COMPARATIVE PERFORMANCE OF TWO ONE-DOSE MYCOPLASMA HYOPNEUMONIAE VACCINES IN A 2,800 SOW MULTIPLIER FARM IN THE PHILIPPINES

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[1]Boehringer Ingelheim AH Phil., Inc.

Introduction

Vaccination against Mycoplasma hyopneumoniae is a common practice in the country to reduce clinical signs and production losses associated with Enzootic Pneumonia (EP) infection, particularly in late growing finishing stage[1]. Different protocols are in place in the field, applying either a one-dose or a two-dose vaccination regimen, more commonly programmed
around weaning[2]. The aim of this study is to evaluate and compare the efficacy of Ingelvac MycoFLEX, a 1 ml-one dose Mhyo bacterin, with another popular one-dose Mhyo brand, focusing on pig performance particularly on ADG, finished weight, and livability improvement.

Materials and Methods

This study was conducted in a 2,800 sow farrow-to-finish genetic multiplier farm. Pigs were weaned at four weeks of age more or less, and transferred to the nursery. Nine hundred thirty four (934) healthy pigs aged 21 days (±3d) on Day 0 were blocked by age and body weight and randomly assigned to treatment groups (Table 1). All test pigs were individually tagged with unique ear tags and housed under equal conditions.

Table 1. Study Design (pigs aged 21 days, ±3 days)

Wilcoxon Rank Sum test was used to compare ADGM and finished weight, while Approximate Z-test was applied to compare mortalities. The entire field evaluation was conducted over a period of five months.

Results

The Ingelvac MycoFLEX® vaccinated pigs showed significantly higher average daily weight gain (ADG) and market weights compared to the control group vaccinated with Brand R. Moreover, there was a 1.43% lower mortality in favor as well of the MycoFLEX group, albeit, just a numerical advantage(Table 2).

Table 2. Effect of Vaccine on Pig Performance

The average ADG and finished weights were higher in the Mycoflex group at 79g and 101.2kg, respectively. On both parameters, 95% confidence intervals of the median did not intersect each other, proving highly significant differences. Boxplots and density curves supported this conclusion as both ADG and weight distributions under the Mycoflex group were situated to the right of the Control (Brand R) group.

Fig. 1

These significant performance differences resulted to 31g ADG and 3.65kgs live finished weight improvements in favor of the Ingelvac MycoFLEX vaccinated pigs. Considering the prevailing production cost in the local farm setting (cost of feed, vet-med, pork selling price, etc.), these more efficient growth and livability results can easily give a very favorable ROI of about 9.49 for a Filipino pig farmer.

In conclusion, these observations further corroborate earlier studies showing that Ingelvac MycoFLEX can significantly improve growth and livability parameters better than most other Mhyo vaccine options in the market[1,2,3,4], and that, pig performance gains must always be prioritized over lung lesions.

References
[1] Hernandez, et al., 2012 IPVS Proceedings, p.698.
[2] Gutiérrez, et al., 2012 IPVS Proceedings, p.701.
[3] Park, et sl., 2017 8th APVS Proceedings, p.242.
[4] Schuttert, et al., 2014 IPVS Proceedings.

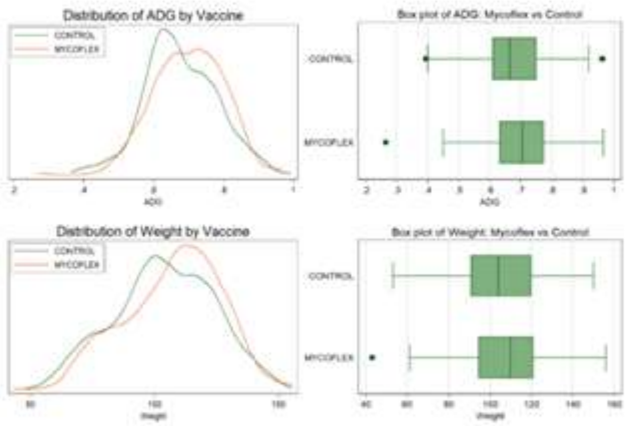
Table 1

GROUP	N	TREATMENT
Control (Brand R)	462	2ml, IM, one-dose
Ing. MycoFLEX	472	1ml, IM, one-dose

Table 2

Parameter	Control (Brand R)	MycoFLEX	*P-value*
Pigs, N	462	472	
ADG, g	0.7562	0.7869	*0.0001*
Weight, Kg	97.55	101.20	*0.0034*
Mortality, %	7.79	6.36	*0.3918*

Graphs or Images 1



P-B-022

COMPARISON AMONG DIFFERENT IMMUNIZATION PROGRAMS OF MYCOPLASMA HYOPNEUMONIAE AND MYCOPLASMA HYORHINIS BIVALENT INACTIVATED VACCINE IN FIELD

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- Introduction

Mycoplasma hyopneumoniae (Mhp) and Mycoplasma hyorhinis (Mhr) are both pathogenic mycoplasma in swine and widely exist on pig farms in Taiwan. Both can transmit horizontally between pigs in a herd and from sows to offspring during the lactation stage. Mhp mainly caused mycoplasmal pneumonia, while Mhr induced polyserositis, arthritis and mycoplasmal pneumonia, affected growth efficiency and survival rate. These two pathogens could lead to significant economic losses in the swine industry, and the diseases control strategies primary rely on respective vaccines and antimicrobials. Monovalent vaccine cannot provide comprehensive protection because there is no cross protection between Mhp and Mhr. While vaccines against Mhp are widely used in Taiwan, research on the use of commercial Mhr vaccines in the field is limited. Thus, the objective of this study was to evaluate the efficacy of a Mhp and Mhr bivalent inactivated vaccine in the field in Taiwan and to compare the efficacy among different immunization programs.

- Materials and Methods

A Mhp and Mhr positive, clinically affected farrow-to-finish farm was selected in this experiment, sow were divided into vaccinated group (VS, n=44) and non-vaccinated group (NVS, n=41). Each of the two groups was subdivided into 1-week-old piglet vaccinated groups (VS-1VP, n=15; NVS-1VP, n=14), 3-week-old piglets vaccinated groups (VS-3VP, n=15; NVS-3VP, n=13) and piglet non-vaccinated group (VS-NVP, n=14; NVS-NVP, n=14), in a total of six groups. The suckling pigs were allocated to 6 groups according to their sows. Sows in VS group were vaccinated intramuscularly at 8 weeks and 4 weeks before farrowing, and sows in NVS group were without vaccination. The vaccine administrated in this experiment was a bivalent inactivated vaccine (Bayovac Mhyo and Mhr bivalent inactivated bacterin, Elanco). Survival rate (4-12w/o), average daily weight gain (4-11w/o, 11-28w/o, 4-28w/o) and lung lesion scores of slaughtered pigs were recorded to evaluate the vaccine efficacy. The survival rate and pleuritis rate were compared among groups using Chi-square test. Parametric statistical analysis (One-way ANOVA) was used to test difference in average daily weight gain among different groups. Non-parametric statistical analysis (Kruskal-Wallis test) was used to test difference in lung lesion score and pleuritis lesion score among different groups.

- Results

The results showed that the survival rates of nursery period (4-12w/o) had no significant difference among 6 groups. The average weight of 5 vaccinated groups at slaughter were all higher than NVS-NVP group. The average daily weight gain of 5 vaccinated groups during 11-28w/o were all significantly higher than NVS-NVP group. The lung lesions score of VS-1VP (2.23 ± 2.14) and NVS-3VP group (2.27 ± 1.85) were significantly lower than NVS-NVP group (3.90 ± 3.54, $p < 0.05$). The pleuritis rate of VS-1VP (10.4%) and NVS-3VP group (9.6%) were significantly lower than NVS-NVP group (32.7%), while pleuritis lesions score of NVS-3VP group (0.13 ± 0.44) were significantly lower than NVS-NVP group (0.85 ± 1.46, $p < 0.05$).

- Conclusions and Discussion

The Bayovac Mhyo and Mhr bivalent inactivated bacterin can improve average daily weight gain and average weight at slaughter and may reduce lung and pleuritis lesions after administration to sows and/or piglets.

- Acknowledgement

We gratefully acknowledge the technician and veterinarian in ADDC of NPUST for their contributions to this study.

- References

[1] Lee J.A. et al., 2018. Vet. Immunol. Immunopathol. 196, 14-17.
[2] Sibila M. et al., 2008. Vet. Microbiol. 127(1-2), 165-170
[3] Lin J.H. et al., 2006. Vet. Microbiol. 115(1-3), 111-116.
[4] Madec F. et al., 1982. Journ. Rech. Porc. Fr. 14, 405-412.

Table 1

Group	Survival rate (%)	Average weight (kg)			Average daily weight gain (kg)		
		weaning	finishing	slaughter	4-11w/o	11-28w/o	4-28w/o
VS-1VP	95.95	7.13 ± 0.48	23.65 ± 1.57	128.13 ± 9.63	0.376	0.740*	0.654*
VS-3VP	93.94	6.94 ± 0.68	23.36 ± 1.61	122.92 ± 10.65	0.373	0.683*	0.612
VS-NVP	96.19	7.13 ± 0.92	24.10 ± 2.83	126.54 ± 11.82	0.386*	0.702*	0.628*
NVS-1VP	97.18	6.58 ± 0.86	24.33 ± 3.81	125.00 ± 10.85	0.409*	0.716*	0.643*
NVS-3VP	98.55	7.50 ± 0.66	24.17 ± 0.31	128.65 ± 8.16	0.379	0.747*	0.659*
NVS-NVP	96.15	6.77 ± 0.56	23.05 ± 0.24	120.38 ± 10.50	0.370	0.665	0.614

* represent significant difference compared with NVS-NVP (control) group

Table 2

Group	N	Lung lesions score % (N)						Pleuritis lesions score	
		0	1-4	5-9	10-15	>15	Mean ± SD	% (N)	Mean ± SD
VS-1VP	48	15 (7)	73 (35)	10 (5)	2 (1)	0 (0)	2.23 ± 2.14*	10.4 (5) *	0.25 ± 0.86
VS-3VP	48	4 (2)	63 (30)	25 (12)	6 (3)	2 (1)	4.38 ± 4.02	45.8 (22)	1.10 ± 1.45
VS-NVP	51	4 (2)	65 (33)	22 (11)	4 (2)	6 (3)	4.41 ± 4.60	47.1 (24)	1.00 ± 1.36
NVS-1VP	48	10 (5)	52 (25)	38 (18)	0 (0)	0 (0)	3.69 ± 2.39	33.3 (16)	0.60 ± 1.09
NVS-3VP	52	4 (2)	81 (42)	15 (8)	0 (0)	0 (0)	2.27 ± 1.85*	9.6 (5) *	0.13 ± 0.44*
NVS-NVP	52	2 (1)	71 (37)	17 (9)	8 (4)	2 (1)	3.90 ± 3.54	32.7 (17)	0.85 ± 1.46

* represent significant difference compared with NVS-NVP (control) group

P-B-023

COMPARISON OF A READY-TO-USE AND A FRESHLY MIXED COMBINATION PCV2 AND MYCOPLASMA HYOPNEUMONIAE VACCINES ON PRODUCTIVITY PARAMETERS IN A WEAN-TO-FINISH PIG FARM IN CHINA

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Introduction:
Porcine circovirus type 2 (PCV2) and Mycoplasma hyopneumoniae (Mhp) are two economically important pathogens causing porcine circovirus-associated diseases and swine enzootic pneumonia, respectively. Complicated co-infections of PCV2 and Mhp with other respiratory pathogens are common, causing porcine respiratory disease complex (PRDC). Vaccination against PCV2 and Mhp remains the most efficient and economical approach to date in pig production worldwide [1-2]. This study aimed to compare the field effectiveness between a ready-to-use PCV2 and Mhp combination vaccine and a freshly mixed monovalent PCV2 and Mhp vaccines in a farm historically reported of PRDC incidence.

Materials and methods:
This study was conducted in a wean-to-finish pig farm located in Southwest China from September 2021 to March 2022. At weaning age of 21 days of age, a total of 2157 piglets were randomly allocated into Group A and Group B. On the same day, pigs in Group A received intramuscularly once a ready-to-use 2ml Fostera PCV MH, while pigs in Group B were vaccinated once intramuscularly with freshly mixed 2 ml of monovalent PCV2 and Mhp vaccines. These pigs were raised under the same management conditions in the same buildings of all-in all-out system by site . Adverse reactions in vaccinated pigs were monitored daily until 3 days post vaccination. Blood samples were collected at several time points for QPCR and ELISA tests. Body weights were collected at weaning age and the end of the finishing phase. Mortality and antibiotic treatment for PRDC were daily recorded.

Results:
The pigs in both groups were diagnosed with wild-type PRRSV positive at the late nursery and co-infected with PCV2 and IAV-S at finishing phase. Throughout all timepoints, there was no statistically significant difference in PCV2 and PRRSV viremia levels and PCV2, PRRSV and IAV-S antibodies levels between the two groups. Productivity parameters are shown in Table 1. Pigs in Group A had fewer systemic adverse reactions than those in Group B. Compared with Group B, the mortality at nursery phase and the wean-to-finish overall mortality of Group A was lower by 1.07% and 3.11%, respectively. The cumulative mortality curve (Figure 1) showed that Group A had lower mortality throughout the study. The treatment rate (number treats/number pigs 100) of Group B was significantly higher than that of Group A ($p<0.01$). Pigs in Group A gained an average of 8 grams more weight daily than those in Group B.

Conclusions and discussion:
The study showed pigs vaccinated with Fostera PCV MH performed better on productive performance in addition to its convenience for use and practicality, without causing the impact of adverse reactions. In both groups, most pigs died during the growing-finishing phase, caused by the co-infections of wild type-PRRSV, PCV2 and IAV-S. Despite the ongoing

PRDC incidence, the Fostera□ PCV MH vaccinated group had lower mortality, lower treatment rate and higher ADG. This study demonstrated that in pig farms with PRDC complications, besides choosing a more effective PCV2 and Mhp vaccine, controlling the primary infection caused by wild-type PRRSV is necessary to reduce the impact of co-infections.

References:
[1] Guo J et al. 2022. Viruses, 14(9): 2005.
[2] Maes D et al. 2018. Transbound Emerg Dis, 65(1):110-124.

Table 1

Table 1. Productivity parameters of pigs according to different vaccination protocols

Vaccines	Group A Fostera®PCV MH	Group B Freshly mixed vaccine
Number of pigs	1130	1027
Anaphylaxis cases (rate)	2(0.18%)	55 (5.36%)
Nursery mortality	2.30%	3.37%
Overall mortality	15.32%	18.43%
Treatment Rate	127.33%[a]	172.07%[b]
ADG	684 g/d	676 g/d

(Statistical significance between a and b, p<0.01)

Graphs or Images 1

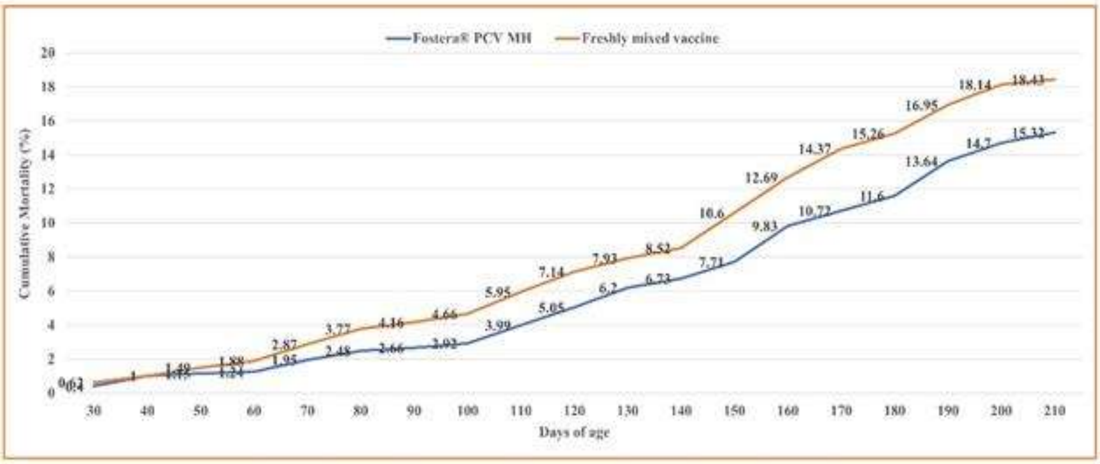

Figure 1. Cumulative mortality at different time points

P-B-024

COMPARISON OF AN INJECTABLE TOLTRAZURIL-GLEPTOFERRON AND AN ORAL TOLTRAZURIL MIXED INJECTABLE IRON DEXTRAN IN THE FIELD IN TAIWAN

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[1]Department of Veterinary Medicine, National Pingtung University of Science and Technology, [2]Animal Disease Diagnostic Center, College of Veterinary Medicine, National Pingtung University of Science and Technology

Introduction:
There are many reasons for the slow growth of piglets in the farrowing unit, some of which are neonatal coccidiosis caused by Cystoisospora suis (C. suis) and iron deficiency anaemia (IDA). Clinical coccidiosis is characterised by yellowish rarely grey liquid, reduced body weight gains and appears frequently at the age of 7–11 days [1]. The primary consequences of IDA are reduced growth rate and increased susceptibility to infectious diseases. Supplementation of iron and treatment against C. suis are common practice in newborn piglets. Since animal manipulation is needed for all these interventions, reduced handling for application of therapeutic compounds at the same time may reduce stress for the piglets. A injectable combination product, Forceris® (CEVA) has been developed and the efficacy was confirmed in comparison with an established reference product by Joachim et al [2]. This study evaluates anemia and coccidiosis in pig farms using this product.

Materials and Methods:
A commercial pig farm located in southern Taiwan was selected. Fifteen sows and their litters were selected. Two days after farrowing, the piglets of each sow were randomly divided into two groups. the Forceris group received 45 mg of toltrazuril and 200 mg of iron/piglet as gleptoferron and the control group was treated with toltrazuril and iron according to the original operating procedures of the farm. Fecal and blood were collected from piglets at the age of 2, 14 and 25 days for fecal score, autofluorescence microscopy detection and hemoglobin concentration detection respectively. And weighed at the age of 2 and 25 days.

Results:
This experiment compared the hemoglobin concentrations of the two groups at each time point, the Forceris group is higher than control group but there is no significant difference (Figure 1.). The fecal collected at each time point were used for fecal scoring, and there was no significant difference between these two groups. Coccidia oocysts were not detected on autofluorescence microscopy in the feces of both groups of pigs. There was no significant difference in body weight at birth and at weaning, but the results showed that the overall weight of the Forcreis group was 40.46 kg more than that of the control group, and the mortality rate before weaning was also significantly lower than that of the control group (Table 1).

Conclusions:
No coccidia oocysts were detected in both two groups, and the hemoglobin concentration of the pigs in the Forceris group was also slightly higher than that in the control group. And in the performance of body weight and pre-weaning mortality rate, the Forceris group was better than the control group.

Acknowledgement:
We gratefully acknowledge the technician and veterinarian in ADDC of NPUST and the team of Ceva Sante Animale for their contributions to this study.

References:
[1] Lindsay D. S. et al., 2012. Disease of Swine. pp.897.
[2] Joachim A. et al., 2018. Parasit Vector. 11, 206.

Table 1

Table 1. The summary of experimental results.

Item	Group		Difference
	Forceris	Control	
Amount of piglets Day 2	76	75	0
Amount of piglets Day 25	74	69	+ 4
Mortality before weaning (%)	2.63	8	**- 5.37**
Hemoglobin Day 2 (g/dl)	9.1	8.7	+ 0.4
Hemoglobin Day 14 (g/dl)	11.41	10.91	+ 0.5
Hemoglobin Day 25 (g/dl)	11.5	11.1	+ 0.4
Average body weight Day 2 (Kg)	1.22	1.25	- 0.03
Average body weight Day 25 (Kg)	6.28	6.15	+ 0.13
Total body weight Day 25 (Kg)	464.85	424.39	**+ 40.46**

Graphs or Images 1

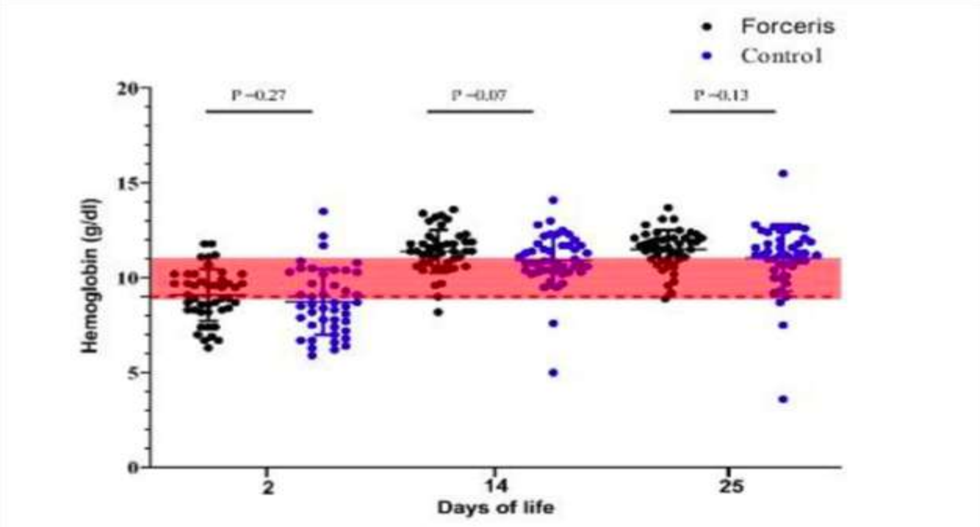

Figure1. The result of hemoglobin.
The red translucent area represents first-stage iron deficiency, and the area blow the red dotted line represents second-stage iron deficiency.

P-B-025

COMPARISON OF LUNG EP-LIKE LESIONS IN SLAUGHTERED PIGS, VACCINATED WITH DIFFERENT COMMERCIAL MYCOPLASMA HYOPNEUMONIAE VACCINES UNDER FIELD CONDITIONS.

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[1]*Ceva,* [2]*CEVA Salud Animal*

INTRODUCTION
Vaccination against Mycoplasma hyopneumoniae (Mh), one of the primary agents involved in porcine respiratory disease complex (PRDC), is a common tool used for prevention and control of Enzootic pneumonia (Ep). Evaluation of lung lesions in slaughterhouse is a common method to assess the efficacy of vaccination. The aim of this study was to investigate the prevalence and extension of Ep-like lesions observed at slaughter in pigs vaccinated with either Hyogen® (vaccine-H) and other 9 commercial Mh vaccines.

MATERIAL AND METHODS.
From January 2016 until October 2021 a total of 2.174 batches and 344.966 lungs from different farms from Spain were scored at the slaughterhouse using the Ceva Lung Program (CLP) score methodology, which assists lung scoring for EP-like lesions.
For each batch the following parameters were calculated:
- Percent of affected lungs with Ep-like lesions
- Percent of affected surface out of all lungs.
- Percent of affected surface of pneumonic lungs.
- Percent of scarring lungs
- Percent of cranial pleurisy

The statistical analysis was performed using the Mann-Whitney test.

RESULTS
Pigs vaccinated with Vaccine-H showed statistically ($p<0.01$) less percentage of affected lungs with Ep-like lesions, less percentage of affected surface and less scars than lungs vaccinated with other one-dose vaccines, two-dose vaccines, or one-dose_bi-valent vaccines.
Batches vaccinated with Vaccine-H showed statistically lower percentage of affected lungs with Ep-like lesions than vaccines 2,3,4,6,7,8,9 ($p<0.01$), and statistically lower percentage of affected surface than vaccines 2,3,4,6,7,8,9($p<0.01$) and vaccine-5 ($p<0.05$). Concerning scarring lungs, Vaccine H showed statically lower percentage than vaccines 2,3,4,5,6,7,8 ($p<0.01$) and vaccines 1 and 9 ($p<0.05$).

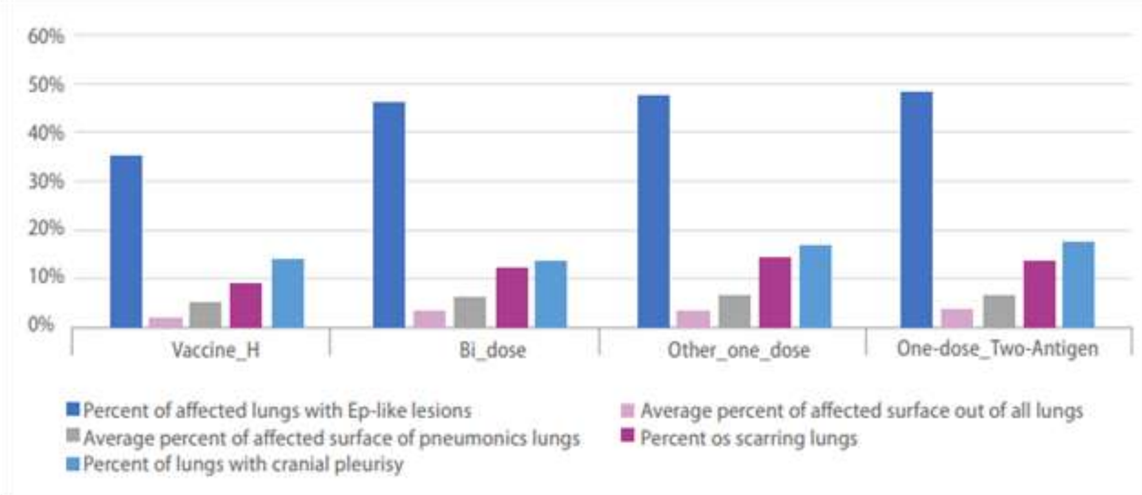
Figure 1. Summary of results of the groups: Vaccine H, Bi-dose, Other-one-dose and One-dose_two-antigen

Figure 2. Summary of results of the 10 different group

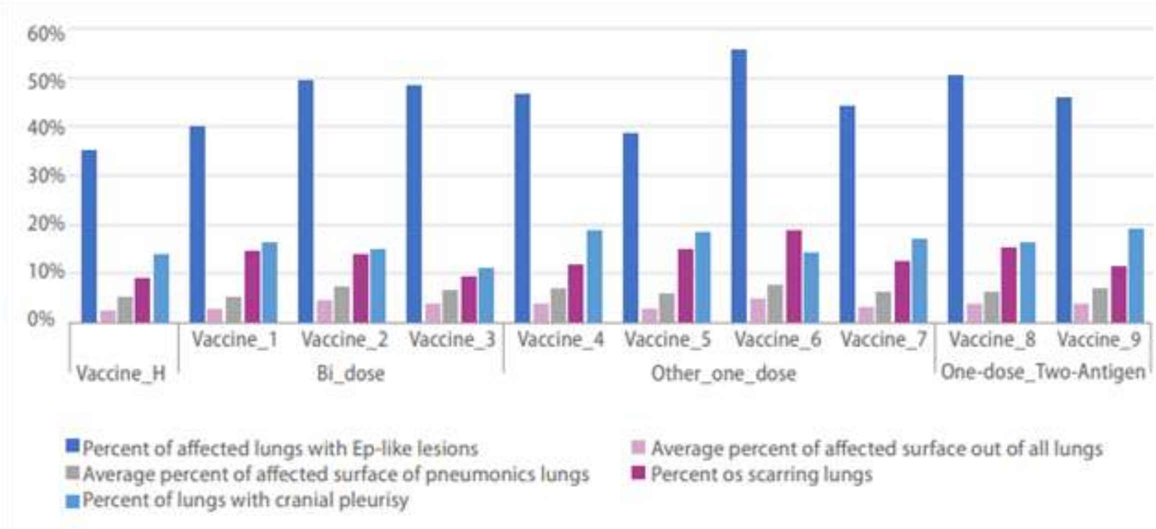
CONCLUSIONS AND DISCUSSIONS
Lungs from farms vaccinated with vaccine-H showed less EP-like lesions than lungs from farms vaccinated with any other commercial vaccine.
The vaccine-H showed, in this study, its superiority in the reduction of lung lesions over the rest of the vaccines included in the study.

REFERENCES
1 Madec F. et al. Journées de la Recherche Porcine, 1982.
2 Christensen G. et al. Acta Vet Scandinavica, 1993.
3 Dottori M. et al. Large Animal Review, 2007.

Graphs or Images 1



Graphs or Images 2



P-B-026

COMPARISON OF PARENTAL TOLTRAZURIL-IRON COMBINATION (FORCERIS®) WITH ORAL TOLTRAZURIL AND PARENTAL IRON DEXTRAN FOR THE CONTROL OF ANAEMIA AND COCCIDIOSIS UNDER FIELD CONDITIONS

Dr Ye Xiong Wong[1], Dr Chiou Yan Tee[2], Dr Wai Cheng Michelle Fong[3], Dr Ye Xiong Wong[2]
[1]Ceva Animal Health Malaysia Sdn Bhd, [2]Rhone Ma Malaysia Sdn Bhd, [3]Faculty of Veterinary Medicine, Universiti Putra Malaysia

-Introduction
Coccidiosis (Cystoisospora suis) is a common cause of diarrhea in piglets resulting in low performance and economic loss. Traditionally, oral toltrazuril is given as a metaphylatic treatment for newborn piglets. Besides, piglets are born subanemic with low iron reserve. Without exogenous iron supplement, fast growing piglets may develop iron-deficiency anaemia (IDA). Forceris® is a single dose combination of toltrazuril and gleptoferron, apply intramuscularly between the 2nd to 4th day of life. This combo product controls the coccidiosis and IDA in neonatal piglets. This study evaluates the efficacy of Forceris® under field conditions.

-Materials and methods
This study was conducted in a farrow-to-finish farm with 800 sows. 30 litters were randomly divided into 2 groups (Forceris® & positive control). On 2nd day of life (SD2), Forceris® group was injected with 1.5mL of Forceris (45mg toltrazuril + 200mg gleptoferron), while control group was given 1mL oral toltrazuril (50mg) and 1mL iron dextran injection (200mg) according to SPC. Post 24-hour treatment reaction were recorded. The litters were weighed and blood haemoglobin level were test using HemoCue machine on SD2 and SD27. Diarrhoea incidence and faecal sample were collected for oocyst count on SD11 and SD17. Data was analysed using T-test and Mann Whitney-U test.

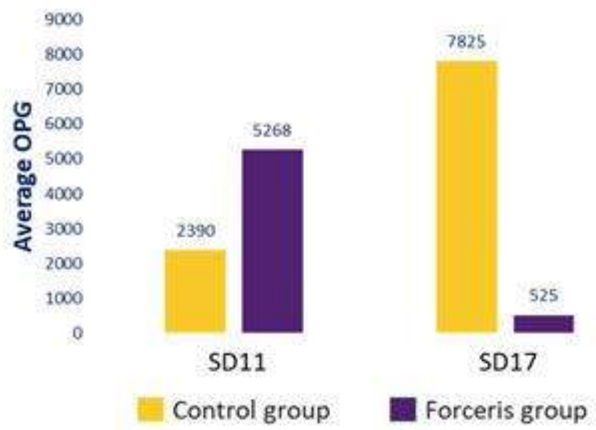
-Results
24 hours after injection, both groups showed minimal reaction. On SD11, oocysts were detected in both groups. One week later, the mean OPG of Forceris® group reduced by 90%, whereas the control group mean OPG increased by three-fold (Figure 1). Treatment group had lower diarrhoea percentage on SD11 (12.84% vs 18.54%, P=0.18) and SD17 (7.94% vs 18.51%, P=0.066). On SD27, 96% of the treatment group piglets blood haemoglobin was at optimal level (>11g/dL) with no anaemic piglet. Control group had 89% of optimal level piglets but 2.2% of anaemic piglets (<9g/dL). The average daily weight gain of treatment group is better (162.77g vs 152.74g, P=0.448).

-Conclusion
Forceris® has better control of coccidiosis and iron deficiency anaemia in piglets compared to traditional method.

-References
1. Joachim, A. et al. Parasites Vectors 11, 206 (2018).
2. Joachim, A., Ruttkowski, B. & Sperling, D. Porc Health Manag 4, 20 (2018)

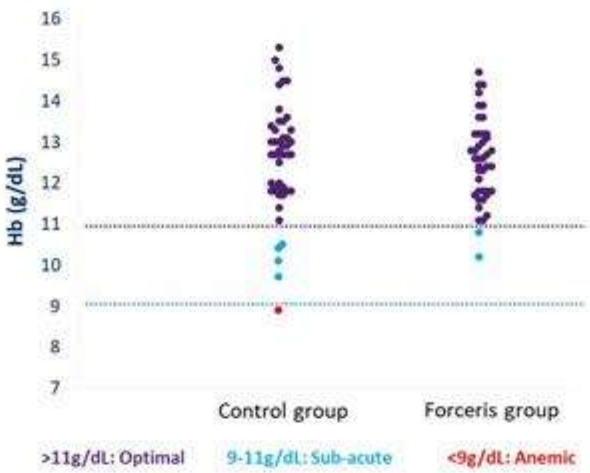
Graphs or Images 1

Figure 1: Average OPG on SD11 and SD17.



Graphs or Images 2

Figure 2: Blood hemoglobin level on SD27.



P-B-027

COMPARISON OF PHARMACOKINETICS OF TOLTRAZURIL AND ITS METABOLITE, TOLTRAZURIL SULFONE, BY ORAL AND INTRAMUSCULAR ADMINISTRATION IN SUCKLING PIGLETS.

Prof. Hung-Chih Kuo[1], Mr. Ta-Wei Yeh[1], Prof. Chi-Chung Chou[2], Prof. Chao-Min Wang[1], Prof. Szu-Wei Huang[1], Ms Ching-Fen Wu[1]
[1]National Chiayi University, [2]National Chung Hsing University

Introduction
Cystoisospora suis (C. suis), previously known as Isospora suis, is a common pathogen that leads to diarrhea and unthriftiness in suckling piglets [Lindsay et al., 2019]. The only registered and effective chemotherapeutic drug available for suppressing the development of C. suis is toltrazuril (TZR). TZR undergoes extensive metabolism to form toltrazuril sulfoxide (TZR-SO) and then to toltrazuril sulfone (TZR-SO2), which exert anticoccidial activity [Lim et al., 2010]. This study aimed to compare the pharmacokinetic and metabolic profile of two different route (oral and intramuscular) of TZR in suckling piglets.

Materials and methods
A total of 20 healthy male suckling piglets were enrolled in this study and allocated to two group randomly. Piglets of the oral group received a fixed dose of 50 mg of TZR per piglet orally on the 3rd day after birth. Piglets of the intramuscular group received a fixed dose of 45 mg of TZR per piglet intramuscularly on the 3rd day after birth (Table 1). Blood samples were collected at 1, 2, 4, 6, 8, 10, 12, 15, 18, 24, 30, 36 days after administration. Serum concentration of TZR and TZR-SO2 at each time point were determined by high performance liquid chromatography (HPLC).

Result
The maximum concentration (Cmax) of TZR of oral group was higher than intramuscular group with a shorter corresponding peak time (Tmax) (14.00±4.48 µg/mL at 34.03±6.97 h post-dose and 5.36±1.26 µg/mL at 120±25.66 h post-dose in oral and intramuscular group, respectively).
TZR was rapidly converted to TZR-SO2. TZR-SO2 was eliminated more slowly when compared with TZR (Average t1/2 of 288.27±104.19 h and 334.80±82.67 h in oral and intramuscular group, respectively).In addition, the levels of area under the serum concentration-time curve (AUC) of both TZR and TZR-SO2 administration in oral group were higher than intramuscular group (Table 2, Figure 1, Figure 2).

Conclusions and Discussion
In this study, oral TZR appears to have a faster absorptive property and a better bioavailability of TZR than intramuscular TZR when tested on 3-day-old piglets. This may be attributed to the apical canalicular system possessed by piglet intestinal enterocytes, which have the ability to non-selectively absorb high molecular weight substances by pinocytosis or endocytosis [Deluco et al., 2022]. Both oral and intramuscular TZR converted to TZR-SO2, which last over 36 days after TZR administration. This result showed both routes of TZR administration exert persistent clinical efficacy against C. suis infection due to the prolonged t1/2 of TZR-SO2 [Lim et al., 2010]. In conclusion, TZR is absorbed better in gastrointestinal tract than in muscle in the 3-day-old piglets. Furthermore, the sum of TZR and TZR-SO2 levels is crucial for prevention and control of C. suis.

Acknowledgement and reference
Deluco B, Wilson HL. Assessment of intestinal macromolecular absorption in young piglets to pave the way to oral vaccination: preliminary results. Vet Res Commun 46: 79-91, 2022.
Lim JH, Kim MS, Hwang YH, Song IB, Park BK, Yun HI. Pharmacokinetics of toltrazuril and its metabolites, toltrazuril sulfoxide and toltrazuril sulfone, after a single oral administration to pigs. J Vet Med Sci 72: 1085-1087, 2010.
Lindsay DS, Dubey JP, Duran MS. Coccidia and other protozoa. In: Zimmerman JJ, Karriker LA, Ramirez A, Schwartz KJ, Stevenson GW, Zhang J, eds. Diseases of swine. 11th ed. John Wiley & Sons Ltd, USA, 1015-1028, 2019.

Table 1

Table 1 Body weight and treatment dose of two groups.

Group	N	Mean body weight	Toltrazuril dose
Oral	10	2.4 ± 0.16 kg Max: 2.58 kg Min: 2.16 kg	50 mg per piglet Mean: 20.9 ± 1.4 mg/kg body weight Max: 22.6 mg/kg body weight Min: 19.3 mg/kg body weight
Intramuscular	10	2.5 ± 0.28 kg Max: 2.99 kg Min: 2.22 kg	45 mg per piglet Mean: 17.7 ± 1.9 mg/kg body weight Max: 20.2 mg/kg body weight Min: 15.0 mg/kg body weight

Table 2

Table 2 Pharmacokinetic (PK) parameters of Toltrazuril and Toltrazuril sulfone following a single 50 mg/pig PO and 45 mg/pig IM Toltrazuril.

PK parameters	PO		IM	
	Toltrazuril	Toltrazuril sulfone	Toltrazuril	Toltrazuril sulfone
$t_{1/2}$	109.62±34.62	288.27±104.19	91.38±19.42	334.80±82.67
C_{max} (μg/mL)	14.00±4.48	14.12±4.19	5.36±1.26	9.92±1.15
T_{max} (h)	34.03±6.97	246.00±16.97	120.00±25.66	330.00±109.42
AUC (h·μg/mL)	1702.28±607.77	8407.40±2440.47	996.45±198.61	6356.80±1103.47
Vz/F (L/kg)	2.26±0.80	NA	2.55±0.66	NA
CL/F (L/kg/h)	0.013±0.003	NA	0.019±0.004	NA
MRT (h)	116.78±24.54	578.74±129.12	182.75±25.36	643.96±105.37

Note: NA, not applicable.

Table 3

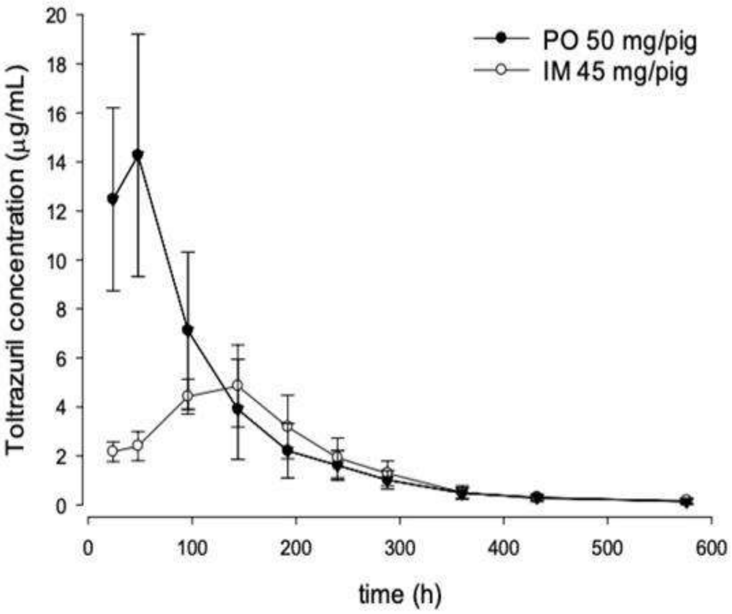


Figure 1 serum concentration-time profiles of Toltrazuril following a single 50 mg/pig PO and 45 mg/pig IM Toltrazuril.

Graphs or Images 1

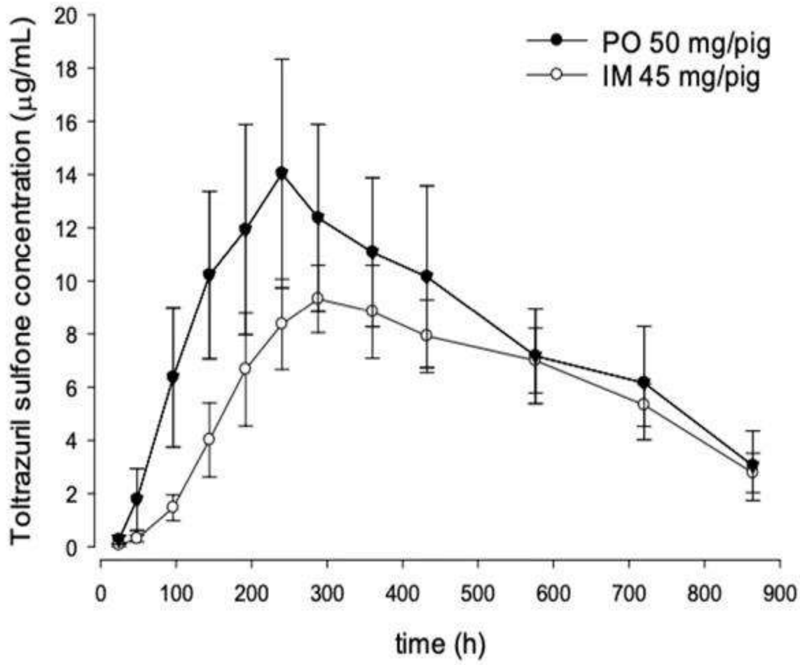


Figure 2 serum concentration-time profiles of Toltrazuril sulfone following a single 50 mg/pig PO and 45 mg/pig IM Toltrazuril.

P-B-028

COMPARISON OF THE PROTECTION AGAINST PCV2 OR MYCOPLASMA HYOPNEUMONIAE EXPERIMENTAL INFECTIONS WITH THE INJECTABLE OR INTRADERMAL PCV2/M.HYO VACCINES

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Introduction
PCVD due to PCV2 virus and Enzootic pneumonia due to primarily Mycoplasma hyopneumoniae (M.hyo) are the most common diseases in current swine herds causing huge and economic losses. Vaccination against those two pathogens helps to reduce their clinical manifestation and decrease of performance (1, 2). Several commercial mono- or bi-valent vaccines are available. The aim of this study was to compare the efficacy of injectable PCV2 and M.hyo RTM vaccines with the intradermal (ID) PCV2/M.hyo RTU product.

Material and Methods
Piglets were vaccinated either with Circovac® mixed with Hyogen®(DUO) - both Ceva or an ID vaccine containing inactivated recombinant M.hyo expressing cpPCV2(Mhyo/PCV ID) at 3 weeks of age (WOA).   Vaccinated and positive control pigs were challenged at 7(WOA) either with the PCV2d strain or with the M.hyo culture. Serum samples were collected prior to vaccination, challenge, and slaughter, and measured and IDVet Mycoplasma hyopneumoniae ELISA kits. Pigs were euthanized 28 days post infection (DPI) and either PCV2 loads in lymph nodes (Lnn) or lung lesions scores (LLS) according to the European Pharmacopoeia 9.0 were measured to assess the efficacy.

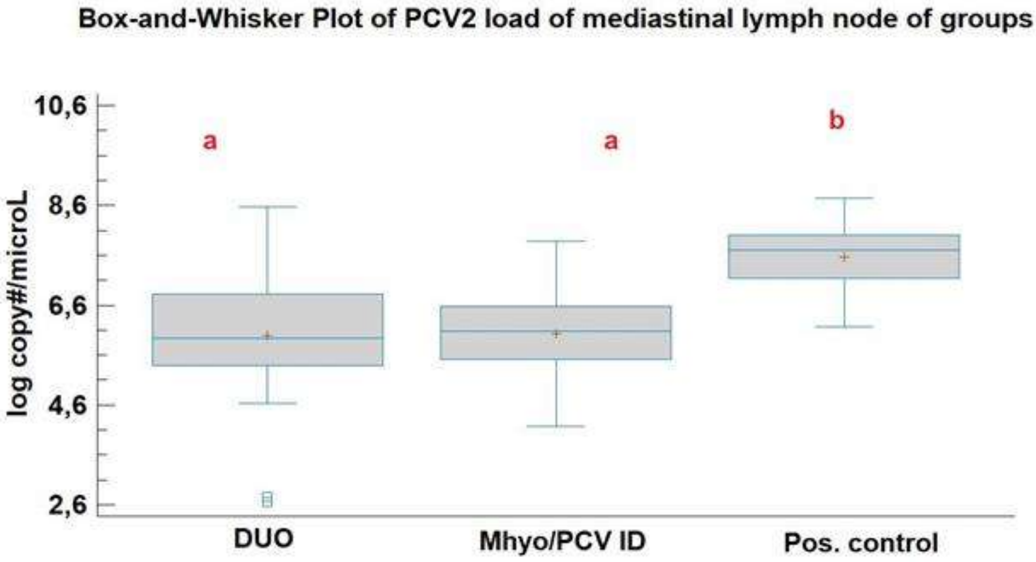
Results
The PCV2 viremia at 28DPI was 0.93 and 0.69 log copy#/microL for DUO and Mhyo/PCV ID respectively(p>0,05).

The PCV2 loads in mediastinal, mesenterial Lnn and tonsillar swabs were 5.99, 5.19 and 2.80 log copy#/microL for DUO and 6.02, 5.05 and 2.59 log copy#/microL for Mhyo/PCV ID respectively(p>0,05).

Mean LLS were 0.63 and 0.86 for DUO and Mhyo/PCV ID respectively (p<0,05 for DUO vs any other groups). Only DUO differed significantly from the positive non-vaccinated control in LLS.

Conclusion
This study demonstrated that whole virus and bacterin based injectable vaccine provided equal PCV2 protection as Mhyo/PCV ID. DUO however significantly outperformed the recombinant intradermal vaccine in the potency to induce strong immune response and in the protection against the development of lung lesions due to M.hyo.
References
Segales J et al. 2014. Expert Rev. Vaccines Early online, 1–15
Maes et al. 2017. Transbound Emerg Dis. 1–15

Graphs or Images 1



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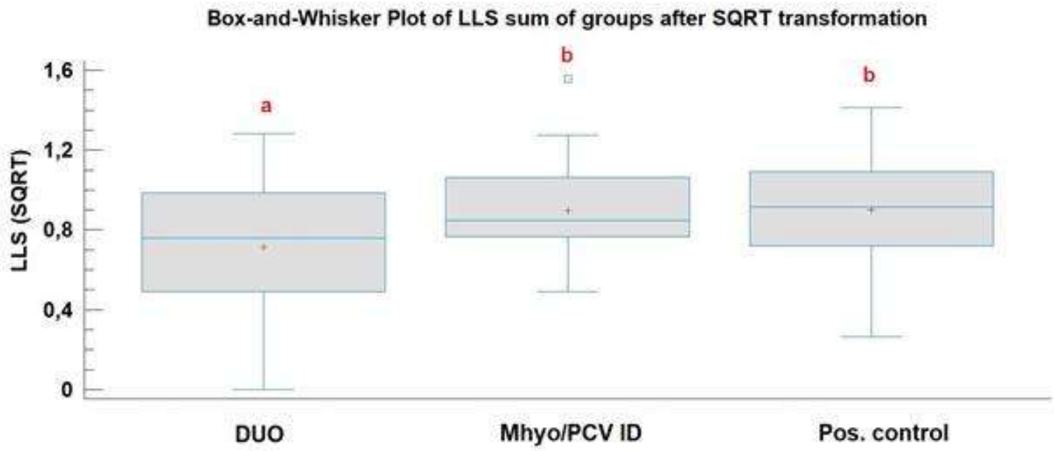


P-B-029

CONTROLLING GLAESSERELLA PARASUIS AFTER VACCINATION OF SOWS AND PIGLETS

Mr. Salvador Romero Aguilar[3], Shixuan Zhu[1], Mr. Wentao Fei[2], Mr. Shixiang Wang[2], Mr. Baohai Feng[2], Mr. Ming Hsun Wu[3]
[1]COFCO, [2]HIPRA CHINA, [3]HIPRA

Introduction
Glaesserella parasuis (GP) is a commensal bacterium of the upper respiratory tract of pigs, some strains of this bacterium can cause severe systemic disease (also called Glasser's disease), especially in nursery pigs (1). It can be found in basically all commercial farms and it's reported to be common etiologic agent in some areas (2). Vaccine could be an effective preventive and control measure for this disease. The aim of this trial was to evaluate the efficacy of vaccination in sows and piglets to control Glasser's disease

Materials and Methods
In September 2021, a farm with 4,800 sows located in the North of China had piglets of 40 days of age suffering from swollen joints and multiple fibrinous polyserositis (Figure 1). Gamithromycin, Tulathromycin and Amoxicillin were used in the feed stuff for treatment. However, medication was less effective and the mortality of nursery pigs gradually increased from 3% to 20%.
From November 2021, sows and piglets were immunized using a commercial GP vaccine (HIPRASUIS® GLASSER, 2 ml, HIPRA). Piglets (one shot at 3 weeks of age) and sows (two shots at an interval of 3 weeks before farrowing).

Figure 1. Swollen joints and fibrinous polyserositis

Results
After immunization, a significant reduction of mortality (from 20.1% to 5.3%) was observed. After one month of continued take such measures, the mortality rate decreased to 2.2% (Figure 2).

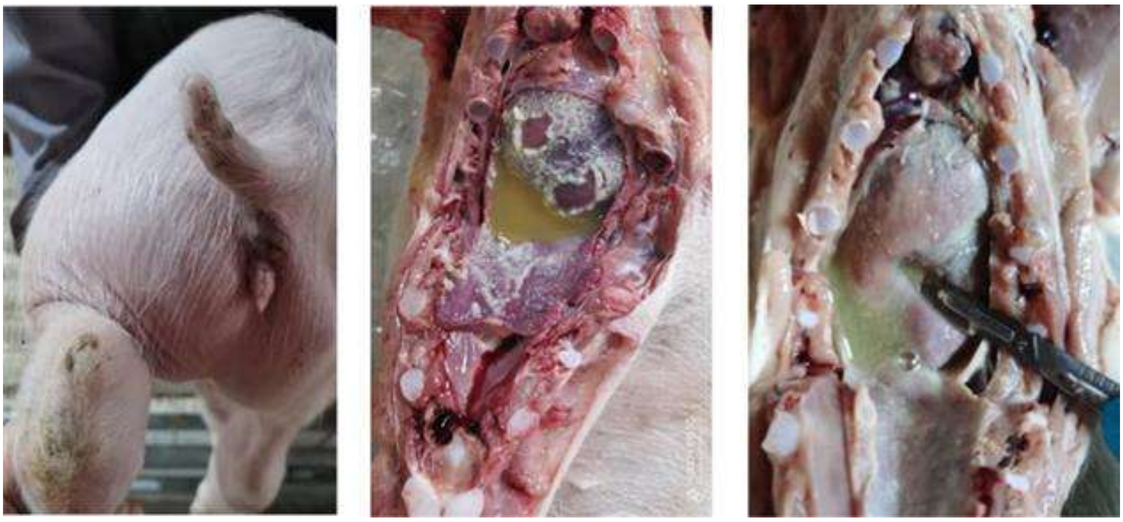
Figure 2. Summary of nursery mortality
p-value <0,001*** Logistic regression was performed

Conclusion and Discussion
Weaned is a predisposing factor for most diseases of nursery pigs. On the one hand, maternally derived antibodies drop sharply while the active protection has not yet been formed. On the other hand, the changes in the environment after weaning, including the weaning process, will cause great stress to the piglets. These two reasons often contribute to Glasser's disease outbreak during nursery period. It has been previously reported that simultaneous protection of sows and piglets is more effective than single immunization of sows or piglets (3). In this study, the simultaneous immunization of sows and piglets with HIPRASUIS® GLASSER was able to control the disease in an acute outbreak by reducing significantly the mortality.

References
1. Aragon et al. 2019. Disease of Swine, Wiley and

Sons, Inc.
2. Kang I et al 2012, Can J Vet Res, 76: 195-200.
3. Yeonsu Oh et al 2013, Can J Vet Res, 77: 183-190.

Graphs or Images 1



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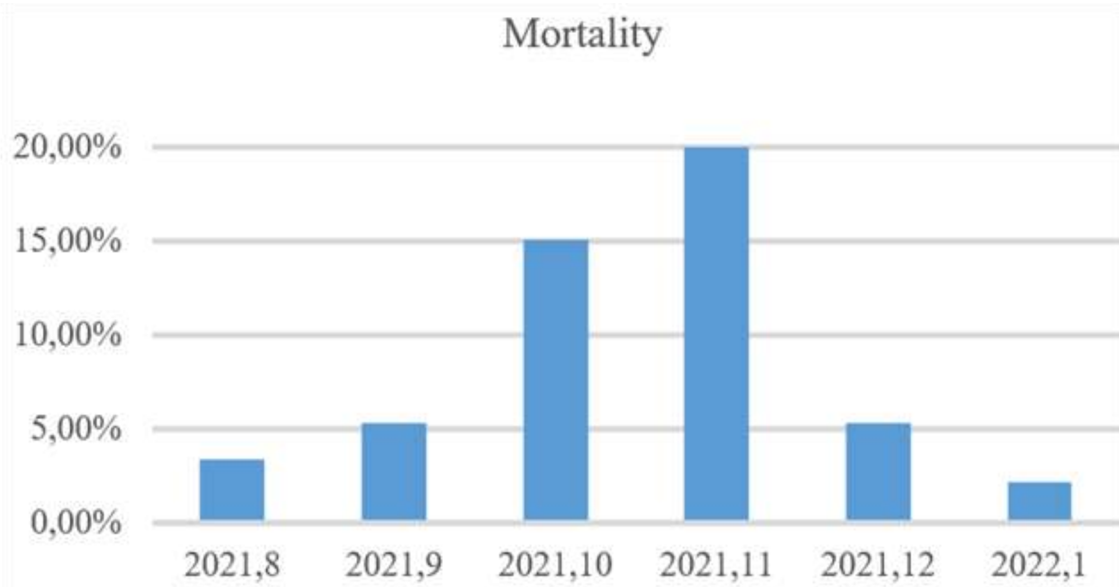


P-B-030

CORRELATION OF SEVERITY OF ATROPHIC RHINITIS AND PNEUMONIA ACCOMPANIED BY POLYSEROSITIS ON KOREAN SWINE FARMS

Mr Noh Hyundong[1], Mr. Joaquín Miguel[1], Mr An Kyohyun[1], Mr Seo Sangwon[1], Mr Byun Jeongjae[1]
[1]*HIPRA*

Introduction
Atrophic rhinitis (AR) caused by Bordetella bronchiseptica and Pasteurella multocida causes damage on pigs' nasal turbinates (1). As nasal turbinates filter the pathogens out from the inhaled air, it is known that pigs with AR are prone to suffering from more respiratory diseases caused by Glasserella, Influenza, PRRS, and others (2, 3, 4), as B. bronchiseptica belongs to the porcine respiratory disease complex (5). Although vaccination against AR is very popular in Korea, the situation of respiratory infection related to AR on Korean farms has not been surveyed well. The objective of this study was to discover the correlation between the severity of AR and pneumonia on Korean swine farms, especially focusing on lung lesions and polyserositis.

Materials and Methods
Necropsy was conducted for 267 dead pigs from 81 commercial swine farms distributed throughout all the provinces of South Korea and animals' lungs and snouts were collected. Nasal lesions of each animal were assessed by transection of the snout at the level of 1st upper premolar, following the guidelines of European Pharmacopoeia which has a maximum total score of 18 points. Lung lesions were evaluated by the scoring method described by Madec and Kobisch, having a maximum total score of 28 points. Information about the existence of polyserositis was also collected. All animal information was divided into three categories representing the severity of atrophic rhinitis (AR), according to the score of the nasal lesion; "mild (0-4 points)", "moderate (5-8 points)", and "severe (9-18 points)". Pearson correlation was analyzed to see the relationship between nasal lesion score and lung lesion score. Binominal regression with Tukey as a posthoc test and linear regression with Tukey as a posthoc test were analyzed to see the correlation of severity of AR and polyserositis and the correlation of severity of AR and lung lesion score, respectively.

Results
The nasal lesion score had a positive correlation with the lung lesion score (r=0.65). As the severity of atrophic rhinitis became higher from "mild" to "moderate" and from "moderate" to "severe", lung lesion score also increased and more polyserositis were observed. The differences were statistically significant.

[Figure 1]. Correlation between nasal lesion score and lung lesion score

[Figure 2]. Correlation between severity of atrophic rhinitis (AR) and lung lesion score

[Figure 3]. Correlation between severity of atrophic rhinitis (AR) and presence of polyserositis

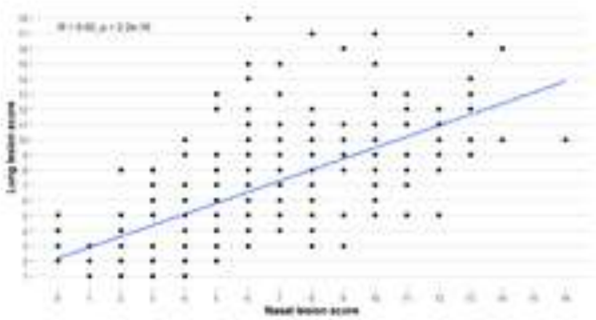
Discussion and Conclusion

Despite the continuous use of vaccines against atrophic rhinitis (AR), many Korean swine farms still seem to have AR in their pigs. As the condition of nasal turbinates has a strong relationship with respiratory problems such as pneumonia and polyserositis (1, 5), it is necessary for Korean swine farms to control AR with a proper vaccination protocol. Following studies
regarding which respiratory pathogen becomes more virulent under the presence of AR would be needed.

Acknowledgments
The authors would like to acknowledge all the veterinarians and farmers who supported them in obtaining the samples.

References
1. De Jong MF. 2006. Diseases of Swine, 10th Edition, 34:577-602
2. Brockmeier S. 2003. Veterinary Microbiology 99 (2004) 75-78
3. Loving C et al. 2010. Microbial Pathogenesis 49 (2010) 237-245
4. Brockmeier S et al. 2000.
5. Hau, S.J., Brockmeier, S. The Pig Site. Review Article. p.1-21.

Table 1



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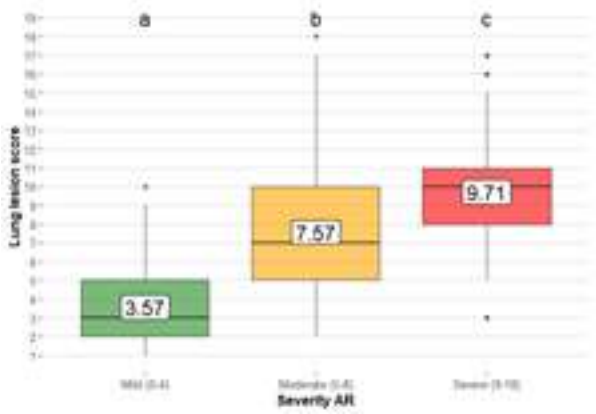

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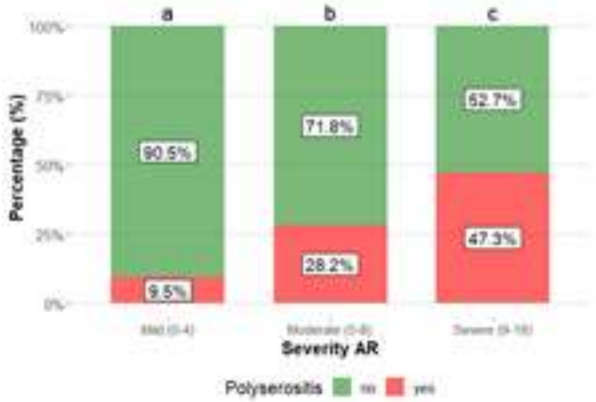


P-B-031

CRUDE F4 FIMBRIAE EXTRACT: A CANDIDATE FOR ESCHERICHIA COLI VACCINE DEVELOPMENT FOR NURSERY PIG

Dr. Luong Thi Yen Nguyet[1], Asst. Prof Puey Ounjai[2], Asst. Prof. Natharin Ngamwongsatit[1,3], Prof. Kampon Kaeoket[1]
[1]Dept. of Clinical Sciences and Public Health, Faculty of Veterinary Science, Mahidol University, Thailand, [2]Department of Biology, Faculty of Science, Mahidol University, [3]Laboratory of Bacteria, Veterinary Diagnostic Center, Faculty of Veterinary Science, Mahidol University

Introduction
Enterotoxigenic Escherichia coli (ETEC) is one of the most important pathogens causing diarrheal disease in neonatal and weaned piglets (1). The F4+ETEC is particularly problematic in pig farms. Using antibiotics to control disease led to increasing selection pressure and resulted in the selection of antibiotic-resistant bacteria (2). The vaccine is a key solution for preventing diarrhea caused by E. coli (3). Virulence factors, such as fimbriae, play an essential role in its pathogenesis and are a candidate antigen for producing vaccine. In this study, we used the crude F4 fimbriae to be exploited as an antigenic determinant for vaccine development against disease caused by E. coli.

Material and Methods
Crude F4 fimbriae were extracted by heat shock along with homogenization from F4+ETEC strain, and analyzed by SDS-PAGE and LC-MS/MS method. Crude F4 fimbriae extracts (150 µg/dose) were used to immunize primary at four-weeks old piglets (n=3) and booster at six-weeks old (unvaccinated group as control group, n=3). All pigs were collected serum and evaluated the level of antibodies by ELISA (4). We conducted challenges on all pigs at two weeks post booster to evaluate the protective ability of vaccines.

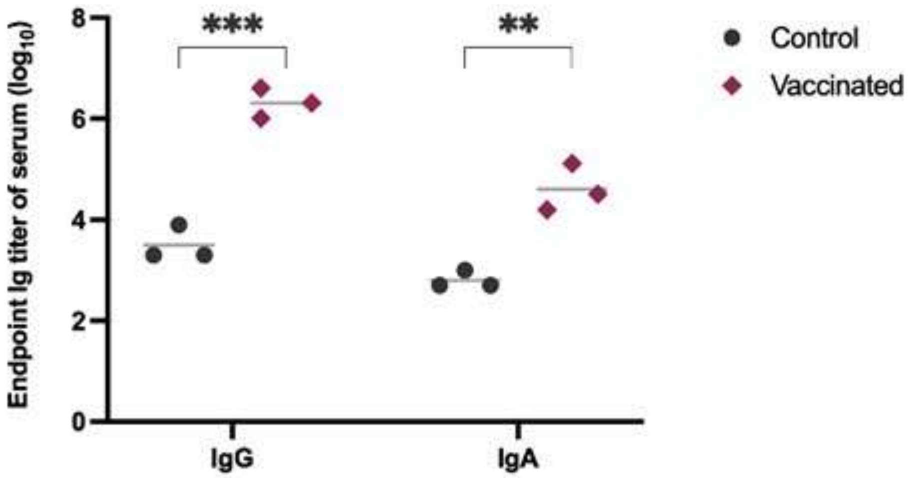
Results and Discussion
The SDS-PAGE and LC-MS/MS analysis revealed that the crude F4 fimbriae extract contains a major band of molecular weight around 24 kDa, corresponding to the size of the major subunit (FaeG) of F4 fimbriae together with several minor bands of different molecular weights. ELISA result showed that the level of serum pig IgG and pig IgA antibodies of vaccinated group were significantly higher than unvaccinated control group ($P < 0.01$) (Fig. 1). In challenge experiment, the control group excreted challenge strain in feces prolonged for 5 days post-challenges, while vaccinated groups were 2-3 days post-challenge only. In present study, we suggested crude F4 fimbriae vaccine is a candidate vaccine to protect piglets against diarrhea diseases caused by E. coli. Furthermore, the result shows that our method used in crude F4 fimbriae extraction can be used for future vaccine development with simplicity, speed, and efficiency.

Acknowledgement
This work was financial supported by Royal Golden Jubilee Ph.D. Programme (RGJ PhD.) under Grant number PHD/0074/2561 and Mahidol University, Thailand.

References
1. Hartadi et al. (2020) Sys Rev Pharm 11, 687-698.
2. Nguyet et al. (2022) Scientific Reports 12, 9038.
3. Zhang et al. (2018) Vaccine 36, 723-728.
4.Vazquez et al. (1996) J Med Microbiol. 44, 453-463.

Figure 1. The level of serum IgG and IgA titer measured by ELISA against F4 fimbriae antigen. Asterisks indicate a significant difference between the given groups (** = P ≤ 0.01, *** = P ≤ 0.001)

Graphs or Images 1



P-B-032

DIAGNOSIS AND PREVENTION OF DIARRHEA IN PIGS

Prof. Wen-Chin Yang[1], Ms Tien-Fen Kuo[1], Ms Greta Yang[1], Prof Yu-Chuan Liang[1]
[1]*Academia Sinica*

Introduction
Pork accounts for 43% of protein food for humans with a yearly value of 250 billion American dollars (1). Pigs of all ages may suffer diarrhea, a common clinical sign of gut disorders. Bacteria, viruses and parasites are causative pathogens that are frequently transmitted through the fecal-oral route (2). Antibiotics are commonly used to prevent and treat diarrhea though their misuse and abuse have public health concerns. The alternatives to antibiotics (ATA) are highly demanded by pig industry. Herbal extracts are emerging as a promising ATA for swine diarrhea (3).

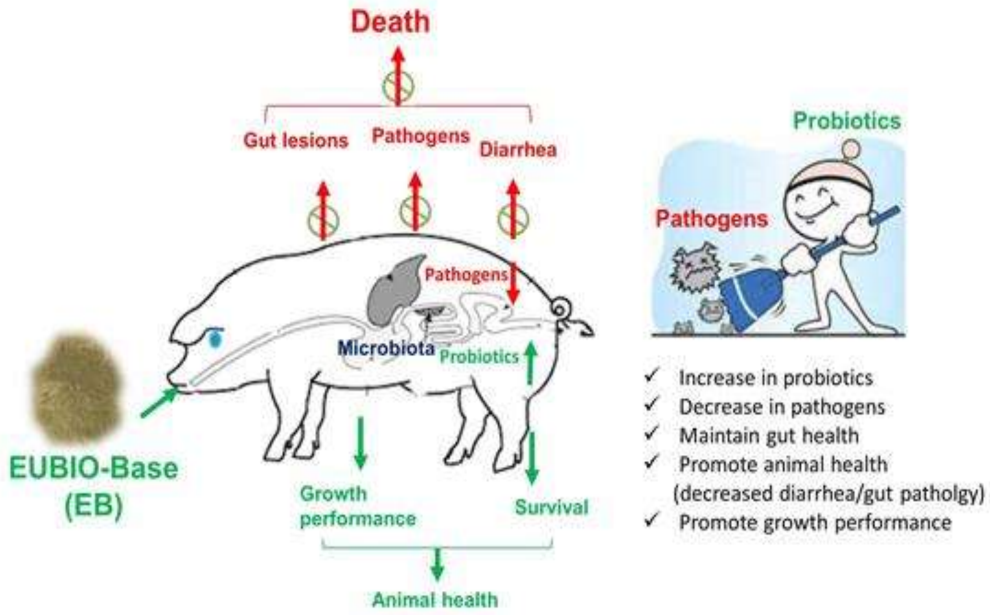
Materials and Methods
Polymerase chain reaction (PCR) was used as molecular diagnosis of specific diarreagenic bacteria and viruses. A herbal formulation, EUBIO-Base (EB), was tested for diarrhea prevention and growth performance in nursery pigs. Fecal score, bacterial counting, next-generation sequencing (NGS) analysis of gut microbiota, body weight, food intake, and feed conversion ratio (FCR) were measured.

Results
In this study, we first developed a fast PCR-based method for some diarreagenic bacteria and viruses. We found that swine diarrhea was caused by co-infections of different pathogens. Since dysbiotic gut microbiota are always related to diarrhea, we subsequently assessed the anti-diarrheal function and mechanism of a herbal formulation, EUBIO-Base (EB), in weaned pigs (4). As a result, EB reduced swine diarrhea as evidenced by fecal score and presence of pathogenic bacteria and viruses. Consistently, NGS data indicated that EB dose-dependently increased proportion of probiotics and reduced diarrheagenic bacteria in the gut of pigs. Moreover, EB dose-dependently elevated body weight gain and lowered FCR in pigs.

Conclusions and Discussion
This proof-of-concept study demonstrates that herbal approach is a promising strategy to prevent diarrhea in pigs of all ages. This work also shows beneficial effects of EB on growth performance in pigs. The mechanism of EB involves regulation of gut microbiota, including increased probiotics and decreased pathogens (Figure 1). The field trial of EB also comes to the same conclucions.

References:
1 Ritchie, H. & Roser, M. https://ourworldindata.org/meat-production (2017).
2 Kylla, H., Dutta, T. K., Roychoudhury, P. & Subudhi, P. K.. Vet World 12, 224-230 (2019).
3 Gadde, U., Kim, W. H., Oh, S. T. & Lillehoj, H. S. Animal health Res Rev 18, 26-45 (2017).
4 EUBIO-Base, http://www.ccpp.com.tw/_tw/01_product/02_detail.aspx?AID=1360 (2023).

Graphs or Images 1



P-B-033

DIFFERENCES IN PARTICLE SIZE AND AGGLOMERATION OF TWO INJECTABLE TOLTRAZURIL AND GLEPTOFERRON FORMULATIONS (BAYCOX® IRON INJECTION, FORCERIS®) AND POTENTIAL IMPACT ON BIOAVAILABILITY

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Introduction
Piglets raised in intensive farming conditions are at risk of developing iron deficiency anemia and coccidiosis caused by Cystoisospora suis. Parenteral (intramuscular) iron administration and oral treatment with toltrazuril in the first days of life is a common practice to prevent these two challenges for piglet health (1, 2). With the introduction of the first patented, injectable combination of toltrazuril and iron (Baycox® Iron Injection, Elanco Animal Health) both, coccidiosis and iron deficiency anemia can be prevented with a single application reducing handling of piglets, optimizing farrowing pen management practices and improving animal well-being. The objective of this study was to compare particle size and agglomeration of two injectable toltrazuril and iron formulations (Baycox® Iron Injection/ Elanco Animal Health, Forceris®/ CEVA) as relevant quality criteria related to bioavailability (3, 4).

Materials and Methods
For both formulations commercially available product was used, shaken well, and mixed with an aqueous dispersing and measuring medium. The measurement was carried out with suitable laser diffraction equipment (Malvern Mastersizer 3000, Hydro LV module). Following initial measurements, ultrasound treatment was applied to dissolve potential particle agglomerations and a second set of measurements was conducted.

Results
The distribution curve of measured particle sizes of Baycox® Iron Injection shows as single peak and lower variability compared to Forceris®, indicating a higher homogeneity of particle sizes (Fig. 1). The second peak in the Forceris® curve, which disappears after deagglomeration with ultrasound (Fig. 2), indicates a tendency of particle agglomeration in the Forceris® suspension. Those agglomerates in Forceris® virtually increase particle size measured. Baycox® Iron Injection does not show any tendency of particle agglomeration. After deagglomeration with ultrasound Baycox® Iron Injection particles remain smaller than the individual particles of Forceris® with D(90) values of 4.8 vs 8.2 µm, meaning 90% of the particles are below the respective size.

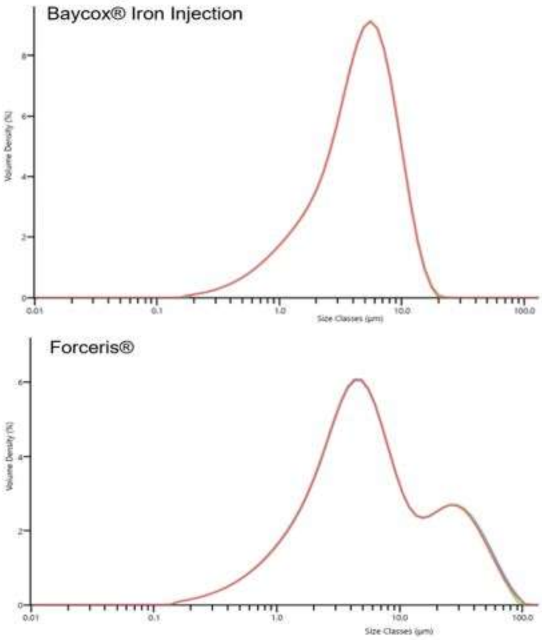
Conclusions and Discussion
The study showed superior formulation characteristics of Baycox® Iron Injection over Forceris® with smaller individual particle sizes of the active pharmaceutical ingredient and no agglomeration. A smaller particle size facilitates faster dissolution (3, 4) and is commonly related to better bioavailability.

References
1. Maes D et al., 2011. Veterinary Record 168: p188.
2. Joachim A et al., 2018. Veterinary Parasitology 253: p102-119.

3. Noyes AA, Whitney WR, 1897. J. Am. Chem. Soc. 19: p 930-934.
4. Nernst W, 1904. Zeitschrift für Physikalische Chemie 47: p 52-55.

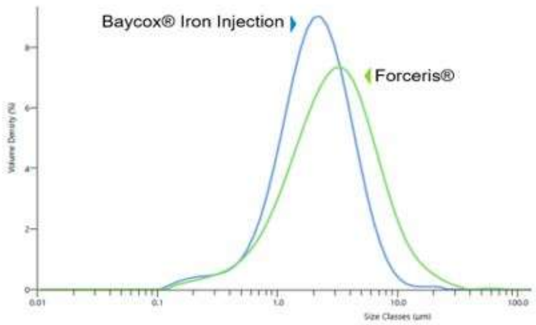
Graphs or Images 1

Figure 1. Particle (agglomerate) size distribution measured by laser diffraction.



Graphs or Images 2

Figure 2. Size distribution of individual drug particles measured by laser diffraction after deagglomeration with ultrasound treatment.



P-B-034

DISTRIBUTION OF ACTINOBACILLUS PLEUROPNEUMONIAE SEROVARS REVEALS A PREDOMINANCE OF SEROVAR 15 IN TAIWAN FROM 2015 TO 2021

Mr. Feng-Yang Hsu[1], Mr. Chao-Nan Lin[1,2], Mr. Ming-Tang Chiou[1,2], Mr. Wei-Hao Lin[1,2]
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Introduction:
Actinobacillus pleuropneumoniae (App) is a major cause of porcine necrotizing hemorrhagic pleuropneumonia, an acute lung disease that causes significant morbidity, mortality, and economic losses in the worldwide pig industry [1]. Serotyping of field isolates is important for App since different serovars have different virulence potentials. Molecular serotyping methods, especially multiplex PCR (mPCR) assays targeting capsular genes, have been described for identifying all 19 known serovars in recent years [2]. Besides, the alteration of serovar prevalence may occur over time in a given country or continent. It is necessary to monitor the geographical distribution of App serovars periodically. Therefore, the aims of the present study were (i) to update the knowledge of the distribution of App serovars from diseased pigs and (ii) to compare the results with previously published data in Taiwan.

Materials and Methods:
App isolates were obtained from diseased pigs sent to Animal Disease Diagnostic Center, National Pingtung University of Science and Technology from 2015 to 2021. Multiplex PCR assays were applied to detect all known serovars [2]. The isolate number and proportion of each serovar were compared with data from 211 App isolates collected during 2002-2007 in Taiwan [3].

Results:
A total of 149 App strains were isolated in the seven-year collecting period. Only serovar 1, 2, 5, 7, and 15 were determined in the present study, with 23 (15.4%), 9 (6.0%), 37 (24.8%), 22 (14.8%), and 58 (38.9%) isolates, respectively. Serovar 15 is the most predominant serovar in this study, followed by serovar 5 and 1 (Figure 1). This distribution is quite different from the last published report [3], in which serovar 1 (65.4%) and 2 (34.1%) were significantly dominant. In the previous study, only one isolate was determined as serovar 5, and both serovar 7 and 15 had not been detected from 2002 to 2007. The fluctuation of proportions of each serovar from 2015 to 2021 is shown in Figure 2. Serovar 15 reached a high in 2019 and remained steady at nearly one-third in 2020-2021. Serovar 7 was first detected in 2017 and increased to nearly 20%. Interestingly, serovar 2 drastically decreased in 2015-2016 and no isolate has been detected for the following four years.

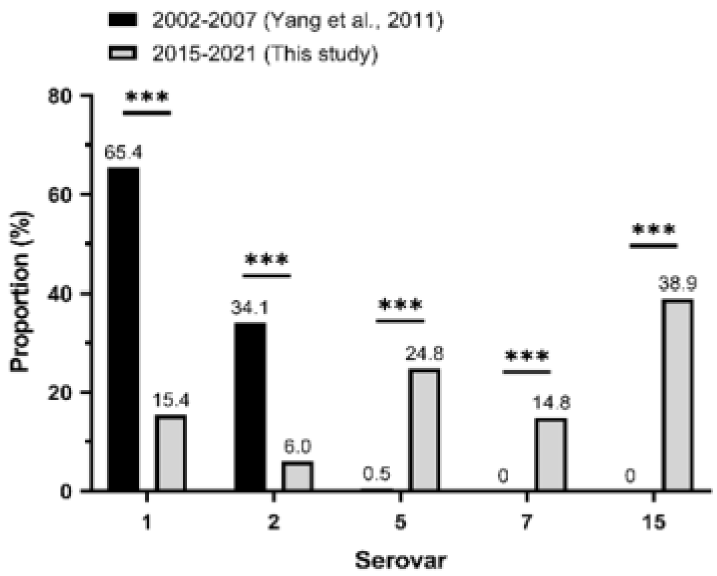
Conclusions:
The distribution of App serovars over seven years revealed the predominance of serovar 15 in Taiwan. The reduction of serovar 1 and 2 isolates may be related to the vaccination control of growing pigs through different programs applied in the field. Further investigation of the effect of bacterin and sub-unit vaccines on serovar shifting occurring in a farm/area should be launched to monitor the serovar prevalence and establish effective control strategies.

References:
[1] Gottschalk M. and Broes A., 2019. Actinobacillosis. Diseases of Swine. 11th ed. 756-758.
[2] Stringer et al., 2021. Vet. Microbiol. 255, 109021
[3] Yang et al., 2011. J. Vet. Med. Sci. 73, 205-208

Figure 1. The comparison of serovar distribution between the previous and current studies.
***: $p < 0.001$

Figure 2. The prevalence of App serovars in Taiwan from 2015 to 2021.

Graphs or Images 1



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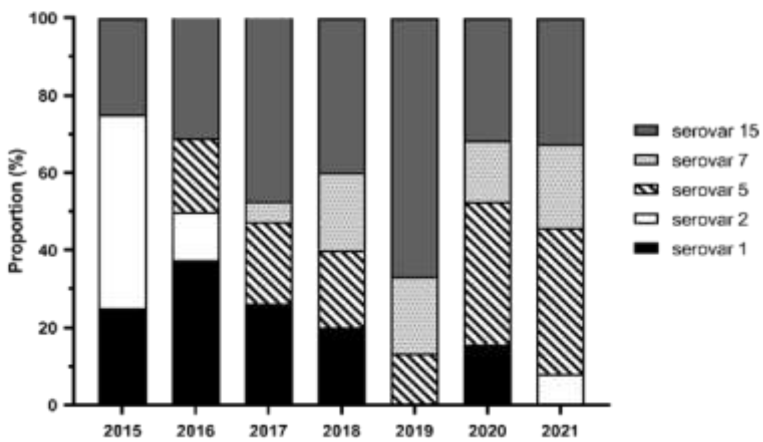

P-B-035

EFFECT OF FORCERIS ON CYSTOISOSPORA SUIS OOCYST EXCRETION AND GROWTH OF NEONATAL PIGLETS

Dr. Daniel Sperling[1], Dr. Bregt Decorte[2], Dr. Stijn Casaert[2], Dr. Nathalie De Wilde[2], Dr. Sara Roose[2], Prof. Dominiek Maes[3], Prof. Peter Geldhof[2]

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Introduction

Cystoisospora suis is the predominant coccidian parasite of swine that causes neonatal porcine cystoisosporosis. Suckling pigs are most susceptible, while older pigs excrete few to no oocysts and show no clinical signs upon infection. Cystoisosporosis is characterized by non-hemorrhagic transient diarrhea and economic losses are mainly due to impaired growth and mortality.

The objective of this study was to assess the effect of a treatment with Forceris, a combination product containing toltrazuril and iron, against iron only on oocyst excretion and growth of piglets at weaning and at the end of the nursery period.

Materials and Methods

The study was carried out on two farms with a confirmed history of Cystoisospora suis infections. Fifteen litters were randomly assigned to either the control or treated group and the piglets of each litter were injected 1-2 days after birth with iron or iron combined with toltrazuril, respectively.

Fecal oocyst shedding was analyzed on days 7, 14, 21 and 10 days post weaning (day 31). For this, individual fecal samples were taken and subsequently pooled per litter. The piglets were individually weighed at the time of injection and at weaning (day 21).

Results

Oocyst shedding was substantially reduced in the treated litters, with only 5 positive tests out of the total of 109 sampling time points (4.6%), with a mean oocyst shedding of 130 (SD=75,8) oocysts/ gram of feces (OPG). In contrast, 64 out of 101 pooled fecal samples tested positive in the control group (63.4%), with an average of 3010,5 (SD= 8328,9) OPG. In terms of growth, across both farms treated piglets showed an average increased weight gain of 346.2 grams ($P < 0.05$) in the period from day 1 to day 21.

Conclusions and Discussion

Treatment of neonatal piglets with a combination of toltrazuril and iron on farms with a confirmed history of C. suis reduced oocyst excretion and significantly increased weight gain during the farrowing period. The long-term effects of the treatment during the nursery phase are currently further investigated.

P-B-036

EFFICACY EVALUATION OF A PORCINE BIVALENT MYCOPLASMA SUBUNIT VACCINE AGAINST MYCOPLASMA HYORHINIS AND MYCOPLASMA HYOPNEUMONIAE CHALLENGE

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Introduction
Mycoplasma hyopneumoniae (Mhp) is the causative agent of swine enzootic pneumonia (SEP) and a major contributor to respiratory diseases. SEP is closely related to retarded growth and low feed efficiency. Mycoplasma hyorhinis (Mhr) is a pathogen causing polyserositis and arthritis in afflicted pigs. It was previously shown that vaccination of piglets with a Mhr subunit vaccine significantly increased the survival rate in two suspected Mhr positive farms (90.6% vs 78.4% and 85.4% vs 77.3%, respectively). It was also found that piglets vaccinated with the Mhr subunit vaccine had fewer lesions in the lungs after Mhp challenge, suggesting that the Mhr vaccine may offer partial protection against Mhp infection in pigs. In the present study, the Mhp antigen was included in the Mhr subunit vaccine to generate a Mhr/Mhp bivalent subunit vaccine and the efficacy of this bivalent vaccine against Mhr and Mhp was evaluated.

Materials and methods
Specific pathogen free pigs were immunized with one dose of Mhr/Mhp bivalent subunit vaccine or normal saline at 5 weeks old. At 9 weeks old, experiment pigs were challenged with either Mycoplasma hyopneumoniae via intra-tracheal route or Mycoplasma hyorhinis via intraperitoneal route. For Mhr challenge, clinical symptoms were observed and recorded daily and animals were sacrificed at 21 days post challenge and tissues were examined for polyserositis (peritonitis, pleurisy, pericarditis) and arthritis [1]. For Mhp challenge, blood samples were collected for antibody analysis using IDEXX M. hyo Ab Test. Four weeks after challenge, animals were sacrificed and lungs were examined for lesion and scores were assigned according to the severity [2].

Results
After Mhr challenge, the clinical score of vaccination group was significantly lower than that of control group on day 11, 12 and 17 after challenge. The Mhr-specific antibody titer in serum in vaccinated pigs rose quickly and was sero-positive one week after challenge, possibly accounting for lower clinical scores in vaccination group. Pathological examination showed that the average lesion score for pleurisy and pericarditis was significantly lower in vaccinated pigs and the average lesion score (polyserositis and arthritis) in vaccinated pigs was also significantly lower than that in control pigs. The vaccinated pigs were positive for Mhp-specific antibody 2 weeks after vaccination and they stayed serum positive for Mhp antibody on the day of Mhp challenge. Pathological examination of the lungs after Mhp challenge showed that the vaccinated pigs had significantly lower lesion score in cardiac lobes, diaphramatic lobes, and intermediate lobes and the average lung lesion score (apical lobes, cardiac lobes, diaphramatic lobes, and intermediate lobes) was also lower in vaccinated pigs. These results demonstrated that vaccinating pigs with one dose of bivalent subunit Mhp/Mhr vaccine at 5 weeks old can reduce clinical symptoms, polyserositis, arthritis, and lung lesions induced by Mhr and Mhp.

References
[1] Magnusson et al. (1998) Vet. Immunol. Immunop. 61, 83-96.
[2] Garcia-Morante et al. (2016) J. Comp. Pathol. 154, 125-134.

Graphs or Images 1

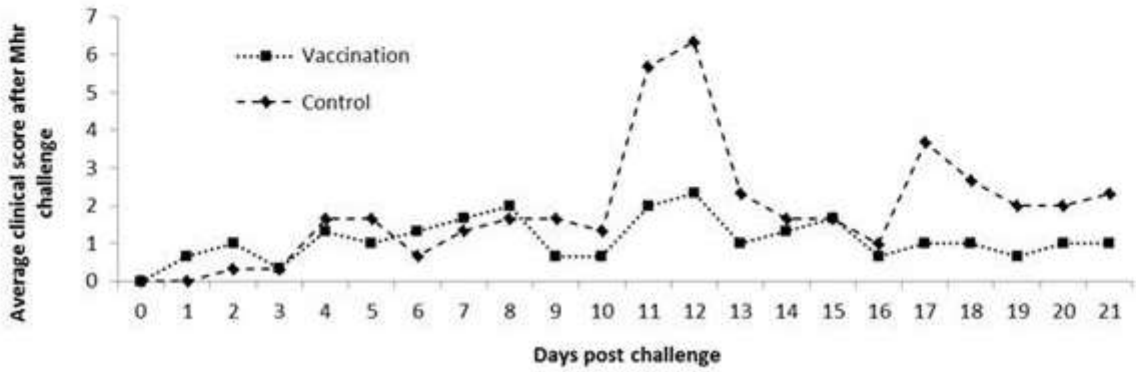

Figure 1. Average clinical score after *Mhr* challenge. Clinical symptoms were observed and scored daily for 21 days after *Mhr* challenge. Data were presented as mean clinical score per treatment group.

Graphs or Images 2

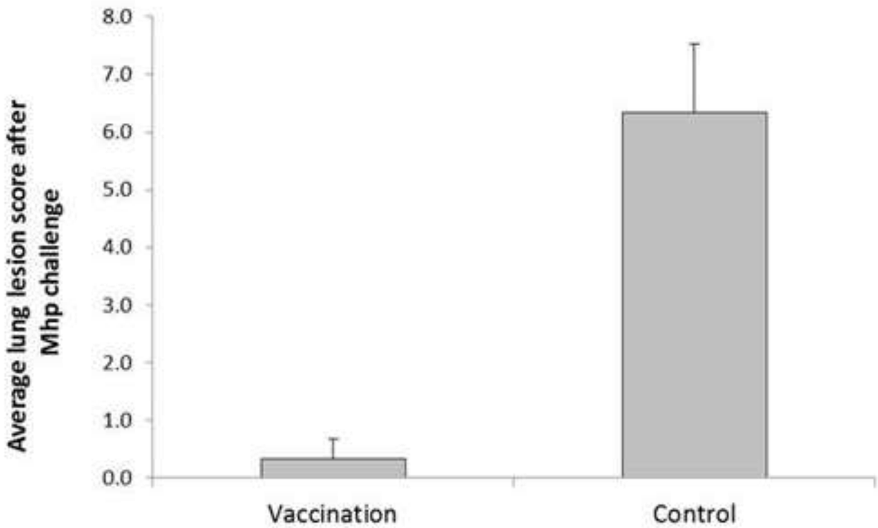

Figure 2. Average lung lesion score after *Mhp* challenge. Lung lesions were examined and scored 4 weeks after *Mhp* challenge. Data were presented as mean lesion score per treatment group.

P-B-037

EFFICACY EVALUATION OF A READY-TO-USE PCV2 AND MYCOPLASMA HYOPNEUMONIAE VACCINE IN A CONVENTIONAL FARM

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Introduction
Porcine Circovirus Type 2 (PCV2) and Mycoplasma hyopneumoniae are economically important pathogens, present in most farms worldwide. Vaccination is the main preventive measure for both infections (1,2). The aim of this study was to evaluate the efficacy of a RTU PCV2 and Mycoplasma hyopneumoniae vaccine in a conventional pig farm.

Materials and Methods
The trial was conducted in a 230-sow farm where both PRRSV and PRV are negative. The farm didn't implement Mycoplasma hyopneumoniae vaccination before as the clinical disease prevalence was low in the farm based on the anamnestic diagnosis. Three weeks-old piglets at weaning were randomly divided into two groups (120 piglets in each group) which vaccinated either with Cirbloc MHyo (Ceva, Hangzhou)- a PCV2 and M.hyo RTU vaccine or PCV2 mono vaccine A that had been used in history. The piglets were marked and fed in the same barn until slaughtering. At the age of 3, 7 and 30 weeks (Slaughtering), the M.hyo ELISA antibodies were detected (Mycoplasma hyopneumoniae Indirect, ID.vet). The pigs from two groups were sent to slaughterhouse and implemented lung lesion scoring using Ceva Lung Program (CLP) at same day. Average daily gain (ADG) and mortality were compared from weaning to slaughtering between groups.

Results
M.hyo antibody in two groups were all negative with low S/P value at 3 weeks old. Positive rate and S/P value increased sharply 4 weeks post vaccination (7 woa) in Cirbloc MHyo group, demonstrating strong immune response. Positive rate increased to 20% and 30% at 7 woa and 30 woa in Vaccine A group, indicating a natural M.hyo infection present in the farm (Table 1).
Cirbloc MHyo group had better production performance compared with Vaccine A group (Table 2).
Both groups showed low lung lesion scores, indicating M.hyo infection and enzootic pneumonia present in the farm (3). However, Cirbloc MHyo group had less enzootic pneumonia-like lesions compared with vaccine A group (Table 3).

Conclusions and Discussion
Mycoplasma hyopneumoniae is the primary pathogen of enzootic pneumonia (EP), a chronic respiratory disease in pigs characterized by a chronic, nonproductive cough, often underestimated by farms. The farm in the trial had relatively low infection pressure confirmed by dynamic ELISA results and Lung lesion scoring. However, the farm still could benefit from Cirbloc MHyo vaccination compared with Vaccine A by improved respiratory health, lower mortality and higher ADG. As a bivalent vaccine including both PCV2 and M.hyo, the farm could also benefit from it by labor saving and biosecurity risk reduction.

Acknowledgement and References

1. Segales J et al. Anim Health Res. Rev. 2005, 6, 119–142.
2. Simionatto, S. et al. Vet. Microbiol. 2013, 165, 234–242.
3. Jun Z. et al. IPVS. 2022:305.

Table 1

Table 1: M.hyo ELISA antibody in two groups

Age	Positive rate %		S/P value	
	Cirbloc MHyo	Vaccine A	Cirbloc MHyo	Vaccine A
3 weeks	0	0	0.115	0.138
7 weeks	100	20	1.101	0.206
30 weeks	60	30	0.594	0.373

Table 2

Table 2: Mortality and ADG in two groups

	Cirbloc MHyo	Vaccine A
Mortality %	3.33	4.17
ADG g	607.65	600.82

Table 3

Table 3: EP-like lesion by CLP in two groups

	Cirbloc MHyo	Vaccine A
Broncho-pneumonic lungs %	17.02	30.61
Affected surface out of sick lungs %	1.47	3.21
EP Index	0.23	0.71

Graphs or Images 1

Fig.1 Serum samples and Lung lesion scoring at slaughterhouse by CLP

P-B-038

EFFICACY OF AN INJECTABLE TOLTRAZURIL-GLEPTOFERRON FOR CONTROL OF COCCIDIOSIS UNDER FIELD CONDITIONS

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Introduction
Cystoisospora suis (C.suis) infection or Coccidiosis is one of major causes of diarrhea in piglets. After ingestion of oocyst, piglets show diarrhea within one week after infection and excrete oocyst to environment. Oral application of toltrazuril is used worldwide to prevent and control coccidiosis but previously published work showed limited efficacy of current control of coccidiosis on affected farms(1). Recently a combined toltrazuril and gleptoferron product-Forceris® has been developed for parenteral application. The aim of the study is to compare oral versus injectable toltrazuril for control coccidiosis under field conditions.

Materials and Methods
This study was conducted in 2,400 sow size located in Eastern part of Thailand. Piglets from 200 sows (2,829 piglets) were divided into 2 groups; group 1 (n=1,367) was treated with a fixed dose 1.5 ml of 45 mg toltrazuril + 200 mg gleptoferron (Forceris®) intramuscularly per piglet on the 2nd day of age (DOA) and group 2 (n=1,462) was treated with 200 mg of parenteral iron dextran on the 2nd DOA + 20 mg toltrazuril/kg body weight orally on the 4th DOA. Feces were collected from 14 randomly selected litters per group at 12 DOA and 24 DOA. Floatation technique was used for detection C.suis oocysts in fecal samples and performance in farrowing unit was recorded. The statistical analysis was performed using Two sample t-test and Fisher's exact test.

Results
There was not significant difference on born alive piglets per litter, birth weight and piglets wean per litter. C. suis oocysts were found in samples in both groups at 12 DOA (3 positive litter in group 2 vs. 1 litter in group 1). There was no shedding detected in group 1 on 24 DOA, compared to group 2 (6/14 positive litters) ($p<0.05$) (table 1). Pre-weaning mortality rate was numerically lower, weaning weight significantly higher and higher average daily weight gain (ADG) was recorded in group 1 as showed in the table 2.

Conclusions and Discussion
Forceris® reduced C.suis oocyst excretion in feces at 12 and 24 DOA when compared to oral toltrazuril. One of the explanation of better efficacy and reduction of shedding of oocysts is fact, that injectable treatment (Forceris®) provide improved pharmacokinetic profile of active substance (toltrazuril and its main metabolite toltrazuril sulfone) both in plasma and target tissue (intestine)(2). Forceris® injection in this study was convenient, reduced stress from handing, effective for control coccidiosis and promoted growth performance of piglets.

Acknowledgement and References
1. Hinney et al., 2020, Cystoisospora suis control in europe is not always effective, Front. Vet. Sci., 7:113.
2. Karembe et al., 2021, Absorption and distribution of Toltrazuril and Toltrazuril Sulfone in plasma, intestinal tissues and content of piglets after oral or intramuscular administration, Molecule, 26: 5633.

Table 1

Table 1. Number of litters with *C.suis* oocyst positive from total litters.

	Group 1	Group 2
12 DOA	1/14	3/14
24 DOA	0/14[a]	6/14[b]

[a,b] Fisher's exact test $p<0.05$

Table 2

Table 2. Performance in farrowing unit.

Performance	Group 1	Group 2
Born alive piglets per litter	13.8	14.6
Birth weight (kg)	1.4	1.4
Weaning piglets per litter	13.0	13.7
%Pre-weaning mortality	5.6%	6.6%
Weaning weight (kg)	6.4[a]	6.1[b]
ADG day 2-24 (g/pig/day)	217.4	204.3

[a,b] Two sample t-test $p<0.05$

P-B-039

EFFICACY OF DIFFERENT PCV2 AND MHYO COMBINED VACCINES AGAINST PCV2 OR MYCOPLASMA HYOPNEUMONIAE EXPERIMENTAL INFECTIONS

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Introduction
Enzootic pneumonia and PCVD remain a severe health problem in pig farms (1). Vaccination against those two pathogens helps to reduce their clinical manifestation and corresponding losses. The aim of this study was to evaluate the efficacy of different PCV2 and M.hyo vaccines in experimental challenge models.

Material and Methods
In two different experiments three weeks old piglets were vaccinated either with Circovac® with Hyogen® (DUO) - both Ceva mixed as RTM or other PCV2/M.hyoRTM vaccines or a PCV2a/b+M.hyo RTU vaccine. In the trial 1) vaccinated and positive control pigs were challenged at 9 weeks of age (WOA) with the PCV2d strain. In the trial 2) vaccinated and positive control pigs were challenged at 8 WOA with M.hyo. Pigs were always euthanized 28 days post infection (DPI) and either PCV2 loads in lymph nodes (Lnn) (trial1) or lung lesions scores (LLS) according to the European Pharmacopoeia 9.0 and Hannan et al., 1982, (trial2) were measured (2,3).

Results
In the trial 1) viremia at 28DPI was 0.55, 0.54 and 0.28 and 3.41 logcopy#/microL for DUO, PCV2/MhyoRTM,PCV2a/b+MhyoRTU and positive control-respectively (p<0,05).
The PCV2 loads in Lnn were significantly lower in vaccinates vs control (p<0,05 control vs others).

Fig 1. PCV2 loads in mesenterial lymph nodes

In trial2) only Duo induced strong serological response, while other two vaccinatd groups seroconverted massively only after the infection.

The mean LLS only for DUO group differed significantly from the positive control (p< 0,05 for DUO vs any other groups).

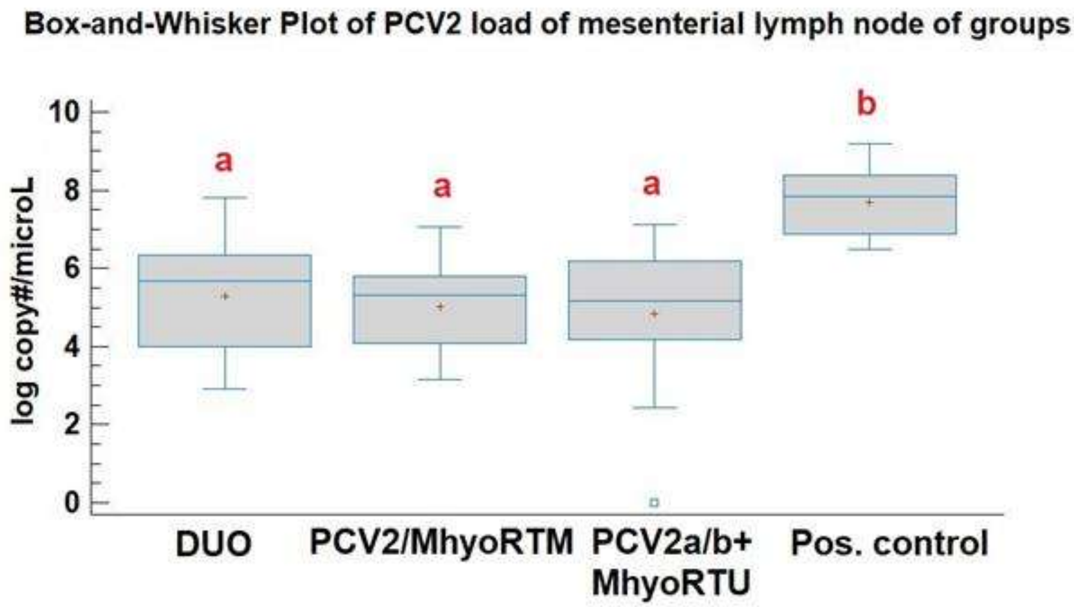
Fig 2. Weighted group lung lesion scores

Conclusion
DUO provided equal PCV2 protection as PCV2/MhyoRTM and PCV2a/b+MhyoRTU. DUO however outperformed those vaccines in protection against the development of lung lesions due to M.hyo. Some of the combined PCV2 and M.hyo vaccines may provide in such a challenge model insufficient protection against M.hyo infection.

References
1. Maes et al. 2017. Transbound Emerg Dis. 1–15

2. European Pharmacopoeia 9.0
3. Hannan et al., 1982, Res. in Vet. Sc. 33, 76-88

Graphs or Images 1



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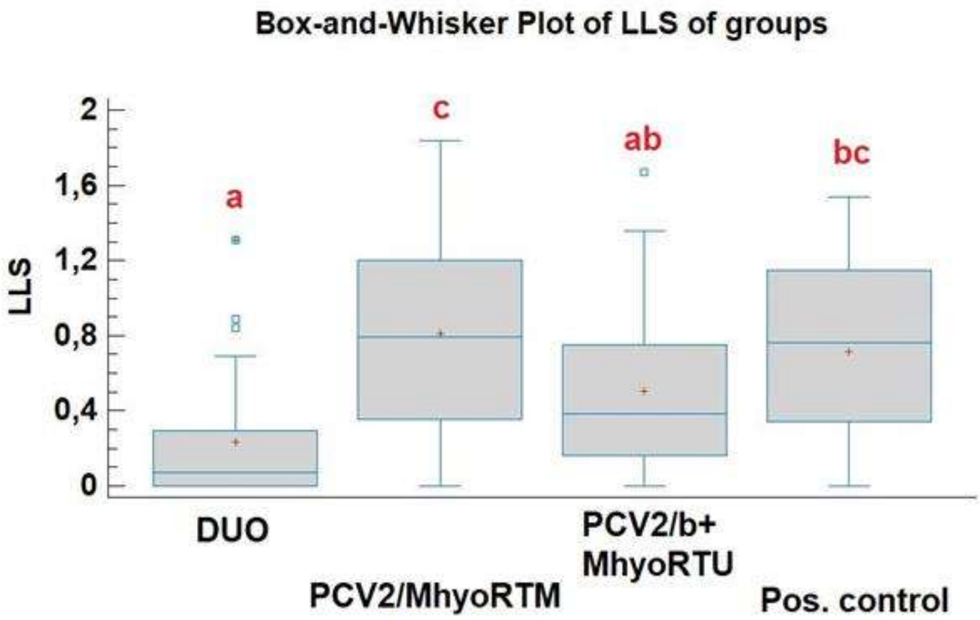

P-B-040

EFFICACY OF PLANT-BASED MIXTURE OF ADDITIVES ON BRACHYSPIRA HYODYSENTERIAE EXPERIMENTALLY INFECTED PIGS

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Swine dysentery (SD) is an important enteric disease described by catarrhal-hemorrhagic diarrhea, and mortality in pigs, and is caused by strong beta-hemolytic Brachyspira spp. (1). The use of nutraceuticals as an alternative to antimicrobials (AB) has been extensively studied in the last few years (2, 3), due to the ban or increased control on the use of AB to which bacteria can develop resistance. Therefore, the aim of this study was to evaluate the efficacy of, the plant-based mixture of additives, DYSGUARD-S® (DYS) on the control of SD in piglets challenged with B. hyodysenteriae.
The study was performed on 23-day-old healthy piglets obtained from an SD-negative farm. The piglets were divided into 4 groups. Positive (PC) and Negative (NC) controls groups, with 10 piglets each, were challenged or not with a pure culture of a pathogenic B. hyodysenteriae, respectively, and received a diet free of antimicrobial and zinc oxide. DYS 1kg (D1) with 8 piglets and DYS 2kg (D2) with 9 piglets were grouped with piglets challenged as PC group and received DYS 1kg/ton or DYS 2kg/ton in the diet, respectively. The piglets were intragastrically inoculated, after 14 days of adaptation to diets, for three consecutive days, using a pathogenic B. hyodysenteriae strain (10^8 organisms). All animals were weighed at three-time points (at arrival, prior inoculation, and prior euthanasia). Every day all animals were clinically evaluated and 14 days after inoculation, all animals were euthanized, and samples were collected for further analysis. The average weight gain, gross lesion, and diarrhea score were evaluated. The Skillings Marck with Conover post hoc tests were used for composite gross lesion score x group x block (heavy or light pigs).
The first onset of diarrhea scores typical for SD in the PC group was at 7 days post inoculation (DPI), while in D1 and D2 groups was 4 days later. At 11., 12., and 13. DPI, diarrhea typical for SD occurred in the PC group in 4, 5, and 4 pigs, respectively, while in the D1 group only once at 11. and 12. DPI, and once in the D2 at 11. DPI. Only in the PC group, did 3 pigs die after the challenge due to the severe SD clinical signs and gross lesions. The D2 group had significantly less extensive and milder gross lesions at necropsy in comparison to the PC group ($P<0.05$) (Fig. 1). There was no difference in average weight gain (D1: 5.79kg ± 2.88, D2: 6.98kg ±1.81, NC: 6.82kg ±0.892, PC: 4.06kg ±3.42).
This is the first study evaluating the efficacy of a plant-based mixture of additives to control SD in B. hyodysenteriae-infected pigs. The earlier onset of diarrhea in the PC group showed that, at some level, supplementation was associated with delaying the appearance of clinical signs, and, at the higher inclusion (D2 - 2kg/ton) significantly reduced gross lesions induced by SD. Despite the superior absolute average daily weight gain in treated groups, no differences were observed probably due to the large coefficient of variation. Overall, the inclusion of DYSGUARD-S® demonstrated a delay in the onset of clinical signs, and reduced gross lesions length and severity of SD in B. hyodysenteriae experimentally infected pigs.
Acknowledgment: The research was financed by PATENT CO. DOO. In some countries, the premixture of feed additives DYSGUARD-S® is registered as PATENTE HERBA® PLUS.
References:
1- Hampson & Burroug, Diseases of swine, 2019.

2- Van Boeckel et al, PNAS, 2015.
3- Vondruskova et al, Veterinarni Medicina, 55, 2010

Graphs or Images 1

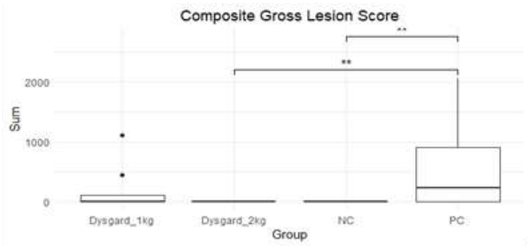

Figure 1. Composite gross lesion score in the large intestines of pigs experimentally infected with *Brachyspira hyodysenteriae*. NC—Negative control group; PC—Positive control group

P-B-041

ELIMINATION OF MYCOPLASMA HYOPNEUMONIAE FROM A 1300 SOW BREED TO FEEDER FARM IN JAPAN

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introduction
Mycoplasma hyopneumoniae is a primary contributor to the Porcine Respiratory Disease Complex(PRDC).Its impact on the cost of finisher pig production makes eradication a serious alternative for profitable production.
Mycoplasma eradication procedures include several steps:
1)load gilts and close herd
2)Mycoplasma exposure
3)blanket vaccination of the whole herd
4)complete depopulation of the nursery
5)medicate sows and piglets on-site with an antimicrobial

materials and methods
A 1300 sow breed to wean farm is two-site system. This farm was PRRS, Actinobacillus pleuropneumoniae and Mycoplasma hyopneumoniae negative.
A Mycoplasma outbreak was confirmed in April 2020 based on serology and clinical signs.
The following eradication protocol and time line:
1)June 2020, blood sampling of herd and lung sampling of gilts on purpuse of confirming infection spread and takeing live Mycoplasma lung homogenate.
2)July 2020, load gilts and close herd. At the same month, blanket vaccination of the whole herd(1st) and Mycoplasma exposure of gilts only.
3)October 2020, Janualy and April 2021, blanket vaccination of the whole herd(2nd,3rd,4th).
4)June 2021, start antimicrobal medication:
plegnant sows: 308ppm Lincomysin in feed
farrowing sows: 200ppm Tilmicosin in feed
suckling and weaning pig: 2.5mg/ body weight kg Tulathromycin intramuscular injection
5)Early July 2021, complete depopulation of the nursery.
6)Late July 2021, finish antimicrobal medication and start to use nursery.
7)September 2021, finish to close herd.

results
After eradication protocol, no mycoplasma of nursury confirmed based on deep tracheal catheters and Mycoplasma real-time PCR(30 ~ 40 samples/time, twise). Mycoplasma status of herd confirmed based on serology(3 times every other month) and clinical signs of sentinel pigs, and mycoprasma eradication of this farm were achieved.

P-B-042

EPIDEMIOLOGICAL INVESTIGATION OF ATROPHIC RHINITIS BY ARTIFICIAL INTELLIGENCE DIAGNOSIS

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[1]*HIPRA*

Introduction
Atrophic rhinitis(AR) is an economic disease. Although it will not directly cause the death of pigs, it will increase the incidence of other respiratory diseases and cause a large economic loss to the farm. AR is divided into two types: progressive and non-progressive. In the past, we evaluated progressive AR in pigs on farms by manually observing turbinate lesions in the slaughterhouse (1). This method is intuitive, but because it is done manually, the results may vary depending on the criteria of different people. To reduce this discrepancy Hipra has introduced an artificial intelligence system to assess turbinate lesions (2).

Materials and methods
Artificial Intelligence Diagnos (AI Diagnos) is a fully automated diagnostic system developed by HIPRA, that uses AI to simplify, objectify, and facilitate the whole process of slaughterhouse assessment. Operation, detection, and classification processes, the output of results, and practical applications were needed to develop this system. From July to October 2022, the HIPRA China team investigated the turbinates of 244 slaughterhouse pigs on 25 farms using the aforementioned system. The prevalence of non-progressive AR was also investigated using Hipra's RHINICHECK PCR testing service.

Results
The results showed that there was progressive AR in all the surveyed areas, and more than 60% of the turbinates had moderate and severe lesions. According to the results of HIPRA China laboratory, it was found that the pathogen of non-progressive AR was also widely found in pigs.

Discussion
The AI system can diagnose AR more accurately and quickly. Owing to this investigation and the results of RHINICHECK, it has been found that the incidence of AR is high on Chinese farms. Rhiniseng is a vaccine produced by HIPRA that can prevent both progressive and non-progressive AR. It is believed that it will be helpful for the prevention and control of AR in China.

References
1. De Jong MF. 2006. Diseases of swine, 10th Edition, 34:577-602. Reference Donko.
2. Artificial intelligence as a new method of assessing enzootic pneumonia and atrophic rhinitis lesions. Jordà R. et al .IPVS 2022. Oral communication

Table 1

Table 1 Evaluation of turbinate lesions of pigs in China by AI diagnosis

Area	No. of noses	Mild (%)	Moderate (%)	Severe (%)
GuangXi	35	20.00%	48.57%	31.43%
East	85	61.18%	24.71%	14.12%
GuangDong	20	20.00%	55.00%	25.00%
Central	35	42.85%	34.28%	22.86%
ShanDong	47	14.29%	42.86%	42.86%
Northeast	27	14.81%	51.85%	33.33%
Total	244	36.07%	38.11%	25.82%

Table 2

Table 2.PCR results of Bb. in HIPRA China Laboratory from 2021 to 2022

Year	No. of samples	Positive rate (%)
2021	206	40.30%
2022	113	34.51%
Total	319	38.24%

P-B-043

EVALUATING THE EFFICACY OF A VACCINE AGAINST ATROPHIC RHINITIS BY COMPARING POSITIVITY OF BORDETELLA BRONCHISEPTICA BY LABORATORY TEST

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Introduction
Bordetella bronchiseptica is one of the predisposing factors causing atrophic rhinitis and respiratory problems by producing several virulence factors (1). In South Korea, most farms apply atrophic rhinitis vaccine to control Bordetella bronchiseptica. However, the efficacy of the vaccine has not been well evaluated due to the inconvenience of necropsy when checking nasal lesions and non-specific clinical symptoms caused by Bordetella bronchiseptica. Evaluating the impact of vaccination against atrophic rhinitis by laboratory test has been also uncommon due to the lack of proper methods. The objective of the present study was to verify the efficacy of atrophic rhinitis vaccine on infection of Bordetella bronchiseptica by comparing the differences in the positivity of the antigen in oral fluids via laboratory test, as an alternative to checking nasal lesions.

Materials and Methods
The experiment was conducted on a commercial swine farm in South Korea, whose herd size is 800 sows (farrow to finish). On this farm, growth retardation and respiratory disease in the nursery had been problems despite the consistent use of a specific atrophic rhinitis vaccine. To improve this situation, a new vaccine containing inactivated Bordetella bronchiseptica cell antigen, Rhiniseng®, was newly applied according to the manufacturer's instruction. 16 oral fluid samples from animals in 4 different batches (5, 6, 7, and 13 weeks old, 4 oral fluid samples from each batch of animals) were collected twice, representing the competitor group (before using Rhiniseng®) and Rhiniseng® group (4 months after using Rhiniseng®), respectively. All oral fluid samples were transferred to FTA cards and a real-time PCR assay targeting gene of Bordetella bronchiseptica was performed (2, 3). The results were interpreted as positive when the Cycle threshold (Ct) value was under 38.5. To evaluate the efficacy vaccine, the positivity of samples in the competitor group and Rhiniseng® group were compared. Binominal regression with Tukey as a posthoc test was performed for statistical analysis to see the differences in the two groups.

[Figure 1]. Collecting oral fluid samples and samples transferred to FTA cards

Results
15 oral fluid samples out of 16 were positive in the competitor group for Bordetella bronchiseptica (positivity=93.8%), whereas only one sample was positive in Rhiniseng® group (positivity=6.2%), which showed a significant reduction of positivity after applying Rhiniseng® ($p<0.05$).

[Figure 2]. Positivity for Bordetella bronchiseptica by group

Discussion and Conclusion

It is a well-known fact that Bordetella Bronchiseptica should be controlled to prevent respiratory problems in pigs (1). However, not all commercial vaccines appear to effectively provide protection against Bordetella bronchiseptica. Laboratory tests for checking the positivity of Bordetella bronchiseptica can be a convenient method to check the efficacy of vaccination against atrophic rhinitis.

Acknowledgments
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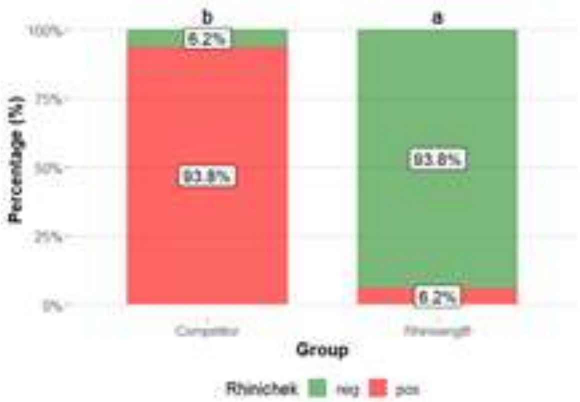
References

1. Brockmeir S. Et al. 2019 Bordetellosis chapter, Diseases of Swine 11th Edition, p. 767-775
2. Nofrarías M et al. 2017. Generallitat de Catalunya
3. Kamp et al. 1996. J Vet Diagn Invest. 8:304-309

Graphs or Images 1

Graphs or Images 2



P-B-044

EVALUATION OF EFFICACY OF GLAESSERELLA PARASUIS VACCINE IN PIG FARMS IN TAIWAN

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Introduction:
Glaesserella parasuis (G. parasuis), a Gram-negative polymorphic rod-shaped bacterium which belongs to family Pasteurellaceae, causes Glässer's disease in swine. Glässer's disease mainly affects nursery pigs and causes clinical symptoms such as wheeze, cough, growth retardation and even death. Polyserositis, polyarthritis and meningitis could be discovered from postmortem examination. It results in huge economic loss in the swine industry. G. parasuis can be categorized into 15 serotypes in current studie. Currently, the main serotypes in Taiwan are type 5/12 (37.6%), type 4 (27.8%) and type 13 (15%). Vaccination can reduce the occurrence of G. parasuis. However, there is limited reports that mentioned about the efficacy and safety of the commercial vaccine after applying to field in Taiwan.

Materials and Methods:
The clinical field trial of three batches in two commercial pig farms (Farm A and B). Three batches, with a total of three groups, were divided into vaccinated sow and vaccinated piglet group (VS-VP), vaccinated sow and non-vaccinated piglet group (VS-NVP) and non-vaccinated sow and non-vaccinated piglet group (NVS-NVP). The vaccine administrated in this experiment was Porcilis® Glässer (Intervet Deutschland GmbH, Unterschleissheim, Germany). Vaccines were immunized twice at 6-8 weeks and 2-4 weeks before farrowing for sows, and 1 and 3 weeks old for piglets. Adverse reactions were observed after vaccination. Blood will be collected from sows before the first vaccination, before the second vaccination and one week after delivery. The colostrum will be collected on the day of delivery. Blood will be collected from piglets at 1, 3, 6, 9, and 12 weeks of age to detect the antibody titers. Mortality, weight and carcass lung pleuritis scores of slaughtered pigs were recorded to evaluate the efficacy of the vaccine.

Results:
Our results showed that there was no severe adverse reaction in vaccinated sows. IgG antibody titers in serum and colostrum of sows showed elevating antibody titer after immunization and it was significantly higher than non-vaccinated group. In addition, IgG antibody titers in serum of piglets that farrow by vaccinated sows was significantly higher than the piglets farrow by non-vaccinated sow at 1 week old. After piglets were immunized, antibody also increased and showed significantly higher than the non-vaccinated group until 9 weeks old. The survival rate in nursery house showed that VS-VP group and VS-NVP group were significantly higher than NVS-NVP group. As for the average daily weight gain (ADWG) and lung pleuritis scores showed that no significant difference between vaccinated and non-vaccinated with sows and piglets.

Conclusions:
The results showed that vaccination of pregnant sows can successfully induce G. parasuis antibodies, and the maternal antibodies can transfer to their offsprings through colostrum, which can further increase the survival rate in nursery house.

Acknowledgement:
We gratefully acknowledge the technical and veterinarian in ADDC of NPUST for their contributions to this study.

References:
[1] Cerdà-Cuéllar M et al., 2010. Vet. Microbiol. 145: 315–320.
[2] Lin WH et al., 2018. PeerJ, 6, e6017.
[3] Oh Y et al., 2013. Nord. Vet. Med, 27(1): 20–25.
[4] Pagot E et al., 2007. Rev Med Vet, 158(5).

Table 1

Farm	Group (n)	Survival rate (%)
A	VS-VP (421)	86
	VS-NVP (727)	84
	NVS-NVP (726)	76 ***
B	VS-VP (316)	82
	VS-NVP (621)	83
	NVS-NVP (656)	75 *

P-B-045

EVALUATION OF FULL AND HALF DOSE OF INGELVAC CIRCOFLEX IN PIG PERFORMANCE UNDER COMMERCIAL FIELD CONDITIONS

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Introduction
Porcine circovirus type 2 (PCV2) is an economically important swine pathogen challenging the global pork industry. The first commercial vaccine against PCV2 for piglets was introduced in 2006 in the U.S. Subsequently, more commercial products are now available. There are several field studies demonstrating PCV2 vaccination is effective in combating porcine circovirus associated disease (PCVAD). Average daily weight gain (ADG) and economic gains in pigs vaccinated against PCV2 is improved in comparison to unvaccinated pigs. Despite this proven effectiveness, when facing high production costs, there is a question from swine producers about the need of applying the full dose of PCV2 vaccine and whether half-a-dose would perhaps provide similar protection. The objective of this study is to evaluate the efficacy of a PCV2 vaccine, Ingelvac CircoFLEX® at full-dose in comparison to half-dose in pig performance under commercial field conditions.

Materials and Methods
A total of 149 pigs were included in this study. Group A was vaccinated at full dose of Ingelvac CircoFLEX® (Boehringer Ingelheim) following label recommendation; group B was vaccinated at half dose (off-label use); and group C received no vaccination, as control. Pigs were individually weighed at initial vaccination and again 155 days later. ADG was determined.

Results
Results are presented in table 1. Starting weights of the pigs in the treatment groups and control group were not statistical different. Pigs vaccinated with full dose had higher weight gain compared to half dose and control group (+9.96kg and +3.25kg, respectively). Group A had also significantly higher ADG compared to control pigs (+64g, $p<0.01$)), and half dose group (+21g, $p<0.05$).

Table 1.

Discussion
Pigs vaccinated with full-dose of PCV2 vaccine (Ingelvac CircoFLEX®) had significantly improved performance as higher ADG when compared to unvaccinated pigs and pigs receiving half-dose. The economic benefit obtained with the full-dose of PCV2 vaccine overcomes by far the suggested cost-reduction of a half-dose scenario, as the pigs receiving full PCV2 vaccine dose generated higher profits. These findings highlight the importance of following label recommendation and applying the correct PCV2 vaccine dose for obtaining the full benefit of protection and performance.

References

1. T. Opriessnig et al (2009). Comparison of efficacy of commercial one dose and two dose PCV2 vaccines using a mixed PRRSV–PCV2–SIV clinical infection model 2–3-months post vaccination. Vaccine 27, 1002–1007.
2. Z. Afghah et al (2017). Ten years of PCV2 vaccines and vaccination: Is eradication a possibility? Volume 206, Pages 21-28.
3. Qing Zhao, Weidong Chai (2018). 25th International Pig Veterinary Society Congress 2018 International PRRS Symposium. Poster Abstracts. page 640.

Table 1

Group	Avg. initial wgt. (Kg)	Avg. final wgt. (Kg)	Avg. weight gain (Kg)	ADG (g)
A-full dose	13.45	189.25	175.80	1134[a]
B-half dose	13.83	186.38	172.55	1113[b]
C-control	13.48	179.32	165.84	1070[c]

Different letters indicate significant differences among groups.

P-B-046

EVOLUTION OF LUNG LESION SCORE AT SLAUGHTER IN TWO CONSECUTIVE YEARS ACCORDING TO MYCOPLASMA HYOPNEUMONIAE VACCINE USED

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Introduction
Mycoplasma hyopneumoniae (Mh) is the primary agent of enzootic pneumonia and many vaccines against Mh are available and used worldwide.
A lot of studies have compared the immediate evolution of lung lesion scoring after the implementation of a Mh vaccine (or a change of Mh vaccine) but the evolution of lung lesion scoring for a long time is rarely described.
It why this study focuses on farms using a same Mh vaccine on a long term, 2.5 years. It aims to describe the lung lesions at slaughter over time according to Mh vaccine used.

Material and Methods
Farms are retrospectively selected among farms of a French cooperative using lung lesion scoring for monitoring pig respiratory health (Madec grid on 6 lobes; score 0 to 4 per lobe) with Ceva Lung Program.
Lung lesion scorings (30 lungs per session minimum) focus on two one-year periods (Y1, Year 1: 01/07/2020 to 30/06/2021; Y2, Year 2: 01/07/2021 to 30/06/2022).
Farms use a same Mh vaccine for 2.5 years (01/01/2020 to 30/06/2022) and have at least one scoring per period. In case of more than one scoring per period, the average score per farm is calculated.
Localization in Brittany, a high pig density area, is considered.
The lung lesion scoring results are described in Y1 and Y2 according to vaccine used.

Results
The study included 239 farms using five Mh vaccines: vaccine A (n=24 farms), B (n=23), C (n=117), D (n=29) and H (Hyogen®, n=46).
Vaccines A, B and H are Mh one shot monovalent vaccines. Vaccines C and D are combo vaccines, Mh and PCV2.
The localization of farms in Brittany varies from 78% (H*) to 50% (A*).
Global results:
- Average Enzootic Pneumonia like Score (EPS) varies from 0.85 (H**) to 1.71 (D**) in Y1 and from 0.79 (H**) to 1.94 (D**) in Y2.
- Percentage of pneumonia free lungs varies from 75.6% (H**) to 61.7% (D**) in Y1 and from 78.0% (H**) to 57.1% (D**) in Y2 (Figure 1).

Figure 1: evolution of % of pneumonia free lungs from year 1 to year 2 according to Mh vaccine used

Severely affected farms:
- Percentage of farms with EPS ≥ 3 varies from 2.2% (H) to 17.2% (D) in Y1 and from 2.2% (H) to 17.2% (D) in Y2.

• Percentage of farms with 20% or more severely affected lungs (scored ≥7) varies from 2.2% (H) to 13.8% (D) in Y1 and from 0% (H*) to 6.9% (D*) in Y2 (Figure 2).
Marks refers to statistical differences (* Chi Square test /**Anova).

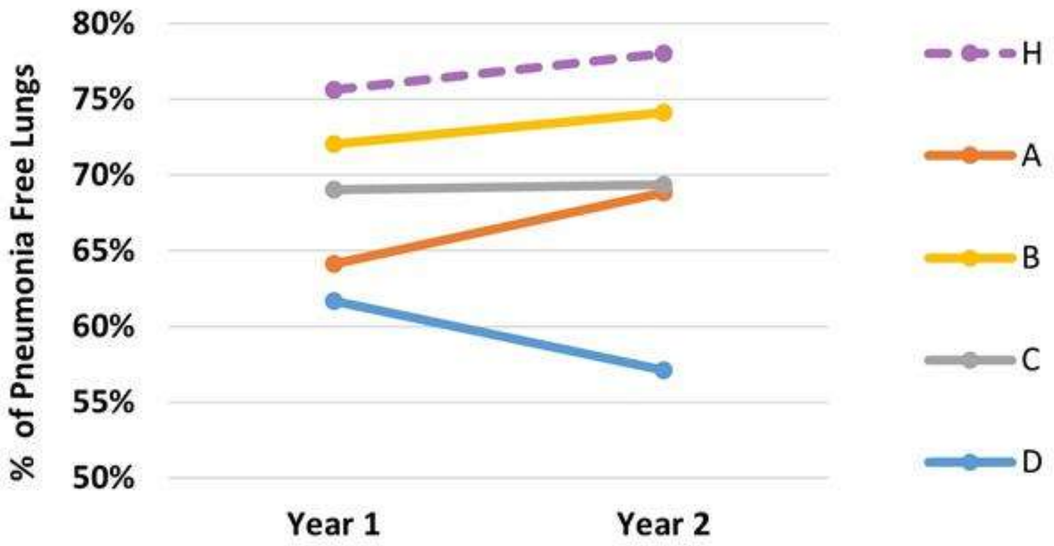
Figure 2: evolution of % of farms with 20% or more severely affected lungs (scored ≥7) from year 1 to year 2 according to Mh vaccine used

Discussion and Conclusion
H group performs better for all criteria and periods, despite a more frequent localization in high pig density. These good results are consisten with other studies focusing on one period, such as those done in Spain (1) or Germany (2)

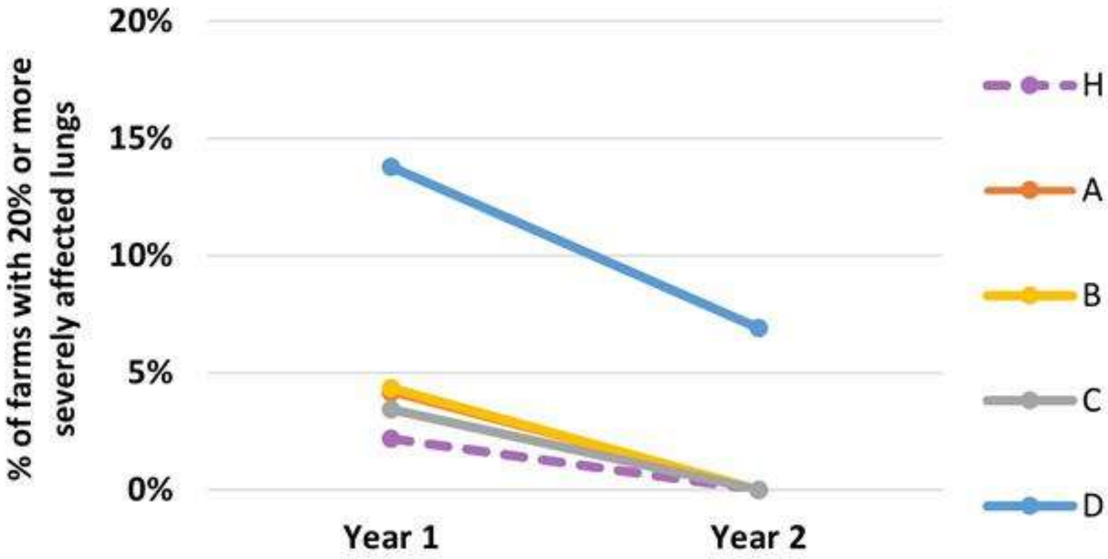
References

1. Lasierra et al., 2021. ESPHM BBD-PP-84
2. Cvjetkovic et al., 2021. ESPHM IMM-PP-3

Graphs or Images 1



Graphs or Images 2



P-B-047

EXCELLENT STABILITY OF AMPHEN® 200 MG/G (FLORFENICOL) IN DRINKING WATER

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Introduction
The clinical outcome of an antimicrobial treatment depends not only on the choice and the quality of the active compound but also on the product formulation. Beside the solubility and bio-availability, the stability in the drinking water also largely determines the efficacy of water soluble antimicrobial products. Florfenicol is a broad spectrum antimicrobial demonstrating fast, substantial serum concentrations and an excellent distribution in different tissues including lungs, joints and central nervous system. Amphen® is a unique patented formulation of 200 mg/g florfenicol for use in the drinking water of pigs. In this study, the stability of Amphen® in the drinking water and containers of different qualities was assessed for 24 hours after administration.

Materials and Methods
The stability in the drinking water was investigated in two batches having the same batch number. The first batch was a recently produced batch while the second batch had been subjected to 25° Celsius and 60% relative humidity stability conditions for at least six months (EMEA/CVMP/540/03-Rev.1). The stability following dissolution was tested at the minimal nominal concentration, determined at 250 mg Amphen® 200 mg/g per liter, under different conditions:
- in drinking water with low and high hardness. Soft water used for the study of the recent and subjected batch contained 20.9 and 20.4 mg/l Calcium carbonate with a pH of 5.1 and 6.3, respectively, at the start of the study. Hard water used for the study of the recent and subjected batch contained 341.9 and 341.2 mg/l Calcium carbonate with a pH of 8.0 and 8.1, respectively, at the start of the study.
- in two types of vessels mimicking the actual conditions at the end-user: PolyEthylene (PE) and PolyVinylChloride (PVC).

During the study period, samples were investigated to monitor three critical product specifications at 0, 8 and 24 h after product administration:
1. The concentration of florfenicol was determined by High Performance Liquid Chromatography (HPLC)
2. The concentration of related substances was determined by HPLC. The acceptance criteria were: Thiamphenicol: ≤0.3%, single unknown impurities: ≤0.2% and total impurities: ≤2.0%.
3. The visual appearance of the solution was observed.

Results
1. The concentration of florfenicol at 8 and 24 h after administration of Amphen® was expressed as percentage of the original concentration (0 h) in water of different qualities (soft and hard water) and stored in excipients of different properties (PE and PVC). The florfenicol concentrations ranged between 97.0 and 100.8% of the original concentrations.
2. The florfenicol related substances were lower than their detection limits in all samples.
3. A clear, colourless solution was observed in all samples.

Conclusion

Amphen® 200 mg/g florfenicol (Huvepharma®) demonstrated an excellent stability for a period of 24 hours in both soft and hard water. Differences in stability between a recently produced batch and an older batch subjected to stability conditions for 6 months were not observed. Furthermore, the properties of the excipients tested did not impact the stability at all. Based upon this ensured stability, a superb clinical efficacy can be expected following Amphen® administration in drinking water under different conditions.

Graphs or Images 1

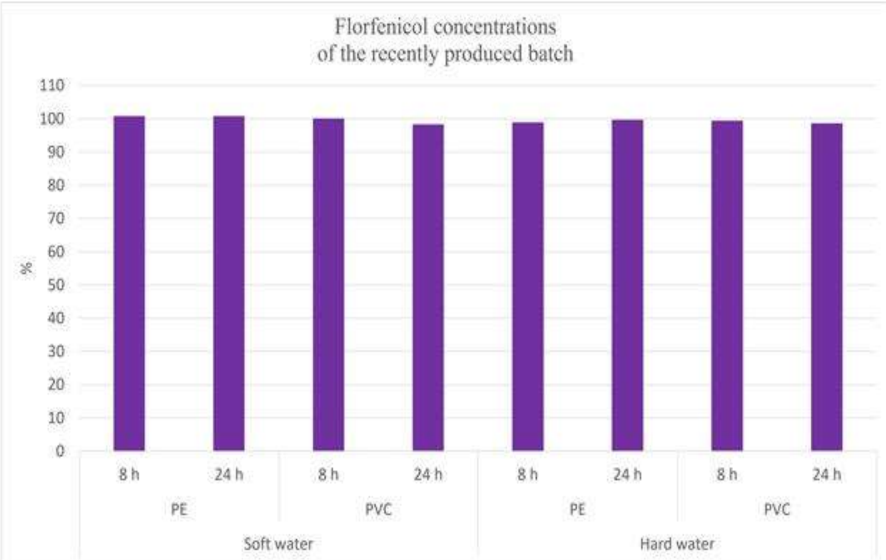

Fig. 1: Florfenicol concentrations expressed as % of the original concentrations (0 h) of the recently produced batch

Graphs or Images 2

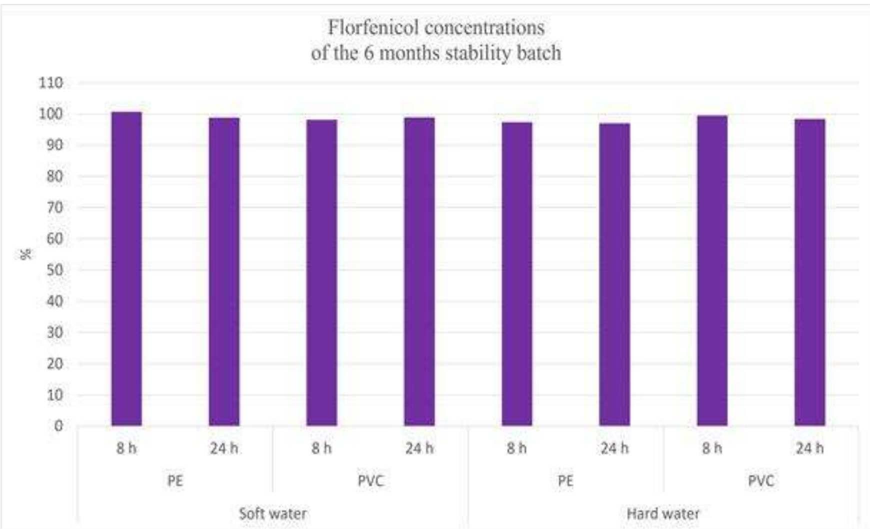

Fig. 2: Florfenicol concentrations expressed as % of the original concentrations (0 h) of the batch which was subjected to 6 months stability conditions.

P-B-049

FIELD OBSERVATIONS THE EFFECT OF ENTERICOLIX SOW VACCINATION AGAINST NEONATAL COLIBACILLOSIS IN THAI FARMS

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Introduction
Postweaning diarrhea by pathogenic Escherichia coli causes significant losses in piglets from birth to weaning to the swine industry worldwide. The causative agents expressed by fimbriae enabling attachment to the enterocyte receptors and produced toxins after colonization resulted in diarrhea phenomenon. The common fimbriae types found in ETEC are F4, F5, F6, F18 and F41. The aim of this field observation was to assess the effect of a novel neonatal diarrhea sow vaccination (Entericolix®) with a broad antigenic E.coli spectrum (F4, F5, F6, F18, and F41 fimbriae) combined with Clostridium perfringens type C on pre-weaning mortality in conventional Thai swine farms with evidenced neonatal diarrhea and edema problems in the weaned pigs.

Materials and Method
Sows from 2 farms (close and open house systems) were assigned into two groups, vaccination (Tx) and non-vaccination (Ctrl) groups. Farm 1 consisted of a close house production system (15 sows TX; 15 sows Ctrl) and farm 2 consisted of a open house system (88 sows TX; 83 sows Ctrl).
The vaccination group received 2 ml of Entericolix at 7 weeks and 3 weeks before parturition. The control group was not vaccinated and exposed to the same management and treatment when compared to the vaccinates. In total 120 fecal samples were randomly taken from each pen of suckling piglets at D2 and D28 (weaning) from both farms to determine the exposure to specific fimbriae type E.coli bacteria (F4, F5, F41, and F18) by using a Multiplex-PCR. The pre-weaning mortality was recorded. Chi-square test was used to determine the difference of preweaning mortality.

Results
Pre-weaning mortality reduced from 25.5% to 20.9% in farm 1 and 12.7% to 8.65% in farm2.
During the pre-vaccination survey, Three Fecal samples were positive for distinct E.coli fimbriae (F41, F4, and F5) using multiplex PCR. In the overall of study, vaccinated sows showed a significant lower percentage of pre-weaning mortality compared to sows without vaccination (11.1% vs 15.5%, p<0.01) (Table 2).
Farm 1 had a tendency towards lower mortality in the vaccinated sows (21.0 % vs 25.6 %; P<0.1) with significant lower mortality due to emaciation (8.9% vs 18.2% p<0.05). Other causes of death (diarrhea and crushing) were not significant.
Farm2 showed a high significant difference towards a lowered mortality in the litters of vaccinated sows (8.7% vs 12.8% p<0.01)
(8.7%) compared to unvaccination group (12.8%) (p<0.05). However, in a close house system, there was no significant difference in pre-weaning mortality between two groups (21.0% and 25.6% for sows with and without E.coli vaccination, respectively) (p>0.05).

Conclusions and Discussion

This study showed that sow vaccination against neonatal diarrhea reduced pre-weaning mortality. This was associated with less incidence of deaths due to emaciation in the vaccinated group of Farm 1. The fact that total mortality reduction in farm one was not significant, is probably due to the low sample size of in total 30 litters, where in farm two 171 litters were involved.

References

1: John Fairbrother, The challenges of Escherichia coli postweaning disease in pigs, P19-23, IPVS202

Table 1

Table1. Mortality performance

	Farm1		Farm2	
	Tx	Ctrl	Tx	Ctrl
Amount of sow	15	15	88	83
%Pre-wean mortality	21	25.6	8.7	12.8

Table 2

Table 2. Effect of sow E.coli vaccination on pre-weaning mortality

	Pre-weaning mortality (%) [1]		P-value
	No vaccine	Vaccine	
Overall	15.5 (157/1013)	11.1 (117/1053)	0.0011
Farm 1 (Close house system)	25.6 (55/215)	21.0 (44/210)	0.0816
Farm 2 (Open house system)	12.8 (102/798)	8.7 (73/843)	0.0046

1 Values in parenthesis represent the ratio of the number of pre-weaning deaths to the total number of piglets born alive.

P-B-050

FIELD TRIAL COMPARING PCV2 AND MHYO COMBINATION VACCINE EFFICACY ON MORTALITY RATE AND AVERAGE DAILY GAIN

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[1]*Msd Animal Health,* [2]*Intervet K.K Japan,* [3]*Eckstein Swine Service*

Introduction:
Porcine respiratory disease complex (PRDC) is a multifactorial disease resulting from the interaction of different infectious agents, management and environmental conditions, and is considered a major health concern. Mycoplasma hyopneumoniae (Mhyo) and Porcine Circovirus type 2 (PCV2) are among the main causes of PRDC1.PCV2 infections later in life have greater economic consequences for swine as this coincides with high growth phases. The aim of this study was to observe and compare the efficacy of Porcilis® PCV M Hyo against a competitor combination vaccine in Japan.

Materials and Methods
This study was performed in a 2000 sow level, farrow to finish commercial farm in Japan. The farm was documented to be PCV2 and Mhyo positive and pigs were weaned every week at 3 weeks of age. 1st to 4th weaned batch, which include 607 to 650 pigs per batch, were vaccinated with 2mL of Porcilis PCV M Hyo and 5th to 7th weaned batch, which include 644 to 698 pigs per batch, were vaccinated with 2mL of Vaccine A at weaning. (Table 1). The number of mortalities was counted, and the average daily gain (ADG) was calculated. Mortality rate was analyzed by Chi-Square test and ADG was analyzed by t-test.

Results
The mortality rate of nursery in P-PCVM group and Group A was 1.4% and 1.0% respectively and there was no significant difference. While the mortality rate of fattening in P-PCVM group was significantly lower than the Group A (1.2% and 3.6% respectively, $P<0.01$). ADG at nursery in P-PCVM group and Group A was 440g/days and 460g/day respectively ($P<0.05$). ADG at fattening in P-PCVM group and Group A was 933g/days and 855g/day ($P<0.01$).
Discussion and Conclusions:
In this study, the efficacy of two commercially available vaccines was compared using mortality and ADG as outcome parameters. Despite Vaccine A achieving better ADG in the nursery stage, vaccination with Porcilis® PCV M Hyo significantly improved mortality rate and ADG in the critical fattening period. This also contributed to a better production result from wean to finish. This result demonstrates that Porcilis® PCV M Hyo was the better option to improve productivity in a farm with endemic PCV2 and Mhyo infection.

References:
1. Pieters M. and Maes D. Chapter Mycoplasmosis (863-883) Diseases of Swine, Eleventh Edition. Edited by Jeffrey J. Zimmerman et al. (2019)

Table 1

Table 1. Number of pigs and treatment

Group	Total No. of pigs	Treatment
P-PCVM	2491	Porcilis® PCV M Hyo
A	1993	Vaccine A

Table 2

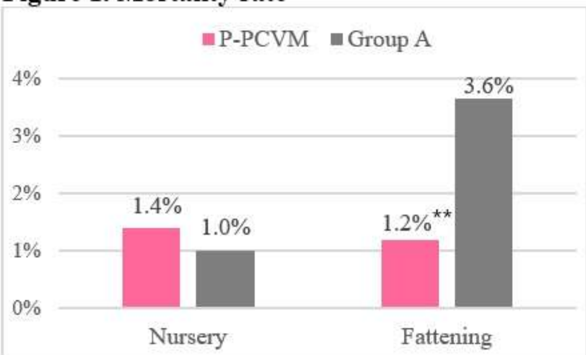
Table 2. Average daily gain (ADG)

ADG (g/day)	P-PCVM	Group A	Difference
Nursery	440	460*	-20
Fattening	933**	855	78
Total	748**	712	36

* P<0.05, **P<0.01

Table 3

Figure 1. Mortality rate



**P<0.01

P-B-051

INFECTION DYNAMICS OF MYCOPLASMA HYORHINIS IN FOUR COMMERCIAL PIG FARMS IN TAIWAN

Mr. Chih-Ming Liao[1], Ms. Sophie Hsieh[1], Mr. Namho Kim[2], Ms. Ching-I Chen[3]
[1]Elancoah, [2]Elanco International Swine Marketing, [3]Division of Animal Medicine, Agricultural Technology Research Institute

Introduction
Mycoplasma hyorhinis (Mhr) is an emerging pathogen associated with polyserositis, polyarthritis, pneumonia, otitis, and conjunctivitis in nursery and early finishing pigs1, 2. Mhr is frequently isolated from lungs with pneumonia caused by Porcine Reproductive and Respiratory Syndrome Virus and Porcine Circovirus type 23. In a previous case report, Mhr was found to be the possible cause of fibrinopurulent meningitis in pigs4. From our customer service experience, we found that Mhr is frequently detected in nursery pigs with pneumonia and polyserositis. These findings necessitated the study on infection dynamics of Mhr in swine farms in Taiwan.

Materials and Methods
Four commercial (A, B, C, and D) farrow-to-finish pig farms with more than 1,000 sows were selected for the study. In each farm, nasal swabs and serum samples were collected from sow and their 3-week-old piglet, and from 6-, 9-, 12-, 18-, 21-week-old pigs and gilts. Ten samples were collected from pigs of each age. The samples were tested for Mhr antigen and antibody at Agricultural Technology Research Institute (ATRI). The nasal swab samples were tested for antigen by polymerase-chain reaction (PCR) and serum samples were tested for antibodies by Enzymelinked immunosorbent assay (ELISA) kit supplied by TaFoong Vaccines & Biotech Co., Ltd. The postmortem examinations were conducted in 3-4 culled nursery pigs in each farm and samples were processed for Mhr isolation and detection by PCR.

Results
Results from testing of swabs showed a high positive rate (70%-80%) in 3-week-old piglet except in farm A (10%). On the contrary, their sows had a low positive rate (0-10%). The Mhr PCR positive rate was the highest at 6 weeks (90-100%) and remained until the age of 9 weeks (50-100%). The Mhr PCR positive rate decreased at 12-week-old (40-70%) but persisted at low detection rate through grower and finisher stage (10-60%). It should be noted that detection rate in gilts was lower (20-40%) (Figure. 1). The results of the antibody positive rate were varied in suckling (20-70%) and nursery (10-90%) piglets. The antibody positive rate dramatically increased at the age of 15 weeks and remained in the finishing period. Gilts and sows also had high antibody positive rate (80-100%) (Figure. 2). These results suggest that maternal antibodies might provide early protection in the suckling period. In farm A, although 3-week-old piglets had a high antibody positive rate (70%), the Mhr PCR positive rate was low (10%). The Mhr PCR positive rate dramatically increased at the age of 6 weeks (100%). In farm B, C, and D, the 3-week-old piglets had low antibody positive rate (20- 50%) with high Mhr PCR positive rate (70-80%). Nursery pigs in these farms had 38-57% of arthritis and polyserositis in the post-mortem examination. The percentage of Mhr isolated and detected by PCR from lung samples were 66% and 100%, respectively.

Conclusion
The results show that nursery pigs in these farms are at a high risk of Mhr infection. There is also a need to monitor gilts which might play a major role in Mhr circulation.

Reference:
1. Lin et al., 1996. Vet Microbiol. 115: 111-116.
2. Pieters MG, and D Maes. 2019. Disease of swine. 863-883.
3. Kobayashi et al., 1996. J Vet. Med. 58(2): 109-113.
4. Bünger et al., 2020. Porc Health Manag 6, 38.

Graphs or Images 1

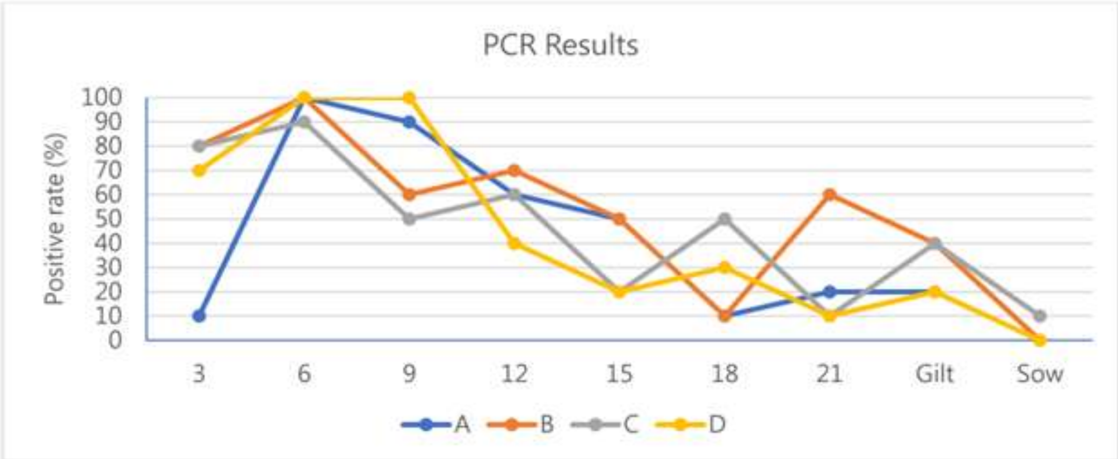


Figure 1. The results of nasal swab PCR for *Mhr* by age from 4 selected pig farms.

Graphs or Images 2

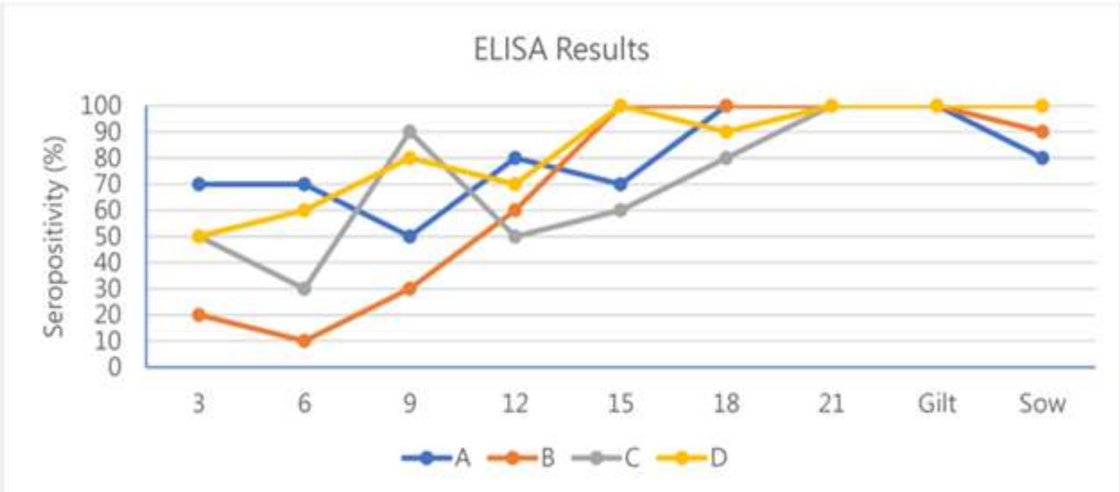


Figure 2. The results of *Mhr* serological examination by age from 4 selected pig farms.

P-B-052

INFLUENCE OF THE APPLICATION METHODS OF IRON/ANTICOCCIDIAL PRODUCTS ON THE BEHAVIOR OF PIGLETS

Dr. Daniel Sperling[1], Dr. Maria Rodríguez[2], Dr. L de Frutos[2], Dr. Joaquin Morales[2]
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Introduction:
The aim of the present study was to evaluate the effect of different treatment protocols for the
control of iron-deficiency anaemia and coccidiosis, one combination injection versus injection of iron and oral application of toltrazuril, on the behaviour of piglets in the time of the treatment administration.

Material and Methods:
A total of 288 piglets were used (from 24 litters; 12 piglet/litter). In each litter, piglets were divided into three experimental treatment groups. So, 96 piglets were kept untreated (control group); 96 piglets received an oral administration of generic anticoccidial agent (20 mg/kg BW) plus intramuscular administration of iron dextran (200 mg/ml; 1 ml/piglet) in the same handling (oral+parenteral group, O+P); and 96 piglets received an intramuscular application of the combination product (parenteral group, P, Forceris®). The total handling time, the vocalisation of the piglets during the administration of the treatment and the reaction to manipulation were determined. Response of the piglets to manipulation was evaluated at the time of the treatments according to the methodology described by Scollo et al. (2020). Blood samples were taken on day -1, on day 0 (after treatment administration) and on day 3 to analyse cortisol and PigMAP, respectively.

Results:
Handling time was higher in the O+P group than in the P group (10.57 vs 4.37 s/piglet; $P<0.05$). Piglets from O+P group emitted more screams during the administration of the treatments than those from P (10.95 vs 4.24% of the animals; $P<0.05$). Moreover, piglets from O+P group reacted worse to manipulation since a higher percentage of animals kept fidgeted even after handling than those from P group (10.92 vs 4.23%; $P<0.05$). Although there was no statistical difference, cortisol concentration was 12.3% lower in P than in O+P group. Moreover, PigMAP concentration tended to be lower in P compared to O+P group (1.31 vs 0.98 mg/ml; $P=0.1$).

Discussion and conclusions:
In conclusion, administering a combination product injection not only decreases the time of administration compared to individual treatments but also decreased the stress during administering in terms of less vocalization and less reaction to manipulation. Consequently, the concentration of blood metabolites indicative of stress was lower. Vocal expression of emotional states has been identified in several species, and is considered as potential indicator of reduced welfare in pigs. A lower frequency of behavioral aversive responses was observed in the P group than in the O+P group suggesting, that one injection only can be considered as welfare friendly approach in piglets management.

References:

1. Scollo A et al. Evaluation of pain and stress in three-week old piglets in relation to route of vaccine administration. Livest Sci. (2020)

Table 1

Image 1. A) i.m. and B) oral treatment administration

P-B-053

INTRODUCTION AND CONTROL OF A NEW ACTINOBACILLUS PLEUROPNEUMONIAE STRAIN IN A GERMAN FARM

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Background and Objectives
A. pleuropneumoniae (App) is a potentially devasting, primary respiratory pathogen commonly inducing acute mortality, pleuropneumonia, and chronic pleurisies. Vaccination measure is an integral part of on-farm App control [1,2]. Commonly three or more different serotypes are circulating App-endemic farms [3].
Serotype-independent protection has been demonstrated previously in a multi-serovar, multi-study analysis for the whole-cell vaccine based on App1 and App2 expressing ApxI, II, and III during production; the CV vaccine of this study [2].
This case report shows the consequences of a newly introduced serotype, taking over the position as the predominant cause of pleuropneumonia, and the consequences of different vaccination strategies.

Material and Methods
The case farm is a 160-sow farrow-to-finishing farm running 3-weeks batch farrowing, located near a street with abundant livestock traffic to the nearby regional slaughterhouse.
In November 2020 an outbreak of coughing and simultaneous sudden deaths in late finishing revealed App serotype 7 (App7) as the cause (cps-multiplex-PCR, IVD GmbH, Seelze-Letter, Germany). An autogenous vaccine (AV) was developed on this strain and implemented for the sows, due to cost-considerations.
In November 2021 a similar outbreak including increased mortality and pleuropneumonia was reported. For this reason the farm-vet decided to apply Coglapix® (Ceva Santé Animale) (CV), at seven and ten weeks of age. Subsequently, from lungs of acutely dead finishers, acute, highly fibrinous-necrotizing pleuropneumonia was demonstrated by histology, and App18 was identified as a new strain to the farm at IVD.
Finisher data gathered from farm production reports and periods of AV and CV was compared.

Results
CV restored clinical respiratory health.
The finishers productive performances in the groups AV and CV, respectively, on mortality rate: 3.5% and 2%; average daily weight gain (ADG): 840g and 860g; days-in-finishing: 107 and 104 days; feed conversion rate (FCR): 2.85 and 2.84 kg feed per kg growth.

Discussion and Conclusion
This case report demonstrates the risks of poor biosecurity, the consequences of introduction of new App-strains, into a common multi-serotype App-endemic farm status. Furthermore, this field study indicated that CV-vaccination not only restored clinical respiratory health, but also improved productive performances, in a heterogenous, multi-serotype (App7 plus App18) environment in line with previous publications [2].

References

1. Taylor D.J., Actinobacillus pleuropneumoniae in: Disease of Swine 11th edition. 2019. 349-354.
2. Mortensen P. et al., Comparative Efficacy in Challenge Dose Models of a Toxin Expressing Whole-Cell Vaccine against Eight Serovars of Actinobacillus pleuropneumoniae in Pigs. Animals, 2022.
3. Renken, C. Seroprävalenz von Actinobacillus pleuropneumoniae sowie zugehöriger Serotypen und Vorkommen von Pleuritiden bei Mastschweinen aus Beständen mit klinischen Anzeichen einer Atemwegserkrankung. Ph.D. Thesis, Ludwig-Maximilians-Universität, München, Germany, 29 July 2017.

Table 1

Group	Mortality rate *%*	Finishing time *days*	ADG *gram growth/day*	FCR *Kg feed/Kg growth*
AV	3.5	107	840	2.85
CV	2.0	104	860	2.84

Table 1: Production parameters = mortality rate, days in finishing till slaughter (finishing time), ADG and FCR compared between groups.

P-B-054

INVESTIGATION OF LAWSONIA INTRACELLULARIS SEROPREVALENCE AMONG PIG HERDS AND THE PRESENCE OF THE BACTERIA GENOME AMONG SLAUGHTER-AGE PIGS IN VIETNAM FROM 2020 TO 2022

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Keywords: Lawsonia intracellularis, seroprevalence, realtime-PCR, pig herds, Vietnam

Introduction:
Lawsonia (L.) intracellularis is a negative bacterium causing Ileitis or Porcine proliferative enteropathy (PPE) in swine industry worldwide causing seriously economic loss. Some research indicates the seroprevalence being high among pig herds in different countries. The aims of this study are (1) To investigate the prevalence of PPE in serum samples among different age groups in pig farms in Vietnam from 2020 to 2022; (2) To detect the presence of L. intracellularis genome in finishing pig at the slaughterhouse by Realtime-PCR method.

Materials and methods:
From 2020 to 2022, the seroprevalence by ELISA method following a commercial kit was implemented from 40 pig farms in all three main regions of Vietnam (the North, the Central and the South) with the scale farm size from 300 to 5000 sows per farm. All of age groups in farm such as sows, gilts, weaned pigs, post-weaning pigs, growing pigs and finishing pigs were included in this investigation. 5 -10 blood samples per age group were taken in each farm. At a pig slaughterhouse in the South of Vietnam, 100 intestinal samples of the ileum part of slaughter – age pigs were randomly collected directly after slaughtering in order to examine the gross lesion of intestinal mucosa and to find the presence of L. intracellularis genome by using realtime - PCR method.

Results:
The total of 1170 blood samples from 40 pig farms were screened for the seroprevalence of antibodies against L. intracellularis. The positive seroprevalence was 100% by farm while there were 46% positive (535/1170 samples) and 4% suspected to be positive (51/1170 samples) by individual pigs. By age group, the lowest positive seroprevalence was found in weaning and post-weaning pigs, 5% and 4% respectively; then there was an increasing trend of the seroprevalence in growing and finishing pigs, 30% and 56% respectively. However, the highest rate was seen in sow (89%) and gilt (87%). The gross lesion showed that 61% (61/100 samples) of the intestinal mucosa of the ileum section was thick. Realtime-PCR result indicated that there were 3% positive (3/100 samples) with Cycle threshold (Ct) value < 36; 2% suspected to be positive (2/100 samples) with Ct value 36,01 to 36,99 and the remaining of 95% was negative.

Conclusions & Discussion:
Using of serum for antibody assessment in this study indicates the past exposure of the pig herd to L. intracellularis in many regions of Vietnam. The highest seroprevalence approximately 90% in sow and gilt indicates this age group seen as a high-risk source of

pathogen excretion and transmitted to their offspring. The presence of L. intracellularis genome was low at the slaughter-age pigs, even the positive seroprevalence of prior pathogen exposure was high.

References:
1. Arnold, M., Crienen, A., Swam, H., von Berg, S., Jolie, R., Nathues, H., 2019. Prevalence of Lawsonia intracellularis in pig herds in different European countries. Porc Health Manag 5, 31. https://doi.org/10.1186/s40813-019-0137-6
2. McOrist S, Jasni S, Mackie RA, MacIntyre N, Neef N, Lawson GH, 1993. Reproduction of porcine proliferative enteropathy with pure cultures of ileal symbiont intracellularis. Infect Immun; 61:4286–92
3. Lindecrona RH, Jensen TK, Andersen PH, Møller K, 2002. Application of a 5' nuclease assay for detection of Lawsonia intracellularis in fecal samples from pigs. J Clin Microbiol. 2002 Mar;40(3):984-7. doi: 10.1128/JCM.40.3.984-987.2002.
4. Vannucci F. A., Gebhart C. J, 2014. Recent Advances in Understanding the Pathogenesis of Lawsonia intracellularis Infections. Veterinary Pathology; 51(2):465–477. doi: 10.1177/0300985813520249.

P-B-055

INVESTIGATION OF PREVALENCE OF CYSTOISOSPORA SUIS IN CENTRAL-SOUTHERN REGION OF TAIWAN

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[1] *National Chiayi University*

Introduction
Piglet coccidiosis caused by Cystoisospora suis (C. suis) is a common disease that leads to diarrhea and unthriftiness in unweaned suckling piglets. The parasite multiplies quickly and effectively under the conditions of a farrowing unit. The only drug licensed for the control of piglet coccidiosis is toltrazuril, which suppresses parasite development and the corresponding diarrhea. The aim of our investigation was to determine the prevalence of C. suis infection in suckling piglets in central-southern region of Taiwan and the relation of C. suis and toltrazuril usage.

Materials and methods
The research was carried out between 2021 and 2022. Overall, 444 litters from 45 farms of suckling piglets aged between 2-3 weeks old were sampled. Each sample was taken from the rectum of at least 5 piglets per litter or feces that were on the ground. Faecal samples were examined for the presence of C. suis oocysts by autofluorescence method [Joachim et al., 2018]. Information of each farm were recorded include farm size, faecal consistency, and anticoccidial drug usage.

Result
C. suis was detected in 17 of the 45 farms (35.5%). Result of each county was presented at table 1. In all, 15.3% of the 444 litters were C. suis positive. Oral and injected toltrazuril were used in 20 and 14 farms and the percentage of C.suis positive was 25% (5/20) and 35.7% (5/14), respectively. Ten farms did not use any form of anticoccidial drug, and the percentage of C.suis positive was 70% (7/10). The other one farm used both oral and injected toltrazuril and C. suis was not detected (Figure 1).

Conclusions and Discussion
In this study, C. suis was detected in 35.5% of the farms and 15.7% of the samples. The data is not similar to the prevalence studies of Europe published previously (Czech: 70.6% of farms, Poland: 67.3% of farm) [Sperling et al., 2020, Karamon et al., 2008]. C. suis with toltrazuril resistance has been confirmed and isolated in Europe [Shresta et al., 2018], which might contribute to higher prevalence of C. suis in Europe than in Taiwan. Of all 45 farms we investigated, 3the C. suis positive rate of 35 farms used toltrazuril as an anticoccidial drug and 10 farms without toltrazuril administration was 28.5% and 70%, respectively. This indicated that even though toltrazuril can not fully interrupt the life cycle of C. suis; nevertheless, it is still an effective method against piglet coccidiosis in Taiwan by decreasing the oocyst amount and exposure to C. suis.

Acknowledgement and reference
Joachim A, Ruttkowski B, Sperling D. Detection of Cystoisospora suis in faeces of suckling piglets–when and how ? A comparison of methods. Porc Health Manag 4: 20-30, 2018
Karamon J, Ziomko I, Cencek T, Sroka J. Modified flotation method with the use of Percoll for the detection of Isospora suis oocysts in suckling piglet faeces. Vet Parasitol 156: 324-328, 2008.
Shrestha A, Freudenschuss B, Jansen R, Hinney B, Ruttkowski B, Joachim A. Experimentally confirmed toltrazuril resistance in a field isolate of Cystoisospora suis. Parasit Vectors 10: 317-325, 2017.
Sperling D, Karembe H, Vanhara J, Hinney B, Joachim A. Suckling piglet coccidiosis on farms in the Czech Republic – A pilot study. Vet Med 65: 427-434, 2020.

Table 1

Table 1. Percentage of positive farm and Prevalence of *Cystoisospora suis* in central-southern Taiwan

County	Farm	*C. suis* positive	Percentage of *C. suis* positive	Examined no.	*C. suis* positive no.	Prevalence
Changhua	3	1	33.3%	30	5	16.6%
Yunlin	8	3	37.5%	79	11	13.9%
Chiayi	19	5	26.3%	189	23	12.1%
Tainan	2	1	50%	20	5	25%
Kaohsiung	6	1	16.6%	60	4	6.6%
Pingtung	7	6	87.5%	66	20	30.3%
Sum	45	17	37.7%	444	68	15.8%

Graphs or Images 1

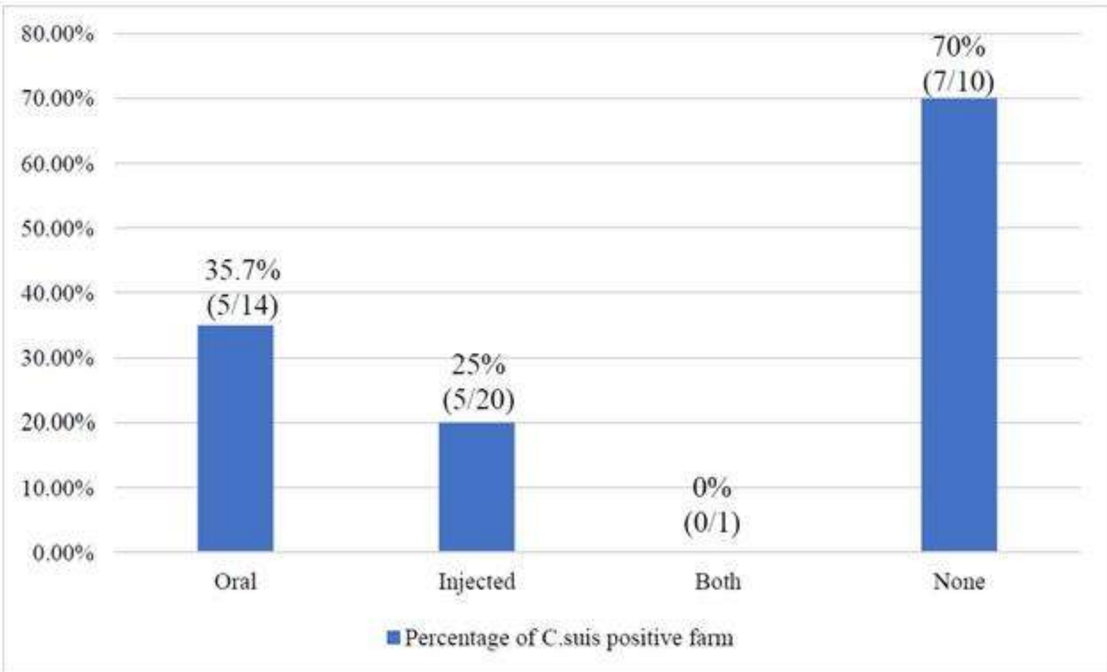

Figure 1. Usage of anticoccidial drug.

P-B-056

LAWSONIA INTRACELLULARIS PREVALENCE SCREENING IN THE COMMERCIAL FARM IN VIETNAM

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Introduction
Porcine proliferative enteropathy (PPE), caused by Lawsonia intracellularis, is the most important enteric disease in the swine industry worldwide. In the United States, a national serological study showed that nearly 100 % of all swine herds had at least one time been exposed to this intracellular organism [1]. There was little information in Asian countries where monitoring of ileitis had not been established as a routine practice. Therefore, the objective of the study was to estimate the prevalence of L. intracellularis infection in endemic swine herds in Vietnam.

Materials and Methods
For each infected swine herd (farm), a cross-sectional study was conducted to determine the prevalence of L. intracellularis. Five (05) serum samples from randomly selected pigs from the following ages – 2, 4, 6, 8, 10, 12, 16, 20, 24 weeks and Breeders (sows at Parity 1, Parity 2, Parity 3, and boars at 1-2 years of age) – 70 samples per farm. The method used the commercial ELISA kit of the Svanova company. The steps followed the protocol of the kit.

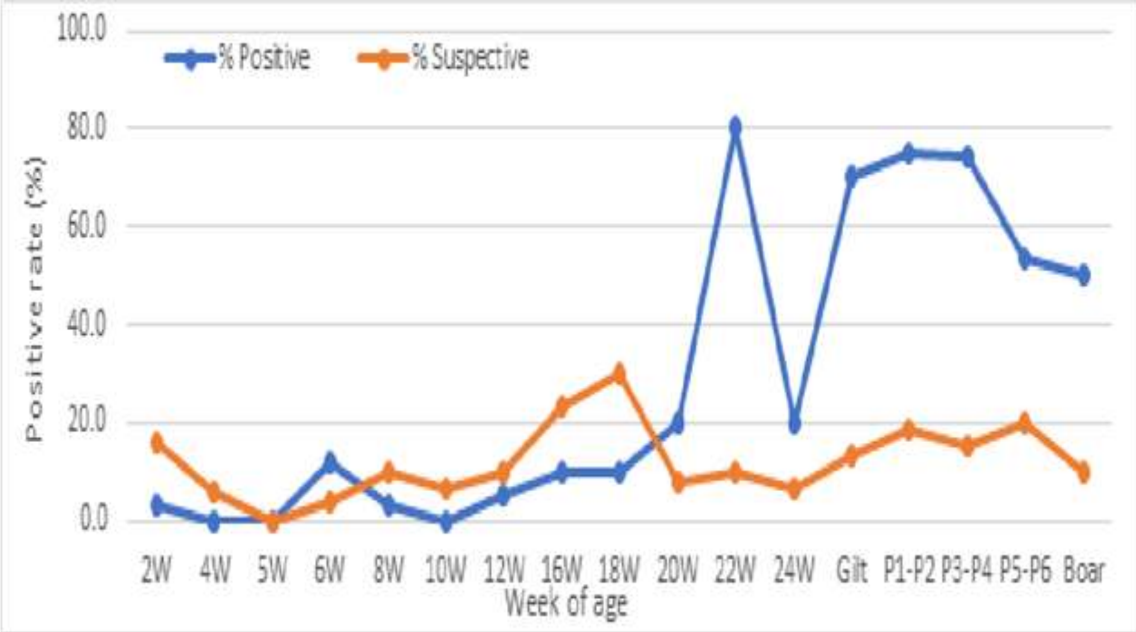
Results and Discussion
A total of 500 serum samples from piglets, growers and sows were collected for sera test. This result showed that the percentage of seropositive samples for ileitis was very low in pigs from 2-10 weeks of age, 3% seropositive at 2 weeks of age and 12% at 6 weeks of age, after which this antibody declined gradually to 10 weeks of age. Previous survey results had demonstrated that maternal antibodies can be transmitted up to 7 weeks of age [2]. Seroconversion began at 12 weeks of age with positive rate was 5%, this positive rate gradually increased with subsequent ages, and by 20 weeks of age, it was 20%. This result was similar to previous surveys when pigs showed strong signs of seroconversion from 12 weeks of age onwards [2, 3]. At 22 weeks of age, seropositivity rate was up to 80%, and 20% at 24 weeks of age. In gilts, boars, and sows, the seropositivity rate was high, ranging from 50-75%.

Conclusion
This study showed high prevalence of ileitis in Vietnam swine herd. The seroconversion rate was concentrated mainly at 12 weeks of age and later. However, in these results, the most common and highest rate was recorded in the group of pigs from 20 weeks of age and later and especially in the breeding group such as gilts, boars and sows.

References
1. Bane, D., Norby, B., Gardner, I., Roof, M., Knittel, J., Bush, E., Prevalence and management risk factors associated with Lawsonia intracellularis seropositivity in the U.S. swine herd, 1997.
2. Gebhart, C, Guedes, R. Proliferative enteropathy: diagnostics and immunity. American Association of Swine Veterinarians. 2001: 353-357.

3. Mirjam Arnold, Annelies Crienen, Hanny Swam, Stephan von Berg, Rika Jolie and Heiko Nathues. Prevalence of Lawsonia intracellularis in pig herds in different European countries. Porcine Health Management (2019) 5:31.

Graphs or Images 1



P-B-057

LESION AFFINITY OF STREPTOCOCCUS SUIS SEROVARS – EVALUATED BY ISOLATION RATES OF SYSTEMIC AND RESPIRATORY INFECTIONS FROM CLINICAL CASES

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Introduction:
Streptococcus suis (S. suis) is one of the most common pathogens of swine systemic and respiratory disease in the worldwide pig industry, causing meningitis, arthritis, pneumonia, and even acute death. It has been classified into 29 serovars based on the differentiation of capsule polysaccharide structures. Some specific serovars have higher pathogenicity and are linked to neurological and systemic infections [1]. Nevertheless, the relationship between S. suis serovars and the preferential lesions has not been fully discussed. The current study aimed to compare isolation rates from different lesions with different S. suis serovars.

Materials and Methods:
S. suis isolates were obtained from diseased pigs sent to Animal Disease Diagnostic Center, National Pingtung University of Science and Technology from 2015 to 2019. Isolation rates were calculated according to the categories of systemic (meninges, pleura, pericardium, peritoneum, and joint) and respiratory (lung parenchymal tissue) lesions. Serovars were determined by PCR assays as previously described [2, 3]. The isolation rates of each serovar were then analyzed by Fisher's exact test.

Results:
A total of 982 S. suis-associated lesions were included in this study. 213 isolates of 573 systemic lesions (37.2%) and 284 isolates of 389 respiratory lesions (73.0%) were obtained respectively. In addition, serotyping PCR assays were applied for 406 isolates and the distribution showed that 22 serovars were detected except for serovars 6, 10, 11, 13, 18, 28, and 30. The serovars with a higher isolation rate in systemic lesions were as followed: serovars 1, 1/2, 9, 14, 24, and 31; while only serovar 1 showed a significant difference ($p < 0.05$). Serovars 2, 3, 4, 8, and 21 contributed significantly higher isolation rates in respiratory lesions ($p < 0.01 – 0.001$) among the remaining serovars, indicating the potential affinity to respiratory tissues. The current results of serovars 1 and 3 were corresponding with other studies [1, 4, 5]. Besides, no report has ever mentioned the tendencies of both serovars 4 and 8 toward respiratory lesions.

Conclusions:
Even though the results of this study revealed the relationship between specific serovars and isolation rates from clinical cases, more investigations including in vitro and in vivo experiments should be conducted to elaborate on the mechanisms of S. suis invasions in different tissues/organs.

References:
[1] Estrada et al., 2019. J. Clin. Microbiol. 57 (9): e00377-19.
[2] Kerdsin et al., 2014. J. Med. Microbiol. 63 (Pt 6): 824-830.
[3] Lacouture et al., 2020. J. Vet. Diagn. Invest. 32 (3): 490-494.

[4] Bojarska et al., 2020. Vet. Microbiol. 240: 108534.
[5] Wei et al., 2009. Vet. Microbiol. 137 (1-2): 196-201.

Table 1

Table 1. Comparisons of isolate numbers and isolation rates with different *S. suis* serovars from clinical necropsy lesions (2015-2019)

Serovar[a]	Number[b]	Isolation rate (%)
	S, R[c]	(S, R)
1	32/45, 2/7	(71.1, 28.6) †
1/2	11/14, 4/6	(78.6, 66.7)
2	6/52, 42/51	(11.5, 82.4) ‡
3	21/108, 79/94	(19.4, 84.0) ‡
4	11/51, 34/46	(21.6, 73.9) ‡
5	11/26, 8/15	(42.3, 53.3)
7	20/38, 19/27	(52.6, 70.4)
8	19/63, 30/41	(30.2, 73.2) ‡
9	35/54, 15/26	(64.8, 57.7)
12	3/8, 5/7	(37.5, 71.4)
21	0/6, 6/7	(0, 85.7) §
24	10/12, 3/5	(83.3, 60.0)
31	8/19, 3/9	(42.1, 33.3)
UT[d]	19/46, 14/22	(41.3, 63.6)
Total	213/573, 284/389	(37.2, 73.0)

[a] Serovars 6, 10, 11, 13, 18, 28, and 30 were not detected; serovars 14-17, 19, 23, 25, 27, and 29 were not shown in this table
[b] Number of isolated *S. suis* strains/Number of sampled lesions
[c] S, systemic lesions (meninges, pleura, pericardium, peritoneum, joint); R, respiratory lesions (lung parenchymal tissue)
[d] UT, untypable
† Significantly different, $p < 0.05$
‡ Significantly different, $p < 0.001$
§ Significantly different, $p < 0.01$

P-B-058

LUNG LESIONS AND PRODUCTIVE PARAMETERS IN PIGS VACCINATED WITH TWO DIFFERENT PCV2/M.HYO RTM VACCINES, IN A FARM CONDITION

Mr. Roman Krejci[1], R Niemyjski[2], M Czerniecki[2], P Zachara[2], R Lewko[3], K Janeczko[3], M Lisgara[4]
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Introduction
Mycoplasma hyopneumoniae (M.hyo) and PCV2 are involved in PRDC, which can cause severe economic losses to swine farmers. Vaccination against those two pathogens helps to reduce their impact in pigs' respiratory health and performance. Using Ready-To-Mix (RTM) vaccines against PCV2 and M.hyo is a common practice. The aim of this study was to compare lung lesions and productive parameters between pigs vaccinated with two different PCV2/M.hyo RTM vaccines, in a Polish farm.

Material and Methods
The study took place in a conventional 3-site farm with confirmed PRDC issues, in Poland. Pigs received either 2.5 ml of Circovac® and Hyogen as RTM (group DUO), or 2 ml of the other PCV2/M.hyo RTM product (group CM), at 4 weeks of age. Each group consisted of 1020 animals. Weight gain, mortality (%) and medication cost were recorded per group. Lungs of 100 pigs per group were scored at slaughter using the Ceva Lung Program methodology.

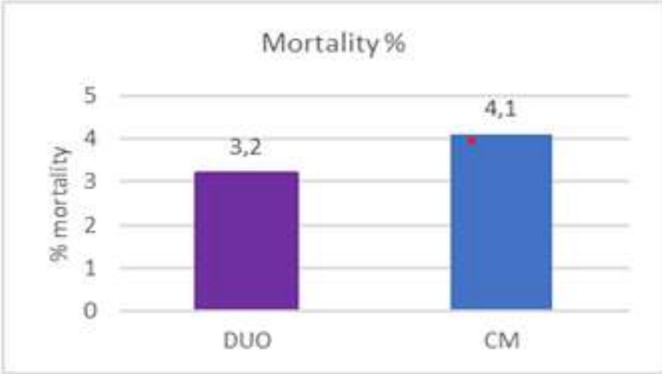
Results
Mortality in the fattening units was 3.2% in DUO and 4.1% in CM group ($p>0.05$).

Average weight (AW) at weaning did not differ ($p<0.05$) between the two groups (DUO: 6.29, CM: 6.35). Pigs from both groups were slaughtered in three different dates. At the first date AW (in kg) was 123 for each group. At the second date, AW was 124 for DUO and 122 for CM and at the third date, it was 117 for DUO and 116 for CM. Frequency of bronchopneumonia-like lesions was significantly lower in DUO group compared to CM ($p<0.05$), with 13% and 30% affected lungs, respectively.

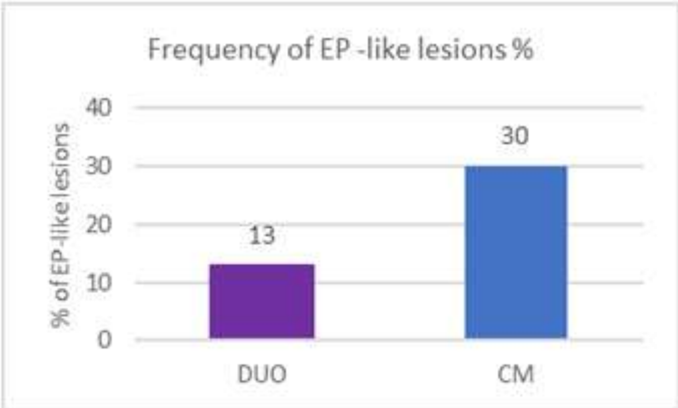
The medication cost per pig was 0.74€ in DUO vs 0.76€ in CM group.

Conclusion
In the present study, pigs which received DUO had better lung health and growth performance compared to pigs which received the other RTM vaccine, resulting in 1670kg more total live weight for DUO.

Graphs or Images 1



Graphs or Images 2



P-B-059

LUNG SCORING SURVEY IN EUROPEAN COUNTRIES IN 2022

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[1]*Ceva*

Background and Objectives
Scoring of lung lesions in the slaughter pigs provides an important information about the respiratory health in the pig population. Lesions suggestive for previous M.hyo or A.p. infections and their scoring were described before. Scoring of those lesions allows quantifying the problems with enzootic pneumonia and pleuropneumonia. The aim of this survey was to collect the results of lung scoring performed in most of swine producing European countries in 2022.

Material and Methods
Ceva Lung Program scoring methodology was implemented to score the lesions at the slaughterhouse. The results were collected EU countries in the 12 months period from December 2021 till the end of November 2022. The median values were calculated for % of lungs with bronchopneumonia (%BP), % of affected lung parenchyma out of sick lungs (% parenchyma), % of dorso-caudal pleurisy (%DP) and APP index (APPI). For A.p. indicators the results from France were not included, because there they were not scored routinely.

Results
The total number of scored lungs was 327588 from 3428 reports with the average of 96 lungs per batch. The median value of %BP was 30% compared to 24% in 2021. The median for % of affected parenchyma was 2.7% compared to 5% in 2021. For % DP the median was 10% vs 8% previous year and APPI index 0.29 vs 0.21 in 2021.

Discussion and Conclusion
The data set from EU countries in 2022 shows similar prevalence of Enzootic pneumonia in swine herds, but lower severity of the lesions. Indicators of A.p-like lesions are stable, similar compared to last year. These data may indicate that control of M.hyo infections seems evolving in a right direction, while A.p. infections still deserve efforts in their prevention.

P-B-061

MOLECULAR SEROTYPING AND PATHOTYPING OF GLAESSERELLA PARASUIS CLINICAL ISOLATES IN TAIWAN

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Introduction: Glaesserella (Haemophilus) parasuis (G. parasuis), is a diverse bacterium that can be found in the upper respiratory tracts of pigs. G. parasuis is responsible for systemic Glässer's disease and bacterial pneumonia, causing severe economic losses in the worldwide swine industry [1]. Serovar classification can evaluate the virulence and pathotype of G. parasuis isolates, but there is no absolute correlation [2, 3]. On the other hand, virulence-associated genes (VAGs) also play important roles in pathogenesis. Ten putative VAGs (V1-10) have been mentioned in a genome-wide association study [4]. Here, the present study aimed to determine serovars and VAGs of G. parasuis isolates from diseased pigs in Taiwan by molecular serotyping and pathotyping methods, and to analyze the relationship between serovars and VAGs.

Materials and Methods: We applied both serotyping and pathotyping multiplex PCR [4, 5] to analyze 112 G. parasuis isolates, which were collected from Glässer's disease cases submitted to Animal Disease Diagnostic Center, National Pingtung University of Science and Technology from 2015 to 2022. According to the description of VAGs in previous study, V1, V2, V4, V8, and V10 were classified as virulence-associated, whereas V3, V5, V6, V7, and V9 were carriage-associated [4]. Thus, V1, V2, V4, V8, and V10 genes were focused in this study.

Results: The dominant serovars of G. parasuis isolates were serovars 4 (29.5%), 5 (29.5%), 13 (13.4%), and 12 (12.5%), followed by molecular serotyping non-typable isolates (10.7%). Our results showed that more than 90% of Taiwanese G. parasuis isolates were positive for V4 gene (Table 1). By contrast, isolates with V1, V2, and V10 genes were significantly fewer and V8 gene was not detected in all isolates. In total 7 kinds of VAG patterns have been discovered and the single patterns (V4 or V10 only) were the most common profiles, occupying 68.8% of all isolates (Table 2).

Conclusions: Virulence-associated gene V4 was likely to be a relatively important indicator in Taiwanese G. parasuis isolates. Further research is needed to demonstrate its function in pathogenesis.

Acknowledgement: We gratefully acknowledge the technicians and veterinarians in ADDC of NPUST for their contributions to this study.

References:

[1] Aragon V., Segalés J., and Tucker A.W., 2019. Glässer's Disease. Diseases of Swine. 11th ed. 844-853.
[2] Lin W.H. et al., 2018. PeerJ. 6:e6017
[3] Schuwerk L. et al., 2020. Vet. Res. 51:137-150
[4] Howell K.J. et al., 2017. J. Clin. Microbiol. 55: 2617-2628
[5] Howell K.J. et al., 2015. J. Clin. Microbiol. 53: 3812-3821

Table 1. Serovars and virulence-associated genes of 112 G. parasuis isolates

Table 1

Serovar	Total	(%)	Virulence-associated genes[a]			
			V1	V2	V4	V10
1	5	(4.4)	2	3	4	3
4	33	(29.5)	1	4	31	5
5	33	(29.5)	0	3	29	2
12	14	(12.5)	2	4	14	2
13	15	(13.4)	0	1	12	0
nt[b]	12	(10.7)	0	1	12	2
Total	112	(100)	5	16	102	14

[a] V8 gene was not detected in all isolates.
[b] nt, non-typable by molecular serotyping PCR

Table 2. Virulence-associated genes patterns of 112 G. parasuis isolates

Table 2

Number of VAGs	VAG patterns	Total	(%)
0	None	9	(8.0)
1	V4	76	(67.9)
	V10	1	(0.9)
2	V2-V4	13	(11.6)
	V4-V10	8	(7.1)
3	V1-V4-V10	2	(1.8)
4	V1-V2-V4-V10	3	(2.7)

P-B-063

PARALLEL FIELD STUDY COMPARING THE PROTECTIVE CAPACITY OF TWO INTERNATIONALLY COMMERCIAL ACTINOBACILLUS PLEUROPNEUMONIAE VACCINES IN TAIWAN

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Introduction

A. pleuropneumoniae (App) is a potentially devasting, primary respiratory pathogen commonly inducing acute mortality, pleuropneumonia, and chronic pleurisies. Vaccination measure is an integral part of on-farm App control. Vaccines containing toxoids adapted from all the ApxI-III toxins could induce protections across all known App serotypes [1,2]. Serotype-independent protection has been demonstrated in a multi-serovar, multi-study analysis for the whole-cell vaccine based on App1 and App2 expressing ApxI, II, and III during production [2]. This vaccine has been compared to the App-subunit vaccine of this study with a significant increase in protection and return on investment in a field trial under European conditions 3. The objective of this study was to compare the above-mentioned two App vaccines available in Taiwan for confirming the increased protection of the whole-cell vaccine based on App1 and App2 expressing ApxI, II, and III during production compared to the App-subunit vaccine under Asian conditions.

Material and Methods

A 2000-sow farrow-to-finish farm with Ap history was selected. 1,040 growers were separated into two groups (G1: n=500; G2: n=540) and raised in separate pens in the same house. At 12 and 15 weeks of age, G1 pigs were vaccinated with Coglapix® (Ceva, France), and G2 with a commercial App-subunit vaccine. The pigs were monitored from 11 to 26 weeks of age for mortality and injectable antimicrobial (AM) treatment. Dorso-caudal pleurisies (DCP) typical of App-induced pleuropneumoniae were evaluated in 200 pigs randomly selected finishers (G1: n=99; G2: n=101) and scored for lung lesions at the slaughterhouse by Professor Chao-Nan Lin according to the Ceva Lung Program (CLP) methodology described previously [4].

Results

Treatment frequencies during finishing (11-26 woa) were lower in G1 (G1=0.36 times/pig, G2=0.64 times/pig; p=0.0598, unpaired t-test) (Fig 1), and so were the mortalities (G1=8.2%, G2=13.0%; p=0.0139, log-rank test) (Fig 1). G1 pigs also showed fewer App-like lesions, DCP, at the slaughterhouse (G1=9%, G2=33.5%; p<0.0001, Fisher's exact test) (Fig 1). p<0.05 between groups are considered significant.

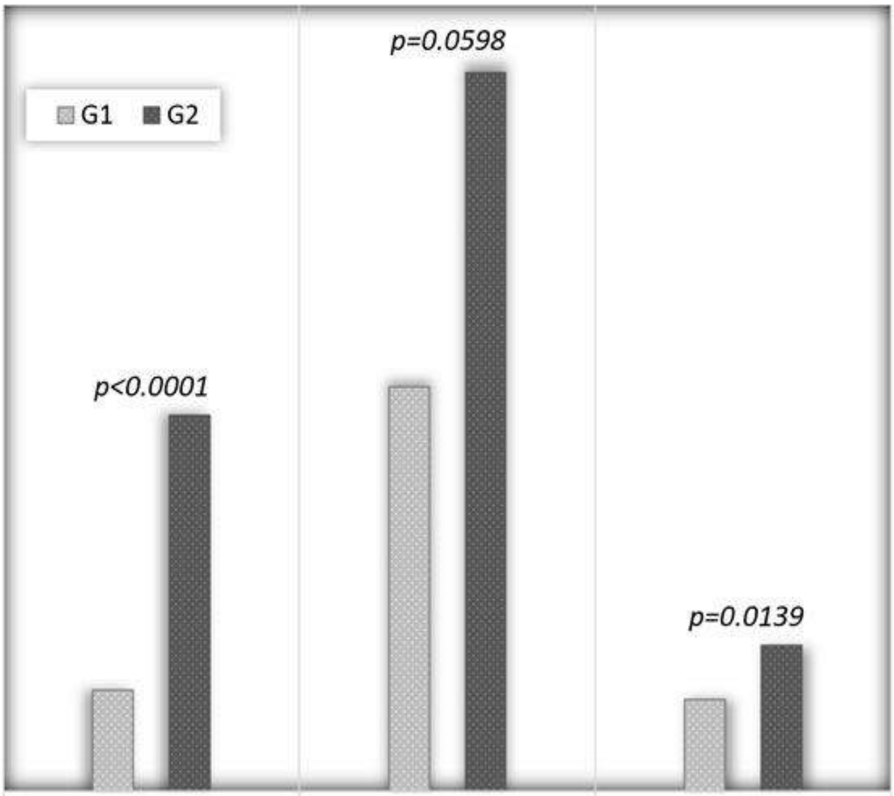
Conclusion and Discussion

App is an economically important respiratory pathogen that could be controlled with proper farm management, susceptible antibiotics, and vaccinations. Here we presented the comparisons of two App vaccines. The group G1 pigs, vaccinated with Coglapix® needed less antibiotic treatment, had lower mortality, and fewer pleurisies at the slaughterhouse. The demonstrated differences in the ability to reduce App-induced disease between the two vaccines were in line with previous studies [3].

References
1. Taylor D.J., Actinobacillus pleuropneumoniae in: Disease of Swine 11th edition. 2019. 349-354.
2. Mortensen P. et al., Comparative Efficacy in Challenge Dose Models of a Toxin Expressing Whole-Cell Vaccine against Eight Serovars of Actinobacillus pleuropneumoniae in Pigs. Animals, 2022
3. Hoelzen P. et al., Comparison of Protectivity and Safety of Two Vaccines against Ap in a field study. Agriculture, 2021
4. Sipos W. et al., Evaluation of the Efficacy of a Vaccination Program against Actinobacillus pleuropneumoniae Based on Lung-Scoring at Slaughter. Animals, 2021.

Graphs or Images 1

Fig 1: **Comparative App-protective capacities**



	Prevalence of lung lesions (DCP)	Overall treatment rate (AM inj/pig/11-26 woa)	Overall mortality rate
G1	9.0%	0.36	8.2%
G2	33.5%	0.64	13.0%

P-B-064

PARENTERAL ADMINISTRATION OF TOLTRAZURIL MAY PRODUCE VARIATIONS IN THE ACTIVATION OF THE IMMUNE SYSTEM DURING TRANSITION.

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Introduction:
Coccidiosis in piglets remains in the populations even though it has been controlled for more than 30 years. Only toltrazuril has shown efficacy against these parasites, which take advantage of the temperature and humidity conditions in farrowing rooms to perpetuate themselves.
Even in the absence of obvious parasite-related clinical signs, there is a possibility that the presence of parasites can lead to an activation of the immune system and thus to a situation of chronic inflammatory and weakened intestine with consequences for both the health and the productive performance of the animals.
The aim of this work was to quantify gene expression for different cytokines as indicators of immune stimulation comparing animals with and without injectable toltrazuril treatment.

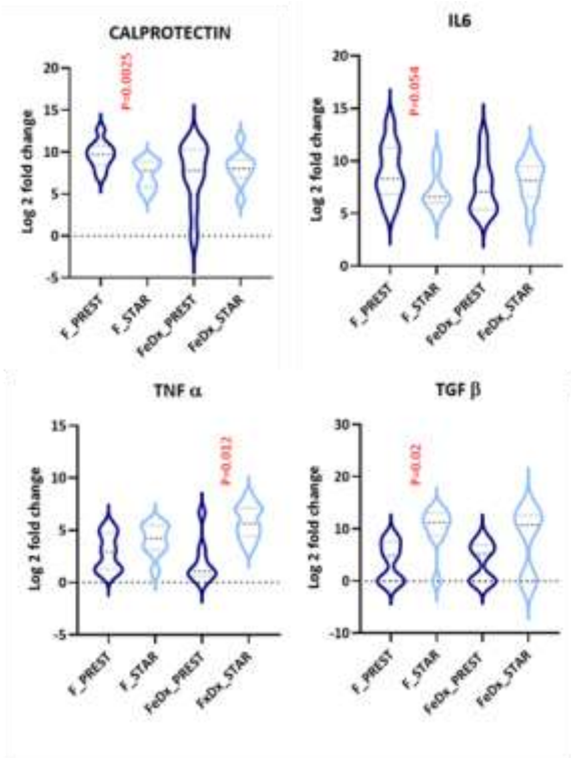
Material and methods:
A total of 162 Duroc piglets were randomly included in two groups: the Forceris® group (N=81) received 1.5 mL of this drug containing 45 mg toltrazuril and 200 mg iron per piglet. A control group (n=81) was set up, which received dextran iron parenterally but not coccidiostat by any route. Piglets were housed in 9-piglet pens with 4 place feeders, with feed ad libitum and water by means of a freely disposable water trough.
Faecal samples were obtained at the end of the prestarter period (PRES; weaning+15d) and at the end of the transition (STA, weaning+45 days), preserved in RNAlater. RNA was isolated and cDNA was synthesized from the mRNA. Relative quantification was performed, using β-actin as housekeeper. Twelve immune-related biomarkers (Calprotectin, IL1α, IL1β, IL6, IL8, IL10, IL12p35, IL12p40, TGFβ, IFNα, IFNγ and TNFα) were analyzed for gene expression in feces. The results were analyzed as paired samples to understand the dynamic of the biomarkers over the time

Results:
The biomarkers that showed different dynamics in both groups were calprotectin, IL6, TNFa and TGFb. While calprotectin and IL6 decreased significantly in the Forceris® group and remained unchanged in the control, a significant increase in TNFα was observed in the control group and TGFβ in the Forceris® group. There were also an increase for IFNα, IFNγ and IL1b but in this case occurred in both groups.

Conclusion:
Even on positive farms where there is no obvious coccidiosis-related clinic, the use of parenteral toltrazuril can produce variations in the activation of the immune system, preventing chronic inflammatory states that could clearly affect the productive performance of piglets.

Figure 1. Violin plot for Calprotectin, IL6, TNFα and TGFβ gene expression .

Table 1



P-B-065

PERFORMANCE OF A MYCOPLASMA HYOPNEUMONIAE (MHP) SERUM ELISA FOR THE DETECTION OF MHP ANTIBODY IN PROCESSING FLUIDS

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[1]Iowa State University, [2]Pampanga State Agricultural University, [3]Carthage Veterinary Service, Ltd

Introduction:
Serum-based Mycoplasma hyopneumoniae (MHP) monitoring in breeding herds is constrained by the labor required to collect blood samples from individual sows and the number of samples required for statistically valid surveillance. Processing fluid (PF), the serosanguineous fluid recovered from tissues collected at the time of piglet processing (3 to 5 days of age) is an easily collected specimen with high diagnostic utility and potential to serve in sow herd surveillance1. The purpose of this study was to evaluate the diagnostic performance of PF samples for the detection of MHP antibody using a commercial ELISA kit.

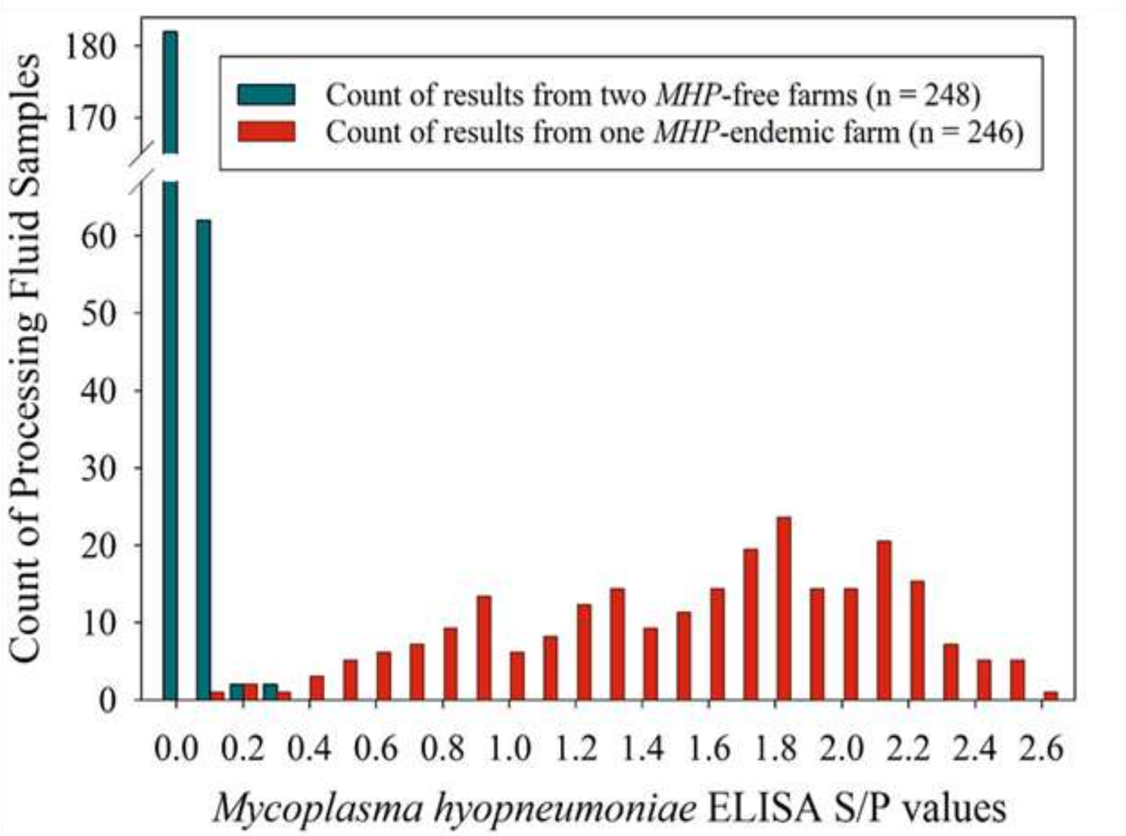
Materials and Methods:
Processing fluid samples (n = 494) were collected from three commercial swine farms. One farm was considered MHP-endemic (n = 246 samples) and two farms were considered MHP-free (n = 248 samples). Samples were tested at 1:10 dilution using a commercial MHP ELISA designed to detect anti-P46 antibody in serum (M. hyo Ab test, IDEXX Laboratories, Inc.). Diagnostic specificities and sensitivities for specific ELISA sample-to-positive (S/P) cutoffs were estimated by receiver operating characteristic (ROC) analysis.

Results:
A frequency distribution of the MHP ELISA S/P responses by farm status is shown in Figure 1. At a cutoff of S/P ≥ 0.4, the ROC analysis estimated diagnostic specificities and diagnostic sensitivities as 100.0% (95% CI: 100, 100) and 97.6% (95% CI: 95.5, 99.2), respectively. That is, all samples (n = 248) from MHP-free farms produced S/P values < 0.4, while 3 of 246 samples from the MHP-endemic farm produced S/P values < 0.4 (i.e., 0.387, 0.344, 0.386).

Conclusion and Discussion :
The findings in this study are consistent with the report by Boettcher et al. (2010) who described ”excellent” agreement between processing fluids and sow serum samples. Further, our results suggested that processing fluids could be supplemental to, or even replace, sow serum sampling for breeding herd MHP antibody surveillance.

References:
Boettcher, J. 2010. Proceedings of the 21st IPVS Congress. International Pig Veterinary Society.

Graphs or Images 1


Count of results from two *MHP*-free farms (n = 248)
Count of results from one *MHP*-endemic farm (n = 246)
Count of Processing Fluid Samples
0
10
20
30
40
50
60
170
180
0.0 0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6
Mycoplasma hyopneumoniae ELISA S/P values

P-B-066

PK/PD AND CLINICAL RELATIONSHIPS OF APRAVET® (APRAMYCIN SULFATE) ADMINISTERED TO PIGS FOR THE TREATMENT OF ESCHERICHIA COLI INFECTIONS

Mr. Ulrich Klein[1], Mariana Karanikolova[2], Alain Kanora[1], Lieven Claerhout[1], Wouter Depondt[1], Mr. Ping-Yi Chen[3], Dimitar Pashov[4], Nuananong Sinwat[5], Narut Thanantong[5], Monticha Suwanwong[5], Somyod Kankuntod[5], Korntip Kanjanavaikoon[6]
[1]Huvepharma NV, [2]Department of R&D, Biovet JSC, [3]Huvepharma Taiwan, [4]Department of Pharmacology, [5]Kasetart University, [6]Huvepharma Thailand

Background & Objectives
The pharmacokinetics (PK) of apramycin sulfate (Apravet® - Huvepharma NV) small intestine content concentration (SIC) based on feed medication was related to apramycin MICs against Escherichia coli (EC) strains from Europe and Thailand (pharmacodynamics –PD).

Materials and Methods
The apramycin SIC was determined in a PK study with twenty 4-5 week old pigs (bodyweight 8.8-10.5kg), which were medicated at dose of 8mg/kg bw.[1] Medicated feed was offered 21 consecutive days.
At day 21 of the PK study pigs were euthanized at 12, 24, 36 and 48 hours after treatment stop to determine the apramycin intestinal contents concentration (HPLC) in the small intestine.
Apramycin MIC data were generated based on susceptibility testing of Escherichia coli strains in a pan-European (B, DK, F, D, NL, E, UK) and in a Thailand study.[2 3] 238 EU and 311 Thailand EC strains generated 2019 – 2020 were MIC tested (MIC dilutions: 0.06 - >1024µg/ml). MIC_{50}, MIC_{90} and MIC ranges were determined.

Results
The apramycin SIC was recorded at 45.0µg/g at 8mg/kg bw dose. 12 and 24 hours after treatment stop the apramycin SIC remained high, respectively 33.2µg/g and 10µg/g.
European EC strains: The SIC concentration directly after euthanasia exceeds the MIC_{90} values (8-16µg/ml) determined for B, D, NL and Pol isolates. For DK, E and UK strains the MIC_{90} values (512µg/ml /1024µg/ml) were higher than the SIC concentration. High apramycin intestine concentrations at registered dose were determined – above MIC_{90} of 95% of tested European E.coli isolates.
European EC strains show country-specific differences in apramycin MIC patterns with narrow MIC ranges (2-16µg/ml) for strains from B, NL, Poland. Broader MIC ranges were determined for D (1-512µg/ml) and DK, E, UK (2-1024µg/ml).
Thailand EC strains: The determined SIC concentration at 8mg/kg bw was higher than the determined MIC_{90} value (16.0µg/ml) for all tested EC strains. 94% of the tested Thailand strains had MICs below the SIC of apramycin.
No differences in the apramycin MIC patterns of the EC strains from the three different Thailand regions were found. The MIC_{90} values for EC strains from Central, Eastern and Western Thailand were the same (MIC_{90} = 16.0µg/ml).

Conclusions and Discussion

An excellent therapeutic and metaphylactic effect of Apravet® (Huvepharma) in the case of oral Escherichia coli infection treatment via feed medication at registered dosage of 8mg/kg bw in European and Thailand farms can be expected based on the available PK/PD data.

References

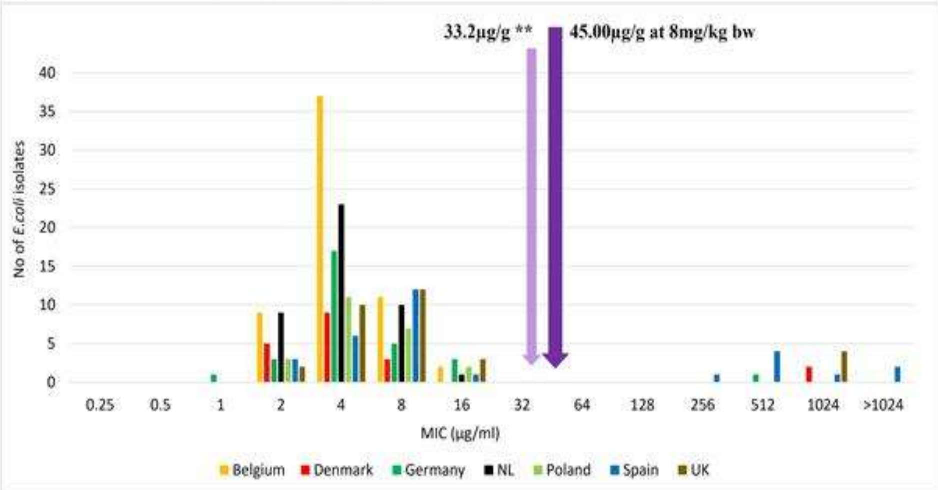
[1] Karanikolova, M. et al. (2016). Pharmacokinetic profile of Apravet® after oral administration. Proc. 24th IPVS Congress/8th ESPHM Congress Dublin, Ireland, PO-PF3-240, p.194.

[2] Hawser, S. (2022). European Collection of Veterinary Pathogens (VetPath V 2018-2020) – Minimal Inhibitory Concentration (MIC) Testing, final IHMA report.

[3] Pariyotom, N. (2021). Antimicrobial susceptibility results of Escherichia coli isolated from clinical pigs in Thailand, final report May 2021.

Graphs or Images 1

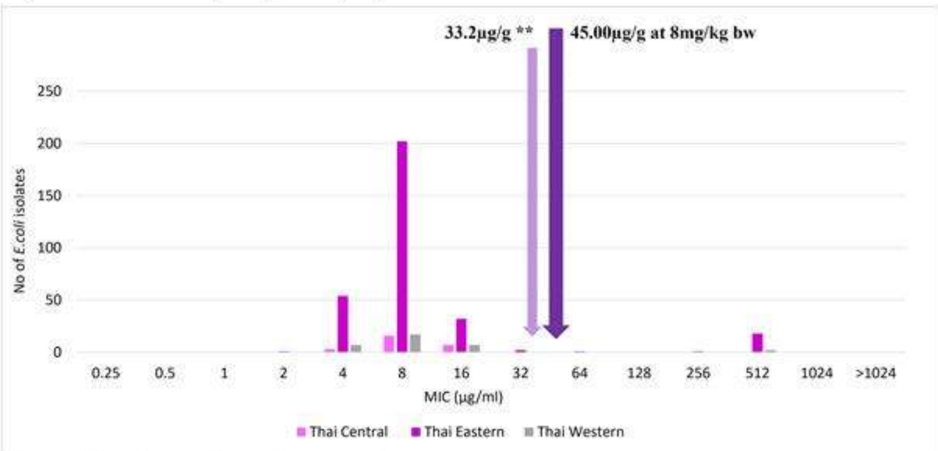
Figure 1: PK/PD relationships of Apravet® apramycin on EU *Escherichia coli* strains



** Apramycin intestine conc. 12 hours after treatment end

Graphs or Images 2

Figure 2: PK/PD relationships of Apravet® apramycin on Thailand *Escherichia coli* strains



** Apramycin intestine conc. 12 hours after treatment end

P-B-067

PK/PD RELATIONSHIPS OF PHARMASIN®/TYLOVET® (TYLOSIN) ORAL ADMINISTRATION FOR THE TREATMENT OF CLOSTRIDIUM PERFRINGENS IN PIGS

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[1]*Huvepharma NV,* [2]*Huvepharma Taiwan*

Introduction
Clostridium perfringens (CP) is a widespread pathogenic bacterium associated with fatal necro-haemorrhagic enteritis seen in 1-5 day old piglets up to 3-4 weeks old. In fattening pigs, C. perfringens causes sudden death and painful gut inflammation.
The purpose of this paper was to place Pharmasin®/Tylovet® pharmacokinetic data in relationship with published data on tylosin pharmacodynamics. Understanding the pharmacokinetics (PK) and pharmacodynamics (PD) of Pharmasin®/Tylovet® is key to predicting its clinical efficacy according to antibiotic prudence guidelines.

Materials and Methods
Two pharmacokinetic (PK) studies were conducted based on oral administration of Pharmasin®/Tylovet® via feed (study No.1) and via water (study No.2).
The pharmacokinetic behaviour of tylosin phosphate (Pharmasin®/Tylovet® Premix) and tylosin tartrate (Pharmasin®/Tylovet® WSG) was investigated after feed and water medication in eleven mixed breed pigs (20-25kg bw) in each study at dosages of 10mg/kg bw.
Medication duration: 5 consecutive days.
On day 5 in both studies, treated pigs were euthanised and blood/organ samples from the respiratory/gastrointestinal tract were collected. PK parameters were determined. The AUC per hour (AUC µg.h/g/hour) was calculated in both studies to determine the tylosin colon concentrations at registered enteric infection treatment dose (5mg/kg =100ppm) and at 10mg/kg (200ppm) dose after feed and water medication.
Tylosin pharmacodynamic data (MICs) were generated based on antimicrobial susceptibility testing of Clostridium perfringens Type A strains originating from Italy (51 strains) and Brazil (50 strains). MIC determination by agar dilution method - tylosin MIC_{50}, MIC_{90}, MIC ranges were calculated.

Results
The colon contents concentration after feed medication calculated, based on AUC per 1 hour, indicate a tylosin concentration at treatment dose of 38.2µg/g (5mg/kg bw) and 76.3µg/g (10mg/kg bw). In the case of water treatment, the colon tylosin concentration was 34.0µg/g (5mg/kg bw) and 68.1µg/g (10mg/kg bw).
Minor differences were determined for tylosin colon concentrations based on feed and water medication.
Different tylosin MIC ranges were determined for the Italian (0.12-128µg/ml) and Brazilian (0.25-32µg/ml) CP strains.
The tylosin PK/PD relationships for the treatment of C. perfringens infections following oral administration were studied based on the determined MIC values for CP isolates from Italy and Brazil and the achieved tylosin colon content concentration at the registered dose levels of 5mg tylosin phosphate/kg bw (feed medication, A) and 5-10mg tylosin tartrate/kg bw (water medication, B).

The tylosin colon concentrations after feed and water medication at therapeutic doses are high and exceed the MIC values for all Brazil C. perfringens strains. In the case of the Italian C. perfringens strains, the tylosin gut concentration exceed the tylosin MICs of 85% of the tested Italian C. perfringens strains.

Conclusions
An excellent therapeutic and metaphylactic outcome of Pharmasin®/Tylovet® in cases of oral C. perfringens infection treatment can be expected based on the available PK/PD data.

Table 1

Table 1: Pharmacokinetics of tylosin in the colon calculated from mean concentration from each time of kill following ad libitum in feed and water application

Parameter unit (feed application)	Colon contents (µg.h/g)	Colon contents (average conc. µg.h/g/hour)	Colon contents (average conc. µg.h/g/hour): tylosin dosage 5mg/kg bw=100ppm	Colon contents (average conc. µg.h/g/hour): tylosin dosage 10mg/kg bw=200ppm
AUC 0-12h µg.h/g	915.90	E 76.3	E 38.2	E 76.3
Parameter unit (water application)	Colon contents (µg.h/g)	Colon contents (average conc. µg.h/g/hour)	Colon contents (average conc. µg.h/g/hour): tylosin dosage 5mg/kg bw=100ppm	Colon contents (average conc. µg.h/g/hour): tylosin dosage 10mg/kg bw=200ppm
AUC 0-12h µg.h/g	816.85	E 68.1	E 34.0	E 68.1

Graphs or Images 1

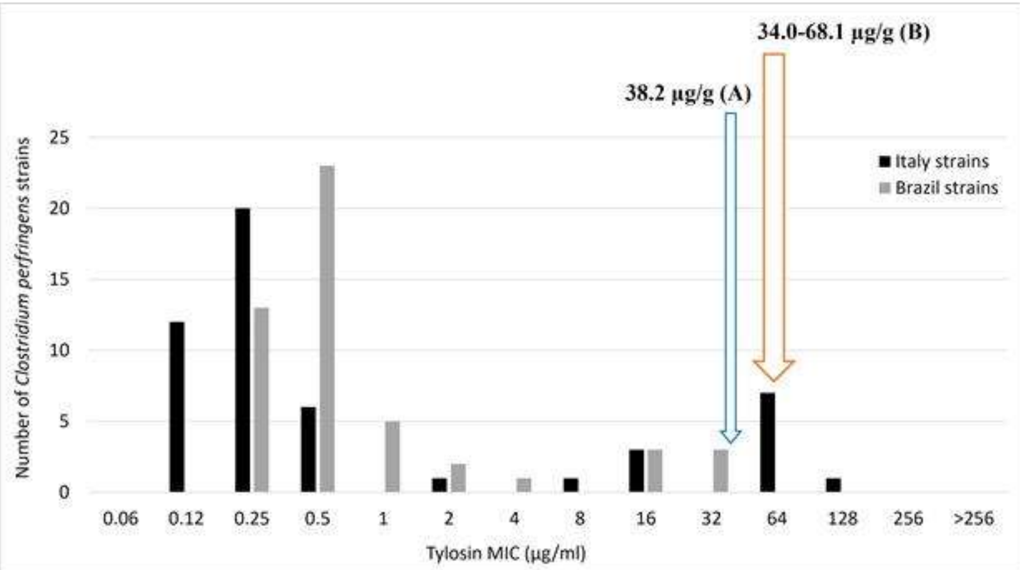

Figure 1: Tylosin colon content concentration after feed medication (5mg/kg bw) and water medication (5-10 mg/kg bw) at registered dosages in relation to the tylosin MIC susceptibility patterns of Italian and Brazilian *C. perfringens* strains

P-B-068

PREVALENCE AND SEVETIRY OF ENZOOTIC PNEUMONIA AND PLEUROPNEUMONIA IN LATIN AMERICAN COUNTRIES: AN EVOLUTION FROM 2018 TO 2022.

Mr. Roman Krejci[1], J Calveyra
[1]*Ceva,* [2]*Ceva Saúde Animal*

Introduction
Respiratory diseases are one of the main causes of economic losses in the swine industry. Monitoring these challenges is important to correct control measures.
To assess the health status of the farm, monitoring by lung lesions has been described (1). Ceva Lung Program (CLP) was confirmed as a valuable tool to establish the prevalence and severity of Enzootic Pneumonia (EP) and pleuropneumonia. The aim of this study is to evaluate the level of EP and A.p-like lesions on Latin American countries pig farms, comparing the periods from 2018 to 2022.

Material and Methods
The survey was conducted on conventional swine farms excluding those with the M.hyo and A.p. SPF status. A total of 916,171 lungs in 13,580 batches of slaughtered pigs were scored using the CLP method. The results were collected from 9 Latam countries in the period of January 2018 to November 2022. Bronchopneumonia lesions (BP), scarring associated with older EP-like lesions were recorded and scored. Dorsocaudal pleurisy (DP) suggestive for previous pleuropneumonia was scored to describe A.p-like lesions.

Results
The median value of % BP in 2018 was 56% and it went down until it reached 43% in 2022. The affected area of the lungs with bronchopneumonia lesions showed a slight reduction, from 6.7% in 2018 to 6.2% in 2022. The percentage of dorsocaudal pleurisy has shown a reduction since 2018, with 10.2% prevalence to 6.3% prevalence in 2021. However, in 2022, the prevalence increased to 7.7%.

Table 1. Evolution of Ep-like lesions and Ap-like lesions from 2018 to 2022: average for Latam countries

Figure 1. Map with LatAm countries indicating the prevalence of bronchopneumonia lesions in the year 2022

Conclusions
The data set from 9 Latam countries from 2018 to 2022 shows very similar distribution of the values during the period evaluated. This confirms CLP as a repeatable, relevant scoring methodology. The incidence of especially EP-like lesions remains high despite the decrease over the years. The control of M.hyo infections seems still to be a major challenge.

Table 1

	2018	2019	2020	2021	YTD 8th, Nov 2022	TOTAL
# CLP	1592	2970	2495	3269	3254	13580
# Lungs	114125	193997	150353	220836	236860	916171
% Bronchopneumonic Lungs	56,1%	50,3%	50,5%	47,6%	43%	50%
%Affected Area Pneumonic Lungs	6,7%	6,5%	6,7%	6,3%	6,2%	6,5%
% Affected Area All Lungs	4,5%	4,1%	4,2%	3,9%	4,0%	4,1%
% Scars	19,0%	10%	10%	11%	13%	13%
% Dorsocaudal Pleurisy	10,2%	9,2%	7,0%	6,3%	7,7%	8,1%
Ap Index	0,31	0,28	0,21	0,19	0,22	0,24

Graphs or Images 1

P-B-069

PREVALENCE OF ENZOOTIC PNEUMONIA IN THE NORTHERN REGIONS OF THAILAND USING A NOVEL ARTIFICIAL INTELLIGENCE LUNG SCORING SYSTEM

Dr. Sittikorn Traiyarach[1], Dr. Napanporn Chowdeekornpun[1], Dr. Alongkorn Buakhiew[1], Mr. Chutipat Duanpen[1], Mr. Kriangkrai Lohaprom[1], Dr. Mongkol Lumyai[1], Mr. Nattawut Deelum[1], Mr. Sarawut Tonoy[1], Ms. Sukonta Jaidee[1], Dr. Ramon Jordà Casadevall[2]
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Introduction:
One of the most prevalent problems in the swine industry in Thailand is the Porcine Respiratory Disease Complex (PRDC) which is caused by multiple bacterial and viral agents. The aetiologic agent that causes enzootic pneumonia (EP) is Mycoplasma hyopneumoniae (Mhyo) and it is an important primary pathogen in the PRDC[1]. The complexity of managing the PRDC problem on farms is both time-consuming and costly. As HIPRA realizes the importance of this problem, it has developed a diagnostic tool called Artificial Intelligence Diagnos (AI Diagnos), an artificial intelligence diagnostic software that aims to identify and assess the Mhyo-like lung lesions in slaughterhouses by automating the process and reducing the subjectivity between different evaluators. The main objective of this study was to assess the prevalence of EP in the northern and north-eastern regions of Thailand using the AI Diagnos system

Material and Methods:
Artificial Intelligence Diagnos (AI Diagnos) is a fully automated diagnostic system developed by HIPRA, that uses AI to simplify, objectify, and facilitate the whole process of slaughterhouse assessment. Operational, detection and classification processes, output of results and practical applications were needed to develop this system.
The system was trained using over 11,000 images inspected and photographed by a smartphone camera under slaughter conditions. These images were evaluated by the system and corrected for learning by five different experts in Mhyo from all over the world, using a modified Madec scoring system for EP lesions and European Pharmacopoeia guidelines[2] for AR lesions. Regarding this trial, 397 lungs were randomly selected from 11 pig farms in the northern and north-eastern regions of Thailand which used different Mhyo vaccines. A whole picture of each lung was captured on a smooth clean surface at the slaughterhouse by a technician. Afterwards, the pictures were upload to the AI Diagnos software. Finally, the algorithm automatically detected (image 1) and classified all lung lesions and generated a report. Different parameters such as incidence (percentage of lungs with lesion), disease index (average lesion grade of all lungs) and lesion index (average lesion grade of affected lungs) were evaluated.
Image 1. Detection of each lobe of the lung

Results:
According to the AI DIAGNOS analysis, the disease lesion was 1.56 and the lesion index 1.58 on average from a total of 397 lungs. The report also shows that there was a 98 percent incidence of EP lesions with an average 16 percent surface area affected.
Table 1. Lung parameters evaluated by AI Diagnos.
In addition, the distribution of lung lesion grades (Figure 1) was greater on the left side of the graph, which means that most of the lesion grades fell into the low severity score. In this case, there were 196 (49.4%) and 165 (42%) in lesion grades 1 and 2 respectively.

Figure 1. Lesion grades result from AI Diagnos assessment

Discussion and conclusions:
Results from the AI Diagnos showed that EP is generally detected in low lesion index results, with a high percentage incidence, on commercial pig farms in the northern and north-eastern regions of Thailand. Since EP is widely distributed, PRDC should be of concern as it is one of the causes of respiratory problems. AI Diagnos is a useful diagnostic tool to facilitate and automate the identification and assessment of Mhyo-like lung lesions at slaughter.

Acknowledgments:
The authors would like to thank the commercial pig farm owners in Thailand who contributed with lungs in this study.

Reference:
1. Diseases of Swine, Eleventh Edition. Edited by Jeffrey J et al. Mycoplasmosis, Pieters M. and Maes D. 863-878
2. Madec F, Kobisch M (1982) Journees de la Recherche Porcine [Swine Research Conference], 14, 405-412.

Table 1

Table 1. Lung parameters evaluated by AI Diagnos.

Parameter	Result
Number of lungs	397
Incidence (%)	98
Disease lesion	1.56
Lesion index	1.58
Average % of affected surface	16

Graphs or Images 1

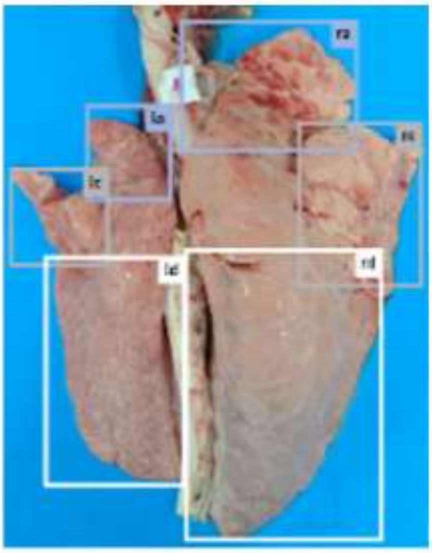

Image 1. Detection of each lobe of the lung

Graphs or Images 2

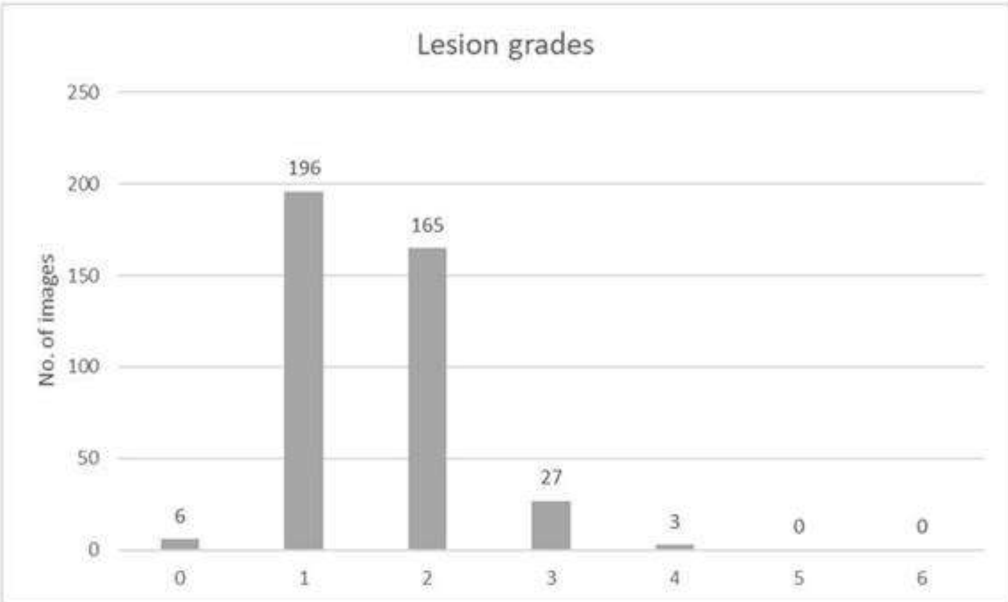

Figure 1. Lesion grades result from AI Diagnos assessment.

P-B-070

PREVALENCE OF ESCHERICHIA COLI VIA DETECTION OF ATTACHMENT FACTORS IN RECTAL SWABS FROM SWINE FARMS IN MALAYSIA

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INTRODUCTION
Worldwide, Escherichia coli is regarded an important cause of wide range of diseases in pigs including neonatal diarrhoea, post-weaning diarrhoea (PWD) and oedema disease [1]. However, the information on the distribution of pathogenic E. coli in Malaysia's swine population is limited [2]. Moreover, in 2021 the Malaysia Department of Veterinary Services recently ban use of colistin, a medically important antimicrobial in food-producing animals [3]. This has provided the necessity to find alternative control strategies such as novel vaccines for pigs. Fimbrial adhesin is one of the virulence factors use to classify pathotype of E. coli [1]. The objective of the study was to establish the prevalence of pathogenic E. coli in Malaysian swine farms via detection of the adhesins in faeces of lactating sows, in neonatal and in nursery piglets.

MATERIALS AND METHODS
The surveillance study was carried out from 2021 to 2022 in 11 swine farms with sow inventories that varied from 200 to 4,500 across Malaysia. In each farm, at least 15 rectal swab samples were collected in lactating sows, 15 pooled samples in suckling and in nursery piglets regardless of presence or absence of diarrhoea using Copan™ sterile swab. Samples were tested for E. coli attachment factors (F4, F18, F5, and F41) using commercial test kit Rainbow piglet scour Bio K 361 following manufacturer's collection, test, and result interpretation protocol [4].

RESULTS AND DISCUSSION
A total of 557 samples were tested. All 11 swine farms were positive for at least one of the E. coli attachment factors. The farm mean prevalence of pathogenic E. coli was 68%. F4 (59.5%) and F41 (74.0%) were the predominant attachment factors involved in neonatal piglets while F4 (23.0%) and F18 (37.2%) were the predominant in the nursery. F41 (45.2%) was still found in newly weaned piglets. The results show similar findings with previous study in Belgium and The Netherlands in 2020 [5].

CONCLUSION
This study confirms that pathogenic E. coli is highly prevalent in Malaysia's swine population. The main attachment factor consistently found in sows and neonatal piglets are F4 and F41 while in post-weaning piglets are F4, F18 and F41. This information has practical relevance in relation to designing farm prevention and control program for diarrhoea in piglets amidst Malaysia strengthening of its antimicrobial policy.

REFERENCES
1. Fairbrother JM, Gyles CL. (2012). Colibacillosis. In: Zimmerman JJ, Karriker LA, Ramirez A, Schwartz KJ, Stevenson GW, editors. Disease of Swine. 10th ed. UK: Wiley-Blackwell, p. 723–747.

2. Ho et al.: Prevalence and characterization of verotoxigenic-Escherichia coli isolates from pigs in Malaysia. BMC Veterinary Research 2013 9:109
3. Feed (prohibited Antibiotics, Hormones and Other Chemicals) Regulations 2012. (Malaysia)
4. https://www.biox.com/en/bio-k-361-rainbow-piglet-scours-p-323/
5. F. Vangroenweghe, A. Luppi and O. Thas. (2020). Prevalence of Enterotoxigenic Escherichia Coli Pathotypes and Virotypes Isolated from Piglets Suffering from Post-Weaning Diarrhea in Belgium and the Netherlands. Archives of Veterinary and Animal Sciences 2(2).

Graphs or Images 1

ANIMAL	Number (percentage) of samples positive for the different *E. coli* attachment factors				
	F4	F5	F18	F41	TOTAL [a]
Sow	10/180 (5.6)	0/180 (0.0)	2/178 (1.1)	103/178 (57.9)	103/181 (56.9)
Suckling	110/185 (59.5)	7/183 (3.8)	37/184 (20.1)	131/ 177 (74.0)	147/185 (79.5)
Nursery	44/191 (23.0)	1/188 (0.5)	71/191 (37.2)	85/188 (45.2)	129/191 (67.5)
TOTAL	164/556 (29.5)	8/551 (1.5)	110/553 (19.9)	319/543 (58.7)	379/557 (68.0)

[a] Number (percentage) of samples positive for any of one of the *E. coli* attachment factors

Table 1: Prevalence of E. coli attachment factors in different age group

P-B-071

PREVALENCE OF SHIGA TOXIN 2E-PRODUCING ESCHERICHIA COLI STRAINS ON TAIWAN SWINE FARMS

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Introduction

Oedema disease (OD) is a Stx2e toxaemia that results in severe oedema at specific sites in pigs that have absorbed Stx2e from the intestine following colonization by EDEC (1). OD mostly occurs in recently weaned pigs. Subclinical OD may also occur, where pigs are clinically normal but may have a decreased growth rate (2). The aim of this study was to analyse the OD status in the nursery and finisher pigs and the correlation with the type of feed in Taiwan.

Materials and methods

From March 2021 to August 2022, from the top 6 pig population provinces of Taiwan, a total of 1200 feces samples of 9 weeks-old and 24 weeks-old pigs were collected from 60 farrow-to-finish farms with feed supply backgrounds recorded. The screened farms were selected randomly. The samples were cultured in Escherichia coli broth, incubated in 40 ℃, 6 hours, tested of Stx2e by Real-time polymerase chain reaction (3).

Results

The detection rate of edema disease virulence factor (stx2e) in fattener (38.5%) is significantly higher than that in nursery pigs (26.7%) individually (Table 1), whereas at farm level, 80% on finishers is higher than 56.7% on nursery pigs (Table 2).
The positive rate of stx2e in the farm where nursery pigs were fed with self-made ingredients was as high as 91.9%, significantly higher than that of farms with commercial feed (Table 3).

Conclusions and discussion

The results of the current study indicate that Shiga toxin 2e-producing Escherichia coli is present nationwide in Taiwan, with a high prevalence at farm level. Moreover, the difference between feed supply affects significantly higher positivity on nursery pigs.
In past decades, there has been no commercial vaccine against ED in the swine industry in Taiwan. Therefore, commercial feeds formulated a crude protein (CP) ratio of around 20% to avoid E. coli over-reproduction. However, it decreases the growth performance, especially in nursery pigs, that is why self-made ingredients compound a higher CP ratio to balance the risk and benefits.

Reference

1. MacLeod DL, Gyles CL. 1990. Infect Immun 58: 1232-1239.
2. F.M. Kausche, et al., 1992. J. Vet. Res., 53: 281-287.
3. M.E. Jacob, et al., 2012. Foodborne Pathog 9: 79-85.

Table 1

	Group	Positive	Negative	Total	Positive Rate(%)	*p*-value
stx2	Nursery	160	440	600	26.67	< .0001
	Finisher	231	369	600	38.50	

Table 2

	Group	Positive	Negative	Total	Positive Rate(%)	*p*-value
stx2	Nursery	34	26	60	56.67	0.0102
	Finisher	48	12	60	80.00	

Table 3

	Feed Supply	stx2		Total	Positive Rate(%)	*p*-value
		Positive	Negative			
Nursery	Commercial feed	12	11	23	52.2	0.001
	Feed compounded by their own formula	34	3	37	91.9	
Finisher	Commercial feed	17	6	23	73.9	0.5081
	Feed compounded by their own formula	31	6	37	83.8	

P-B-072

PROBIOTIC BACILLUS LICHENIFORMIS TO SUPPORT FATTENING PIGS UNDER HIGH CLOSTRIDIUM PERFRINGENS STRESS

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Introduction
With the increased occurrence of intestinal challenges in modern swine production, feed additives that can support growth performance and disease mitigation have gained interest over the past few years. Probiotics are a good example thereof: these viable microorganisms can confer health benefits to the host, if administered in adequate amounts (FAO/WHO, 2002). To test a unique strain of probiotic Bacillus licheniformis (DSM 28710) in commercial conditions, a trial was set up on a commercial fattening farm.

Material and Methods
A total of 208 fattening pigs were randomly allocated to one of two treatments, each with two replicates. All animals had an average weight at the start of the trial of 29,5 kg. The first treatment was an AGP group, fed a commercial basal diet supplemented with 300 g BMD® (bacitracin methylene disalicylate) / mton of feed. The second treatment was based on the same basal diet, this time supplemented with 500 g B-Act®/mton of feed (1.6 x 10^{12} CFU Bacillus licheniformis/mton of feed) - but no BMD®. The probiotic is based on viable spores of Bacillus licheniformis DSM 28710.
As the trial was conducted under commercial conditions, the starting date was determined by the historical records of the farm to coincide with a high Clostridium perfringens challenge period. Animals were supplemented for 70 days, starting when they arrived on-farm. Average daily gain (ADG), average daily feed intake (ADFI) and feed conversation ratio (FCR) were evaluated on day 40 and 70, whilst mortality was recorded daily.

Results
Throughout the trial, there were no statistically significant differences between the two treatments, for none of the parameters evaluated (Table 1, Table 2, $P<0.05$).

Discussion and Conclusion
In this trial, B-Act® was as efficacious as AGP bacitracin in terms of maintaining technical growth performance during a period of high Clostridium perfringens stress. As such, B-Act® provides an interesting tool to support profitable animal production on-farm, whilst reducing the use of antimicrobials. This ties in with the wider global effort of reducing the prophylactic use of antimicrobials, confirming the contribution probiotics can make.

References
FAO/WHO (2002). Guidelines for the Evaluation of Probiotics in Food. Food and Agriculture Organization of the United Nations and World Health Organization Working Group Report. Rome: Food and Agriculture Organization.

Table 1

Table 1: Growth performance parameters for the two treatments, from day 0 to 40

Day 0-40		
Parameter	**BMD®**	**B-Act®**
ADG (kg)	0.750	0.779
FI (kg)	1.612	1.665
FCR	2.134	2.146
Mortality (%)	0	0

Table 2

Table 2: Growth performance parameters for the two treatments, from day 0 to 70

Day 0-70		
Parameter	**BMD®**	**B-Act®**
ADG (kg)	0.856	0.928
FI (kg)	1.767	1.871
FCR	2.063	2.016
Mortality (%)	7.69	7.69

P-B-073

PROTECTION AGAINST RESPIRATORY DISEASE AND LOSSES OF PRODUCTIVITY COMPARED BETWEEN FOUR DIFFERENT COMMERCIAL VACCINES AGAINST ACTINOBACILLUS PLEUROPNEUMONIAE UNDER FIELD CONDITION IN SOUTH KOREA

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Introduction
Actinobacillus pleuropneumoniae (App) can cause severe pleuropneumonia which is a highly contagious disease of swine often characterized by sudden onset, short clinical course, high morbidity, and high mortality1, but also less apparent losses due to subacute and subclinical pleuropneumoniae. All those manifestations potentially developing into chronic App3. The aim of this study was to compare the protective capacity of four App-vaccines evaluated on lung lesions at the time of slaughter, and finishing performance measured on average daily growth (ADG) and mortality.

Materials and Methods
This study was carried out in a farrow to finish farm with history of App serotype7 (App7) issues. A total of 3,576 pigs allocated to one of four App-vaccine groups were vaccinated at 7 and again at 9 weeks of age. Groups were, A: App1+2+3+4+5+7 bacterin (n=906), B: App1+3+5+7 bacterin including Apx I-III (n=900), C: App1+2+5 bacterin including ApxI-III and Pasteurella multocida (n=705), and D: Coglapix® (Ceva, France) (n=1065).
by different 4 groups in the same barn, the same system and. Pigs in the four groups were reared different rooms but under the same conditions. All had same, genetic and production (sanitary, nutritional, etc.) conditions and were slaughtered at the same slaughterhouse.
Mortality was recorded from May 2022 to October 2022, and average mortality rate per group was calculated. Days from birth to slaughter (D) and body weight at slaughter (W) was recorded. ADG was calculated by dividing W with D.
Lung lesions were evaluated in 15 pigs each group at the time of slaughter according to the Ceva Lung Program (CLP)3.

Results
Production parameters key to return on investment are presented in Table 1 and respiratory health parameters are presented in table 2. Differences (Δ) vs best group for all parameters are calculated and shown in both tables. Group D stands clearly out as the best in all parameters.

Conclusion and discussion
In this study, it was shown that pigs in group D, vaccinated with Coglapix® were efficiently protected against the heterologous App7, endemic at the farm. A protection better than that of the vaccines in the all the other three groups, of which two (A and B) contained App7. This was demonstrated by a significantly better performance than the three other vaccine groups in terms of ADG, pig-flow-efficacy, morbidity, lung health, mortality, and related costs. The farm informs an overall return on investment on the Coglapix® vaccination of at least 300%.

References
1. Taylor D.J. Actinobacillus pleuropneumoniae In: Disease of Swine 11th edition. 2019. 349-354.
2. Mortensen et al., Comparative Efficacy in Challenge Dose Models of a Toxin Expressing Whole-Cell Vaccine against Eight Serovars of Actinobacillus pleuropneumoniae in Pigs. Animals, 2022
3. Sipos et al., Evaluation of the Efficacy of a Vaccination Program against Actinobacillus pleuropneumoniae Based on Lung-Scoring at Slaughter. Animals, 2021.

Table 1

Table1: *Production parameters: days till slaughter, ADG and mortality rate compared between groups.*

Group	Pigs at slaughter n	Age at slaughter days		ADG gram bodyweight/day		Mortality rate %	
			Δ vs best.		*Δ vs best.*		*Δ vs best.*
A	845	176.7	*+6.1*	637.8	*-28.4*	6.7	*+2.5*
B	825	182.9	*+12.3*	615.1	*-51.1*	8.3	*+4.1*
C	635	175.9	*+5.3*	622.3	*-43.9*	9.9	*+5.7*
D	1020	**170.6**		**666.2**		**4.2**	

Table 2

Table2: *Lung lesion scores compared groups*

Group	Severity index of lung lesions APPI		Prevalence of lung lesions % Dorsocaudal pleurisy	
		Δ vs best		*Δ vs best*
A	1.07	*+0.94*	47	*+40*
B	1.20	*+1.07*	40	*+33*
C	1.33	*+1.20*	53	*+46*
D	**0.13**		7	

P-B-074

QUALITY OF THE EMULSION AFTER MIXING PCV2 AND M.HYO INACTIVATED VACCINES

Mr. Roman Krejci[1], K Toth[2], L Naszalyi-Nagy[2], P Mazerolles
[1]*Ceva*, [2]*Ceva Phylaxia*

Introduction
Porcine circovirus diseases (PCVD) and Enzootic pneumonia (EP) remain the most widely spread diseases in swine industry due to PCV2 and M.hyo. Vaccines against PCV2 and M.hyo are the most frequently used biologicals for pigs. Circovac® (C) and Hyogen® (H) (both Ceva) are vaccines licensed for ready-to-mix use in piglets (DUO TM). Mixing them prior to the administration facilitates one shot injection of both antigens. The aim of the study was to assess and present the physical properties of the mixed emulsion ready to be injected.

Material and Methods
Vaccines were reconstituted and mixed according to the manufacturer's instructions, that means first Circovac® was reconstituted in its own adjuvant and then mixed with Hyogen®. The distribution of particle size was measured by Malvern Mastersizer 3000. Viscosity was measured by Brookfield viscosimeter (S00 spindle, 25 °C, 60 rpm stirring speed). Syringeability was determined by measuring the flow-out time of 10 ml vaccine pressed through a 21G needle under the vertically acting force of 3,3 kg a weight.

Results
Particle size distribution of mixed emulsion showed a narrow profile very similar to the profile of each particular vaccines before mixing. There was no population of big particle size.
The characteristics of the mixture remain unchanged after 7 days. The viscosity of the final emulsion was 2,1 compared to 5,2 and 2,0 for C and H vaccines respectively (all values in centipoise).
Regarding syringeability, time to pass 10ml of C, H and DUOTM vaccines through the needle was 6,2; 4,8 and 4,9 sec.

Conclusion
Mixing those two vaccines together in the recommended ratio provides a homogenous, smooth and stable emulsion. The physical properties demonstrated that DUOTM is easy to be used for the injectable administration also in the field condition for the mass vaccination of piglets.

Table 1

Tab 1. Viscosity of tested vaccines (centipoise)	
Circovac®	5,2
Hyogen®	2,0
Circovac®+Hyogen® RTM (DUO TM)	2,1

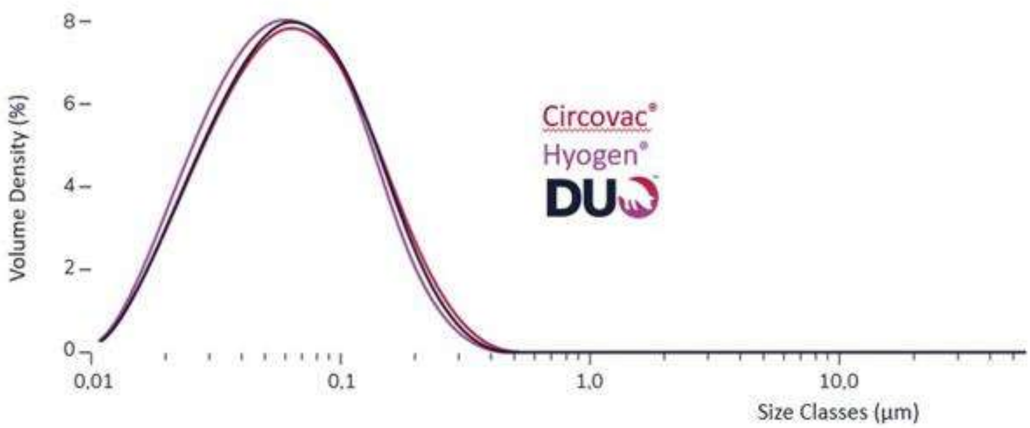
Table 2

Tab 2. Syringeability of tested vaccines (seconds)

Circovac®	**6.2±0.1**
Hyogen®	4.8±0.1
Circovac®+Hyogen® RTM (DUO TM)	4.9±0.1

Graphs or Images 1

Fig 1. Particle size distribution



P-B-075

QUANTIFICATION OF TIGHT JUNCTIONS PROTEINS GENE EXPRESSION AFTER TOLTRAZURIL PARENTERAL ADMINISTRATION

Dr. Daniel Sperling[1], Dr. Francisco Murciano[2], Dr. Ana Gonzalez-Guijarro[2], Dr. Elisa Hernández-Rodríguez[2], Dr. David Espigares[1], Dr. Daniel Serrano[3], Dr. Eva Llamas[2], Prof. Guillermo Ramis[2,3]

[1]Ceva Sante Animale, France, [2]Departamento de Producción Animal. Facultad de Veterinaria. Universidad de Murcia. Spain, [3]Departamento de Sanidad Animal. Facultad de Veterinaria. Universidad de Murcia. Spain, [4]Instituto Murciano de Insvestigación Biomédica (IMIB). Murcia. Spain

Introduction:
Cystopisospora suis is a parasite that infests piglets in industrial production very frequently. Replication of the parasite in the epithelium causes damage resulting in a yellowish pasty diarrhoea. Although efforts have been made to control the parasite for several decades, it remains in populations and continues to be a problem in farrowing rooms. The aim of this study was to determine the differences in gene expression of three tight junction proteins (TJ) in animals administered parenterally with toltrazuril compared to a control group.

Material and methods:
A total of 162 Duroc piglets were randomly included in two groups: the Forceris® (Ceva Sante Animale, France) group (N=81) received 1.5 mL of this drug containing 45 mg toltrazuril and 200 mg iron per piglet. A control group (n=81) was set up, which received dextran iron parenterally but not coccidiostat by any route. Gene expression was determined for three tight junction proteins (Zonulin, Occludin and Claudin) in faeces using a previously described technique (Ramis et al., 2022), comparing both groups. Samples were taken at weaning and at the end of transition.

Results:
Differences were found in Ocluddin (p=0.001) and trend for Zonulin (p=0.079) at the end of the prestarter, in favour of the Forceris® group (Figure 1), while at the end of the transition, the control group had higher quantification for Claudin (p=0.035) with no differences in the other proteins. When doing a related samples analysis (Figure 2), there was an increase in quantification when comparing end of prestarter and end of transition for Ocludin (p=0.017) and a trend in Zonulin (p=0.066) in the control group. There were no differences in Forceris® group.
The quantification of occludin and zonulin does not vary in the Foreris group, while both are increased in the control group, which had a lower quantification at the end of the PRE period, indicating a recovery of integrity that is not necessary in the Forceris® group.
Figure 1: Relative gene expression for occluding and zonulin at the end of prestarter (weaning+15 d)
Figure 2: Violin plot for gene expression of Occludin and Zonulin comparing both periods

Conclusion:
The use of Forceris resulted in better intestinal integrity as showed the gene expression for Ocludin and Zonulin. The fact that there were no differences for TJ proteins in Forceris® groups comparing prestarter and end of nursery suggests a better initial TJ status.
References:

Ramis, G.et al., Oral and Parenteral Vaccination against Escherichia coli in Piglets Results in Different Responses. Animals 2022, 12, 2758.

Figure 1

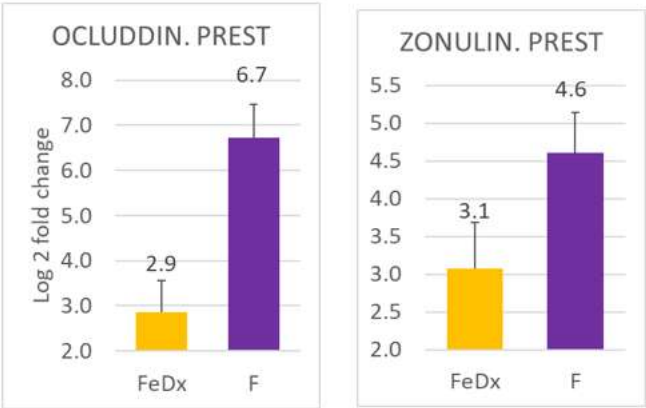
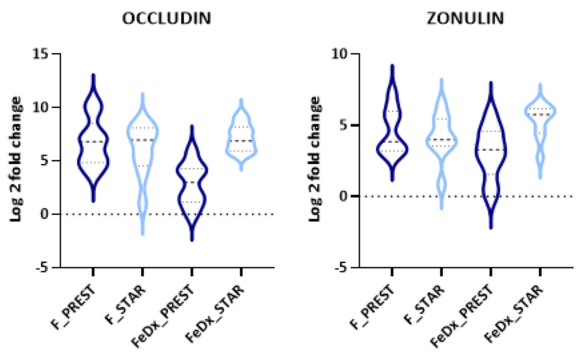


Figure 2



P-B-077

RECOMBINANT VEROTOXIN 2E (VT2E) VACCINE REDUCES THE COST OF MEDICINE ON A FARM WITH SUBCLINICAL SWINE OEDEMA DISEASE

Mr. Gonzalo García Sánchez[1], Mr. Hyundong Noh[2], Mr Kyohyun An[2], Mr Jeongjae Byun[2], Mr. Sangwon Seo[2], Mr Emili2 R. Barba[1]
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Introduction

Swine oedema disease is caused by verotoxin 2e (Vt2e), a toxin that induces systemic degenerative angiopathy. Swine oedema disease can be classified into three categories: clinical, chronic and subclinical forms. Compared to the clinical and chronic forms, which can be accompanied by sudden death or neurological signs, it is not easy to recognize subclinical swine oedema disease which only shows non-specific manifestations such as delayed growth and decreased uniformity. At the same time, it is not easy either to evaluate the benefits of controlling subclinical swine oedema disease. The objective of this study was to assess the reduction in economic losses resulting from vaccination against Vt2e by analysing the cost of medicine before and after administration of the vaccine.

Materials and Methods

A Korean commercial swine farm with 850 sows (farrow to finish) was confirmed as a verotoxin-producing Escherichia coli (VTEC)-positive farm through prior testing by real-time PCR assay. Despite PCR positive results for VTEC from all samples at different ages of the animals, no sudden deaths or neurologic signs were observed, which implied that the farm could suffer a subclinical form of swine oedema disease. The health status of the herd was not very good due to the continuous impact of various respiratory and digestive diseases, which caused huge economic losses to the farm. To control swine oedema disease which could aggravate the situation of the farm, a vaccine with recombinant Vt2e antigen (VEPURED®) was applied from July 2021 onwards, in accordance with the manufacturer's instructions. Information was collected on the cost of the medicine to the farm for two years (from March 2020 to March 2022). The periods from March 2020 to June 2021 and from September 2021 to March 2022 were defined as "Prevaccination" and "VEPURED®", respectively. The period from July to August 2021 were defined as "transition" which meant the time needed for the vaccinated piglets to generate immunity. Expenses considering all costs for medicine were defined as "Total". Amongst these, treatment costs that can be variable depending on the herd's health status were classified into three categories: "Injectable antibiotics", "Antibiotics in feed" and "Additives". For each category, the fluctuation of monthly cost over time and average monthly cost by period were calculated. For statistical analysis, Wilcoxon tests were performed. Herd size throughout the whole period was stable.

Results

Comparing the "Prevaccination" period to the "Vepured®" period, the average monthly cost for the "Injectable antibiotics" category decreased by 46% with and "Additives" altogether, the average monthly cost decreased by 22%, from 24.27 million KRW to 19.01 million KRW showing significant differences (P-value=0.02).

Table 1. Average monthly costs by category

Fig 1. Monthly cost of all antibiotics and additives over time

Fig 2. Average cost of all antibiotics and additives by period

Discussion and Conclusion
Even though there is no mortality, subclinical swine oedema disease can still induce economic losses on the farm. Vaccination with recombinant Vt2e antigen can effectively prevent losses caused by subclinical swine oedema disease by reducing the cost of therapeutic treatments. To estimate the benefit of controlling subclinical swine oedema disease more accurately, further study of other factors such as decreased days to market and increased weight gain will be needed.

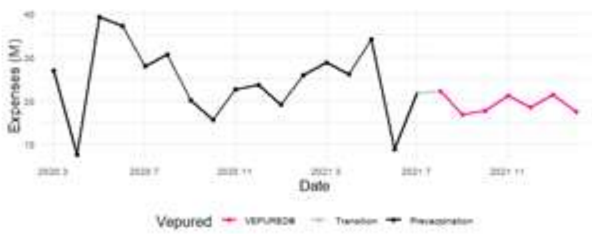
Acknowledgments
This work was supported by Hipra Korea, Inc., Republic of Korea.

References
1 Casanova, N.A., et al.,2018, Overview of the role of Shiga toxins in porcine edema disease pathogenesis, Toxicon.
2.-Do, Kyung-Hyo, et al., 2019, J Vet Sci, 20(1), 43-5.

Table 1

Monthly expenses (Unit : millions KRW)	Prevaccination	VEPURED®	P-value
Antibiotics in feed	116	104	0.32
Injectable Antibiotic**	**502**	**269**	**0.006**
Additives	190	261	0.17
Additives & all antibiotics*	**809**	**634**	**0.02**
Total	1,556	1,532	0.21

Graphs or Images 1



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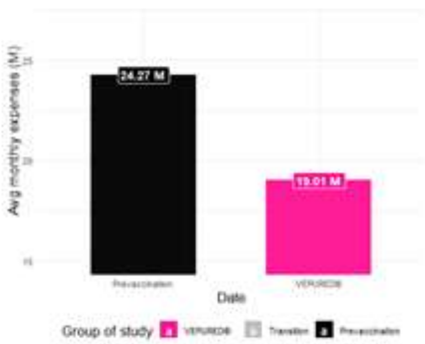

P-B-078

REDUCTION IN ANTIBIOTIC USAGE DUE TO THE COMBINATION OF VITASEM Z AND DICOL

Mr. Adrian Soria, Mr Juan Jose Mena[1], Mrs Noelia Mendoza[1], Mrs Ursula Alvarez[1], Mrs Raquel Ausejo[1]
[1]*Magapor SL*

Introduction.
Seminal doses contamination is known to be frequent so broad-spectrum antibiotics are added to the extenders. Bacterial antibiotic resistance has increased from the past few years, for that reason it is intended to reduce the use of antibiotics to decrease the antibiotic resistance. Dicol is a semen collection medium, which contains a mixture of bactericidal antibiotics, effective against a wide range of bacteria. It is used to preventatively treat porcine semen with antibiotics and limit bacterial contamination before dilution with regular extenders. Seminal doses are usually produced with extenders containing antibiotics such as Vitasem. We aim to try Vitasem Z an antibiotic-free medium for semen extension, which allows to diminish antibiotics in the final dilution by 35.4% when using this extender in combination with Dicol. The objective of the present study is to evaluate if the combination of Dicol and Vitasem Z can mimic regular extender conditions, which are no bacterial contamination and maintenance of semen quality.

Material and methods.
In the present study ejaculates from eight boars were used. Semen was collected with Dicol, afterwards it was diluted 1:10 with Vitasem (control) or Vitasem Z and stored in a cold chamber (16.5ºC). Each extender treatment was analyzed with at least two replicates of the same boar ejaculate. The samples were examined at days 1, 3, and 6 to assess the quality of the spermatozoa. Total motility, progressive motility, short hypoosmotic swelling test, and osmotic resistance test were carried out. Additionally, a microbiological test was run to determine the degree of contamination in the doses. ANOVA with least squares test was performed to analyze the effect of the treatment and day in the evaluated variables.

Results.
Throughout the evaluation days, all the measured parameters were found to be within the ranges of acceptability for the two treatments (Total motility>70, progressive motility>50, short hypoosmotic swelling test>50% and osmotic resistance test>70%). Total motility was high for both treatments, 91.6, 90.3, 88.7 and 88.9, 87.6, 86.0, control and Vitasem Z respectively. Progressive motility was similar between the two treatments. There were not significant differences among extenders for short hypoosmotic swelling test (77.5 % control and 76.5% Vitasem Z, $p>0.05$) and osmotic resistance test (82.0% control and 82.3% Vitasem Z; $P>0.05$). A microbiological test was done to see whether the treatment's effects were comparable to that of extender with higher antibiotic concentration. Both extenders can restrain bacterial development, keeping the Colony Forming Unit (CFU) at the lowest level, suggesting that both treatments maintain the Colony Forming Unit at a minimum.

Conclusions.
To conclude, the seminal quality of Vitasem Z and Dicol and Vitasem (control) extenders was similar. Furthermore, both treatments kept Colony Forming Units and bacterial contamination at their lowest levels. Vitasem Z and Dicol combination enables a 35.4% reduction in antibiotic usage while maintaining seminal quality.

Table 1

Table 1. Extender samples microbiological analysis.

CFU/mL	Day			
	0	1	3	6
Control	10	10	10	10
Vitasem Z + Dicol	10	18	10	10

<300 CFU/mL indicates there is no harmful bacterial contamination.

P-B-079

SAFETY AND EFFICACY OF PCV2 AND MYCOPLASMA HYOPNEUMONIAE (M.HYO) VACCINES MIXED TOGETHER IN THE PROTECTION AGAINST EXPERIMENTAL INFECTIONS.

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[1]Ceva, [2]Ceva Phylaxia

Introduction
PCVD (Porcine Circovirus Diseases) and enzootic pneumonia (EP) remain a major health problem in most swine farms. Different mono-valent or combined vaccines are available on the market. The aim of the study was to assess the safety and efficacy of PCV2a and M.hyo vaccines mixed prior to use (ready-to mix RTM) against PCV2 and M.hyo experimental infections.

Material and Methods
Circovac® (Ceva) and Hyogen® (Ceva), one dose each, were mixed in one vial (DUO) before the administration. In the laboratory safety study, 10 piglets were vaccinated with 2.5ml of DUO and 10 piglets with PBS. Local and systemic reactions were observed for 14 days.
In the efficacy studies piglets were vaccinated with DUO at 3 weeks of age (WOA) or not vaccinated as controls. Animals were infected intranasally at 26WOA with either PCV2a isolate or with M.hyo strain. Pigs were always necropsied 4 weeks post infections. PCV2 virus loads by qPCR and M. hyo lung lesion (LLS) (European Pharmacopoeia) were measured to assess the efficacy.

Results
Safety: no piglet had a fever and the average body temperature was 40.0 °C in vaccinates and 38.9°C in controls. No significant difference in and ADG (0.32 vs 0.33kg/day vaccinates vs controls) were found.
Efficacy: The mean PCV2 viral loads (log10 copies/ml) in different lymph nodes were 2.5-3.1 for group DUO and 4.3-5.3 for the controls ($p<0.05$).

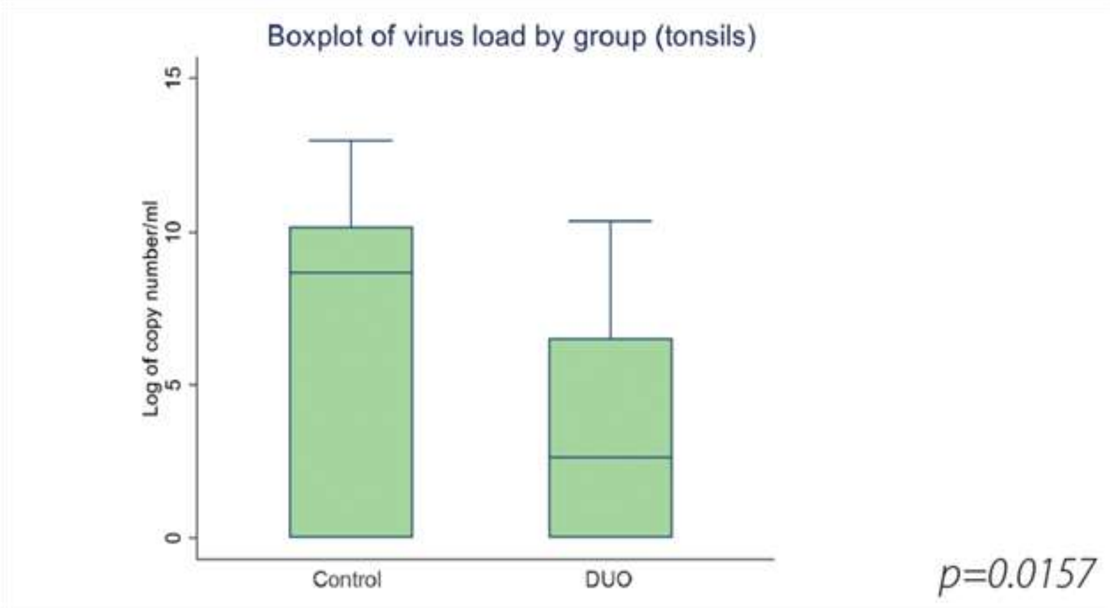
Fig 1. PCV2 PCR loads in tonsils

In terms of M.hyo efficacy, the mean weighted LLS were 33.2 for DUO group and 72.3 for the control. The distribution of the weighted lung lesion scores is shown in Figure 2. Lung scores were significantly different between groups (p= 0. 0110, Wilcoxon rank-sum test).

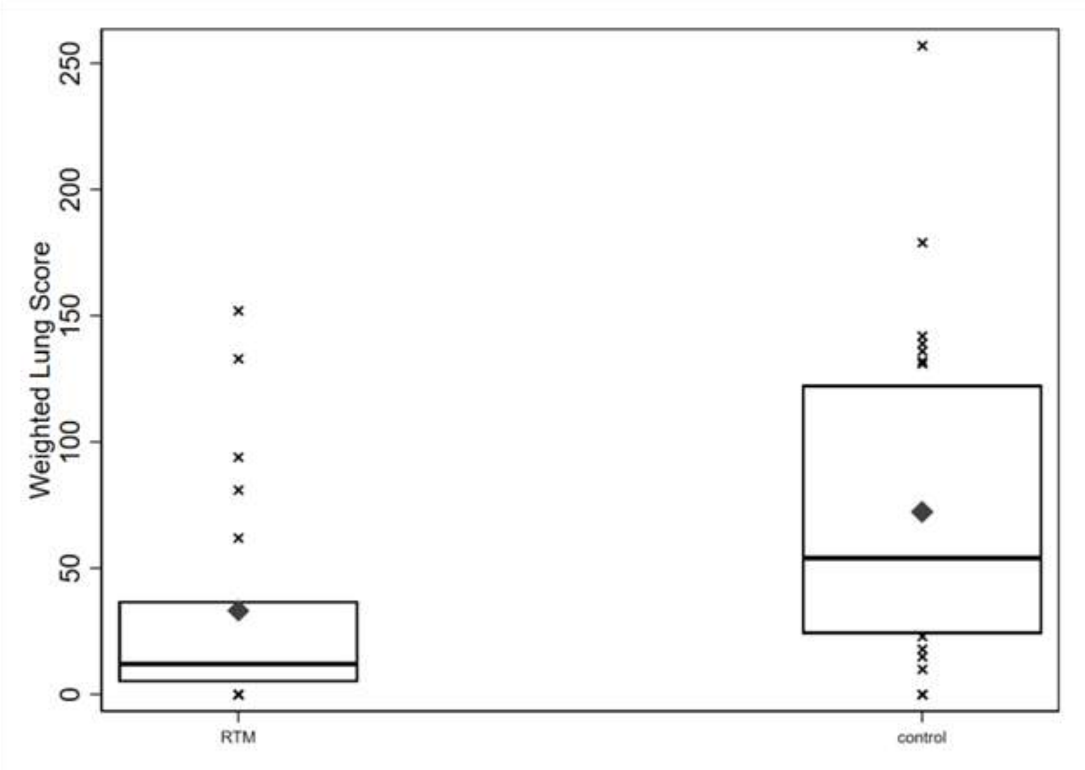
Fig 2. Weighted lung lesion scores

Conclusion
This study confirmed that Hyogen® mixed with Circovac® as RTM (DUO TM) into one dose was safe for piglets. The duration of protection after vaccination for 23weeks was demonstrated against the experimental infection with PCV2 as well as against M.hyo. DUO TM proved to be a safe and highly efficient tool in the protection against PCVD and EP as the RTM product.

Graphs or Images 1



Graphs or Images 2



P-B-080

SAFETY OF THE SIMULTANEOUS ADMINISTRATION OF A PCV2 VACCINE AND A MULTIVALENT RESPIRATORY VACCINE

Dr. Eric Bousquet[1], Dr Xuan Doan[2], Dr Hung Bui[2], Dr Vo Duc Dao[3]
[1]Virbac, [2]Virbac, [3]Tho An hamlet

Introduction
Combined vaccination against different pathogens is increasing for piglets in order to protect them at the same time against these pathogens and to reduce the number of animal handlings. This study was performed to assess the safety of two vaccines (a PCV2 vaccine and a multivalent respiratory vaccine) when injected separately on the same day in piglets at 3 weeks of age. Safety and efficacy of both vaccines have been reported when administered alone in Asia and Latin America (1-2-3-4-5).

Materials and methods
The study took place in a farrow to finish farm owning 400 sows in Vietnam. Forty piglets of 3 weeks of age were allocated to 4 groups of 10 piglets each (5 male and 5 female piglets per group). These piglets came from 6 sows of parities P3 or P4, each group containing the same number of piglets from each sow. The control group received an intramuscular (IM) injection of a saline serum on each side of the neck (1 ml per injection). Another group received a single IM injection of a PCV2 vaccine (Suigen® PCV2, Virbac, 1 ml). A third group received a single IM injection of a multivalent respiratory vaccine (Suigen Donoban® 10, 1.5 ml). This vaccine contains bacterins and toxoids of Pasteurella multocida, Bordetella bronchiseptica, Actinobacillus pleuropneumoniae and bacterins of Streptococcus suis, Haemophilus parasuis and Mycoplasma hyopneumoniae. The fourth group received a single IM injection of the PCV2 vaccine (1 ml) and a single IM injection of the multivalent vaccine (1.5 ml). All injections were made in the neck (on opposite sites for the group receiving the two vaccines), after warming the products at ambient temperature. Piglets were then individually examined daily during 7 days according to a standardized scoring for general and local reactions. The rates of post injection reactions were compared between groups by the Fisher's exact test.

Results
Piglets body weight ranged between 5.7 kg and 6.5 kg on the day of injection. No local reaction was noticed at injection site whatever the group. The only general reaction observed was a transitory shock for one piglet receiving both vaccines on the day of injections. This piglet behaviour returned to normal status from one day after vaccination, without any complementary treatment. The post injection reaction rates were not significantly different between this group and the control group (p=1).

Conclusion
Both vaccines were well tolerated when administered alone or at the same time, with only one reversible general reaction for one day in one piglet receiving both vaccines. The simultaneous injection of both vaccines in separate injection sites from 3 weeks of age could be a valuable approach to protect piglets against PCV2 and bacterial respiratory infections, provided that further studies confirm the field efficacy of this vaccination program.

References
1. Aguirre LM et al IPVS 2016.

2. Bousquet E et al APVS 2019.
3. Oh Y et al APVS 2019.
4. Bousquet et al IPVS 2020.
5. Bousquet et al IPVS 2022.

P-B-081

SEROLOGICAL MONITORING OF MYCOPLASMA HYOPNEUMONIAE VACCINATION UPTAKE USING A NEW ELISA KIT

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[1]Ceva, [2]IDvet – Innovative Diagnostics, [3]SSIU Ceva-Phylaxia

Introduction
Mycoplasma hyopneumoniae (Mhyo) is the primary pathogen of Enzootic pneumonia in pigs. Humoral response to the infection can be measured by different available types of ELISA (1). The aim of this study was to assess the potency of different Mhyo vaccines using serology and propose the optimal sample testing for semi-quantitative evaluation.

Material and Methods
In total, 250 samples from pigs vaccinated with Hyogen® (H), (Ceva) or vaccines A and B were analyzed using the ID Screen® Mycoplasma hyopneumoniae Competition ELISA (cELISA) test (IDvet).
To highlight post vaccination monitoring and immune response, several serial dilutions of positive vaccinated sample were tested. Standardized values were calculated and the percentage of positive samples for each dilution was compared among differently vaccinated groups of pigs. Positivity at dilutions 1:1 and 1:5 (out of positives in dilution 1:1) were evaluated to better objectivate seroconversion.

Prevalence of true positive responders was determined as follows:
- Number of positive (nP dil1) samples at the dilution 1:1 (S/N % ≤ 50%) was counted
- Prevalence Index (Prev. Index) value of true positive responders was calculated :

Magnitude of the humoral immune response.
Among the positive samples population obtained by the nP dil1
- Determine the number of positive (nP dil2) samples at the dilution 2: (S/N % ≤ 50 %)
- Calculate the Magnitude Index (Mag. Index) value to estimate the magnitude of the humoral immune response

Results
With only two dilutions per sample, it is possible to assess a semiquantitative interpretation. The first dilution (1:1) measures the overall immune response in a population, and the second dilution (1:5) determines the strength of the immune response. Analysis with the Mhyo cELISA allows to measure the seroconversion with pigs' samples vaccinated with vaccine H (79% and 54% positivity in dilutions 1:1 and 1:5). Such strong seroconversion is not observed with other commercial vaccines (45%, 32% in dilution 1:1 and 16%, 0% in dilution 1:5 for vaccines A and B).

Tab 1. Prevalence and Magnitude index for different vaccines

Vaccine	Prev. Index (nP dil1/n)	Mag. Index (nP dil2/nP dil1)
Hyogen and Hyogen/Circovac	0,79	0,54
Vaccine A	0,45	0,16
Vaccine B	0,32	0,00

Conclusion
Based on available results analyzing blood serum samples in dilutions 1:1 and 1:5 can be proposed. This allows not only to define the prevalence of true positive responders but also to estimate the magnitude of the humoral immune response. In this study Hyogen® provided stronger serological response than other tested vaccine. The IDvet Mhyo cELISA can be potentially used for this vaccine uptake monitoring.

Bibliography
Thacker E.L., 2004; JSHP, 12, 5

P-B-082

SEROTYPE DISTRIBUTION AND ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF GLAESSERELLA PARASUIS ISOLATED FROM DISEASED PIGS IN CENTRAL-SOUTHERN REGION OF TAIWAN

Prof. Hung-Chih Kuo[1], Ms. Chia-Yu Hsu[1], Prof. Chao-Min Wang[1], Prof. Szu-Wei Huang[1], Prof. Ching-Fen Wu[1]
[1]*National Chiayi University*

Introduction

Glaesserella parasuis (GP) is Gram negative bacillus and belongs to family Pasteurellaceae. GP infection causes Glasser's disease and diseased pigs show fever, coughing, swollen joints with lameness and symptoms of central nervous system. Polyserositis with fibrin is the main gross lesion observed during necropsy [1]. Antimicrobial agents are commonly used against GP infection; however, occurrence of antimicrobial resistance results in failure of treatment. In this study, we aim to investigate serotypes and antimicrobial susceptibility of GP in Taiwan. The data will provide veterinarians with useful information in treating GP infection.

Materials and Methods

A total of 333 isolates of GP was collected from diseased pigs at Yunlin-Chiayi-Tainan of Animal Disease Diagnostic Center from January 2013 to July 2021. Isolation sites included lung, bronchus, pleural effusion, pericardial effusion, ascites, synovial fluid and cerebral spinal fluid. Most isolates were isolated from 4 to 9 weeks-old pigs. Multiplex polymerase chain reaction and broth microdilution test were performed to identify the serotype and minimum inhibitory concentration (MIC). MIC breakpoint was checked following the guideline of Clinical and Laboratory Standard Institute [2].

Results

The percentage of serotype 1, 4, 5, 6, 7, 9, 11, 12, 13, 14 were 1.8%, 35.1%, 22.8%, 0.3%, 2.7%, 1.8%, 0.3%, 6.6%, 10.2% and 5.1%, respectively. Besides, 44 isolates were non-typable (Table 1). The resistance of penicillin, amoxicillin, ceftiofur, clarithromycin, florfenicol, tiamulin, sulfamethoxazole-trimethoprim and enrofloxacin were 30.6%, 74.8%, 1.8%, 61.9%, 7.8%, 12%, 84.4% and 72.7% (Table 2).

Conclusions and Discussion

The prevalent serotypes of the GP strains isolated in the diseased pigs in Taiwan were mainly serotype 4 and 5, followed by 12 and 13, which are consistent with the research reported in other countries. The current commercial vaccines exert protective effects on the prevalent serotypes, but the efficacy may be reduced in the outbreaks from strains with other serotypes. Multi-drug resistant strains are common in this study; nevertheless, ceftiofur and florfenicol could be chosen for treatment.

Acknowledgement

Many thanks to the Animal Disease Diagnostic Center of National Chiayi University for providing the bacteria, and professor Kuo and seniors for supporting the MIC test.

References

[1] Aragon et al., 2019. Diseases of swine, 11th ed, 844-853.
[2] Clinical and Laboratory Standards Institute (CLSI), 2022. CLSI M100.

Table 1

Table 1 The isolate number of Glaesserella parasuis serovars in different isolation sites.					
Isolation site / Serotype	Lower respiratory tract	Synovial fluid	Body cavity fluid	Cerebrospinal fluid	Total
1	2	1	2	1	6
4	92	13	10	2	117
5	47	21	7	1	76
6	1	0	0	0	1
7	6	1	2	0	9
9	1	3	1	1	6
11	0	0	1	0	1
12	14	4	3	1	22
13	20	8	4	2	34
14	14	2	1	0	17
NT[a]	30	5	8	1	44
Total	227	58	39	9	333

[a]: non-typable

Table 2

Table 2 MIC distribution profile of GP isolates from January 2013 to July 2021 (n = 333).

Antimicrobial agents	MIC_{50}	MIC_{90}	Resistance rate (%)
penicillin	0.25	8	30.6
amoxicillin	4	256	74.8
ceftiofur	0.125	0.5	1.8
clarithromycin	64	128	61.9
florfenicol	1	4	7.8
tiamulin	4	32	12
sulfamethoxazole-trimethoprim	16	512	84.4
enrofloxacin	2	8	72.7

P-B-083

SHIGA TOXIN STX2E PRODUCED BY STEC E.COLI STRAINS INHIBITS PORCINE IMMUNE CELLS UNDER THE IN-VITRO CONDITIONS

Dr. Daniel Sperling[1], Dr. Hana Stepanova[2], Dr. Anne-Kathrin Diesing[1], Dr. Han Smits[1], Dr. Martin Faldyna[2]

[1]Ceva Sante Animale, France, [2]Veterinary Research Institute, Czech Republic

Introduction:
Oedema disease (OD) is one of the most important pathologies, causing significant losses in nursery piglets. There is also subclinical form of OD which was described under the challenge trial set up, but also under the field conditions of the commercial farms. Stx2e toxin, produced by Shiga-like toxigenic Escherichia coli, is considered a main factor of virulence. Stx2e toxin damages endothelial cells, the histological hallmark of diseases. However, there is growing evidence that Stx's also target immune cells. The aim of the presented study was to assess in vitro effect of different concentrations of Stx2e toxin on piglet immune cells.

Material and Methods:
Porcine peripheral blood mononuclear cells isolated by gradient centrifugation were pre-incubated with Stx2e at three different concentrations: 10, 500 and 5000 CD50/ml or with negative control (E. coli K12 AG). Cells were then stimulated with polyclonal mitogens: concanavalin A, phytohemagglutinin, pokeweed mitogen or lipopolysaccharides. Cell proliferation was assessed by BrdU incorporation into newly created DNA according to manufacture recommendation.
CD25 was assessed by flow cytometry. Percentage of CD25 positive cells were evaluated for particular subpopulations defined as CD3+CD4+CD8+/-, CD3+CD4-CD8+, CD3+γδTCR+ and CD3-sIgM+.

Results:
The toxin significantly decreased mitogen-driven proliferation activity in cultures with all mitogens. Effect of Stx2e was partially dose-dependent. The toxin has significant impact on both T and B populations. Percentage of CD25+ cells was slightly lower in the presence of Stx2e on all defined T cell subpopulations (CD4+, CD8+, and γδTCR+) in dose-dependent manner. B cells defined as sIgM+ seemed to be the most affected populations by Stx2e and the toxin induced significant decrease of percentage of IgM+CD25+ cells.

Conclusions:
Stx2e had negative effect on proliferating activity of both populations of porcine lymphocytes. Described negative effect of different concentrations of Stx2e on immune cells may particularly explain negative impact of subclinical course of OD. The toxin had significant negative impact of cell viability in all types of culture, showing significantly lower percentage of live cells with minor dose-dependent relationship. Further research needs to be performed in order to understand such effect in-vivo.

Tab 1: Proliferation of particular lymphocyte subsets assessed by EdU incorporation and flow cytometry

Table 1

		CD4+ cells					CD8+ cells					gdTCR+ cells					IgM+ cells				
		ctrl neg	ctrl 5000	Stx 10	Stx 500	Stx 5000	ctrl neg	ctrl 5000	Stx 10	Stx 500	Stx 5000	ctrl neg	ctrl 5000	Stx 10	Stx 500	Stx 5000	ctrl neg	ctrl 5000	Stx 10	Stx 500	Stx 5000
ConA	mean	1	1,06	0,27	0,18	0,09	1	1,18	0,50	0,38	0,26	1	0,98	0,50	0,40	0,25	1	1,30	0,22	0,22	0,13
	S.D.	0,00	0,29	0,17	0,10	0,07	0,00	0,58	0,38	0,35	0,22	0,00	0,16	0,12	0,14	0,08	0,00	0,91	0,15	0,14	0,12
PHA	mean	1	0,92	0,41	0,27	0,11	1	1,01	0,62	0,43	0,22	1	1,02	0,59	0,48	0,25	1	2,02	0,44	0,39	0,47
	S.D.	0,00	0,23	0,19	0,10	0,04	0,00	0,07	0,04	0,04	0,03	0,00	0,12	0,08	0,02	0,06	0,00	0,43	0,16	0,18	0,27
PWM	mean	1	1,02	0,35	0,29	0,20	1	0,93	0,55	0,47	0,37	1	1,08	0,61	0,57	0,40	1	1,21	0,31	0,32	0,25
	S.D.	0,00	0,15	0,06	0,06	0,03	0,00	0,15	0,17	0,12	0,10	0,00	0,25	0,17	0,16	0,07	0,00	0,13	0,14	0,20	0,11
LPS	mean	1	1,26	0,59	0,55	0,52	1	0,90	0,28	0,16	0,10	1	1,05	0,32	0,31	0,18	1	1,24	0,11	0,06	0,05
	S.D.	0,00	0,22	0,31	0,36	0,42	0,00	0,25	0,11	0,09	0,10	0,00	0,20	0,23	0,23	0,16	0,00	0,22	0,06	0,02	0,03
NS	mean	1	1,06	0,45	0,41	0,46	1	1,86	0,89	0,54	0,15	1	3,87	1,57	1,22	0,63	1	6,28	0,29	0,23	0,34
	S.D.	0,00	0,30	0,20	0,35	0,43	0,00	0,85	0,58	0,45	0,22	0,00	2,29	1,27	0,75	0,38	0,00	1,49	0,13	0,10	0,29

P-B-084

SPECIFIC REAL-TIME PCR ASSAY TO SURVEY CYSTOISOSPORA SUIS INFECTIONS OF PIGLETS IN CHINA FARMS

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[1]Ceva Sante Animale, [2]Ceva SSIU, [3]Ceva Sante Animale

Background
Cystoisospora suis is an important coccidial protozoan parasite causing varying degrees of diarrhea in young piglets resulted in economic loss. Diagnostics and counting parasite oocysts in piglet feces with microscopy by flotation is a traditional method to identifying pathogens. Specific species real-time qPCR assay based on target pathogen gene sequence analysis is a new technology which is more sensitive method helping pig farms to find sub-clinical infection. This study shows results of an epidemiological survey of coccidiosis infection of piglets in China by the new PCR method.

Materials and methods
From January to October 2022, 380 litters of 7-21 days old piglets' feces from 24 industrial pig farms in different regions of China was randomly sampled. Coccidial DNA was extracted using Power Fecal DNA Kit (QIAGEN). Parameters of mechanical disruption and enzymatic lysis had been optimized. Mitochondrial ribosomal ITS region has been used for detection of coccidians (1).

Result
91.7% of farms was positive and 35.8% of total samples were positive (Figure 1). The positive rate of samples from the surveyed 24 farms ranged from 0-100%, and of which the positive rate of samples from 8 farms exceeded 50% (Figure 2).

Conclusions and Discussion
The coccidiosis infection rate of piglet is very high in Chinese pig farms. The developed qPCR assay provides a specific and sensitive tool for detection and quantification of Cystoisospora suis for veterinaries to take necessary measures.

Acknowledgement and References

(1) Samarasinghe B ,et al. Phylogenetic analysis of Cystoisospora species at the rRNA ITS1 locus and development of a PCR-RFLP assay[J]. Experimental Parasitology, 2008, 118(4):592-595.

Graphs or Images 1

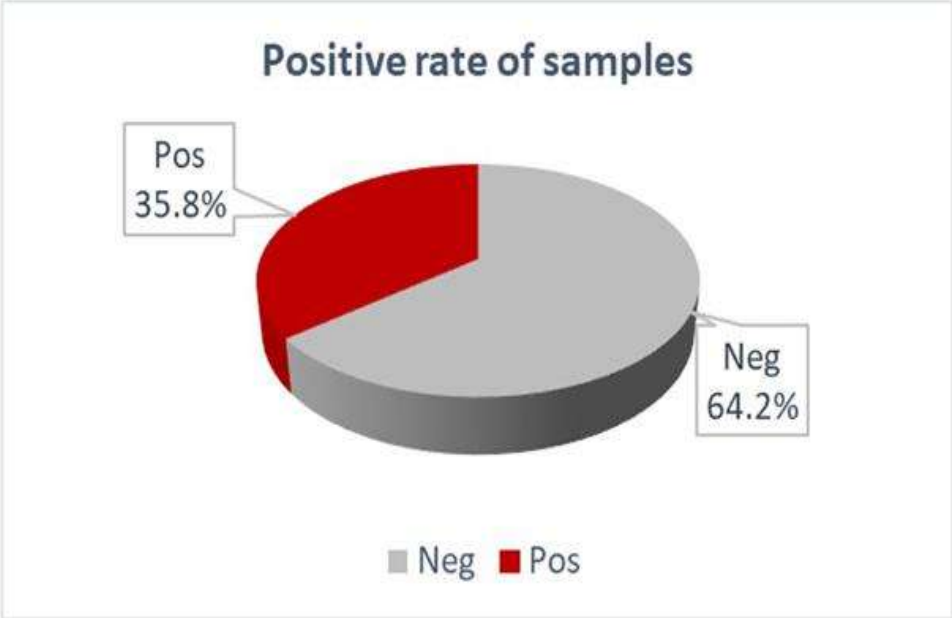

Figure 1 Positive rate of samples

Graphs or Images 2

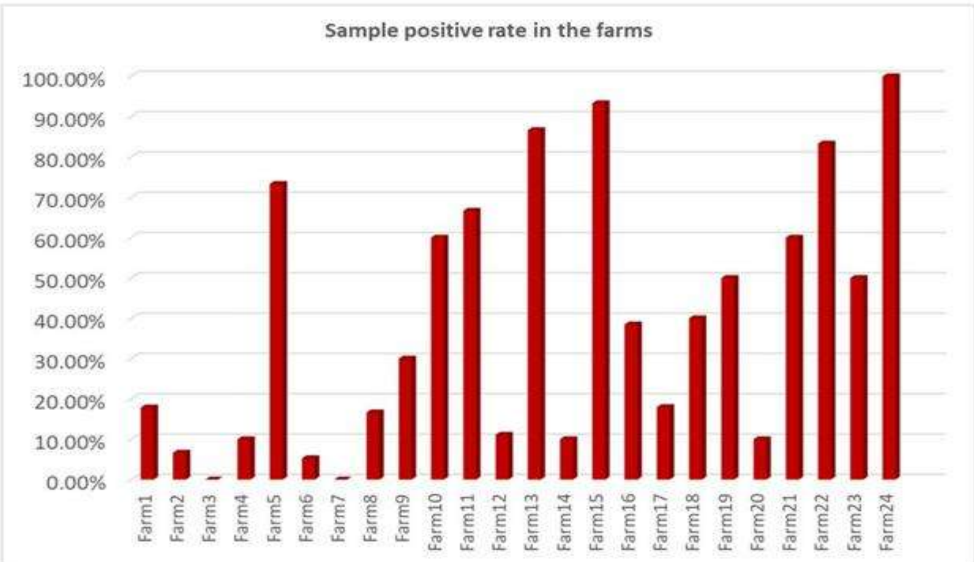

Figure 2 Sample positive rate in the farms

P-B-085

STUDY OF MULTILOCUS SEQUENCE TYPING OF GLAESSERELLA PARASUIS FROM DISEASED PIGS IN CENTRAL-SOUTHERN REGION OF TAIWAN

Prof. Hung-Chih Kuo[1], Ms. Chia-Yu Hsu[1], Prof. Chao-Min Wang[1], Prof. Szu-Wei Huang[1], Prof. Ching-Fen Wu[1]
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Introduction
Glaesserella parasuis (GP) belongs to normal microbiota of the upper respiratory tract of pigs, but virulent strains cause systemic infection commonly known as Glässer's disease that leads to significant economic losses in the swine industry. To date, 15 serovars of GP have been recognized. Serovar has been commonly used as an indicator of virulence. However, it is observed that virulence of field isolates with the same serotype can be significantly differ; therefore, analysis of genotypes is considered for characterization of GP population. In this study, multilocus sequence typing (MLST) is used to investigate the epidemiology of GP population in Taiwan.

Materials and Methods
A total of 333 isolates was collected from diseased pigs at Yunlin-Chiayi-Tainan of Animal Disease Diagnostic Center from January 2013 to July 2021. Isolation sites included lung, bronchus, pleural effusion, pericardial effusion, ascites, synovial fluid and cerebral spinal fluid. Serotyping was performed by polymer chain reaction (PCR) according to the literature [1]. Based on the serotype distribution, we selected 87 isolates for MLST and chose seven housekeeping genes, including atpD, infB, mdh, rpoB, 6pgd, g3pd and frdB, as potential target genes for the MLST assay [2]. The data was analyzed by using BioNumerics version 7.6.3 software.

Results
Among the 87 GP clinical isolates, 16 new alleles and 67 new sequence types (STs) were detected. Burst analysis revealed that STs were clustered into 3 clonal complexes and 69 singletons (Figure 1). All the GP strains in PubMLST database, including strains from the diseased pigs in the study, were defined into two main clusters by Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Most isolates in this study and virulent isolates from the database were mainly located in cluster 2, while cluster 1 included a high percentage of nasal isolates from asymptomatic carriers.

Conclusions and Discussion
The MLST results showed new genotypes, demonstrating the diversity of GP strains. The main strains isolated in this study were in cluster 2, which is in agreement with previous literatures that the pathogenic strains are mainly located in cluster 2; in contrast, only one strain from diseased pig and most of the isolates from healthy pigs belonged to cluster 1. Further study focusing on the relationship between the expression of GP virulence genes and the distribution of genotypes is required to elucidate main factors that dictate the pathogenicity of Glässer's diseases.

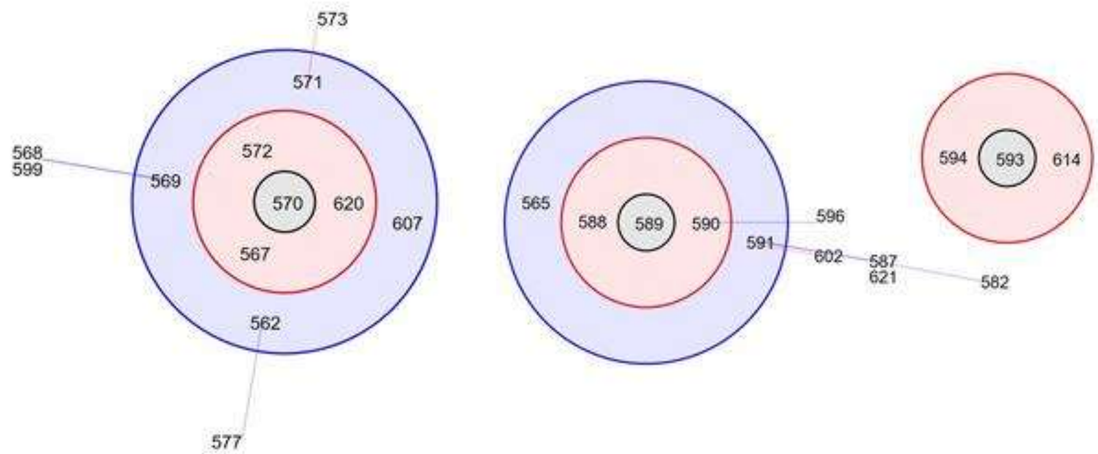
Acknowledgement

This work was supported by the Animal Disease Diagnostic Center of National Chiayi University, and I would like to express our thanks to all the members of the Bacteriology Laboratory in the department of Veterinary Medicine, National Chiayi University, Taiwan.

References

[1] Oliveira et al., 2001. J Vet Diagn Invest 13: 495-501.
[2] Mullins et al., 2013. Vet Microbiol 162: 899-906.

Graphs or Images 1



P-B-086

STUDY OF MULTILOCUS SEQUENCE TYPING OF STREPTOCOCCUS SUIS FROM DISEASED PIGS IN TAIWAN

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Introduction
Streptococcus suis (SS), a gram-positive coccus with capsule, is one of the most important pathogens in the swine industry. Different serotypes of SS can be isolated in the same herd, which suggests that similar clinical symptoms may not be caused by SS strains with identical serotypes. In addition, traditional typing methods only give single serotype results or represent specific geographic locations, which renders the findings difficult to be compared across laboratories. Multilocus sequence typing (MLST) has been successfully used in many studies of molecular epidemiology in bacteria. It is often used for identifying the SS genotype in order to confirm the differences between the sequence type (ST) and ST clonal complexes (CC) of SS strains. In this study, we focus on the epidemiology and laboratory findings of clinical isolates of SS in diseased pigs in Taiwan, investigating the correlations among the strain distribution, serotypes, and genotypes of SS, for better understanding of the epidemiology and development of prevention strategy.

Materials and Methods
From March 2017 to October 2021, bacteria were isolated from the diseased pigs submitted to the Animal Disease Diagnostic Center (ADDC), Department of Veterinary Medicine of National Chiayi University (Taiwan) for necropsy. According to the serotype distribution, we selected 80 isolates for MLST and chose seven housekeeping genes, including aroA, cpn60, dpr, gki, mutS, recA, and thrA, as potential target genes for the MLST assay. Then, PCR and sequencing work were performed, and we used BioNumerics version 7.6.3 software to analyze the data.

Results
Eighty SS clinical isolates in numbers proportional to their serotypes were selected for the MLST; that is to say, 6, 7, 17, 11, 8, 7, 2, 3, 3, and 2 strains were selected for serotype 1, 2, 3, 7, 8, 9, 1/2, 4, 5, and 21, respectively, and 14 unidentified strains. The result of the 80 SS strains showed that aroA, cpn60, gki, dpr, mutS, recA, and thrA genes exerted 16, 15, 13, 9, 11, 9, and 12 different alleles, respectively, forming 28 different STs. The ST27 (12/80, 15.0%), ST94 (13.8%) and ST1831 (13.8%) were the major STs, followed by ST28 (10.0%), ST1832 (8.7%), ST1 (6.3%), ST1833 (3.7%), ST117 (2.5%) and ST1175 (2.5%). Burst analysis revealed STs were grouped in 4 clonal complexes (CC) and 9 singletons (Figure 1). When UPGMA dendrogram was constructed, four major clades were defined.

Conclusions and Discussion
To compare the MLST result with serotypes and pig production stages, it was apparent that the pigs at the suckling stage were mainly infected with SS serotype 1, which corresponded to the main sequence type ST1 (n=5) and CC1 complex. The pigs at the nursing stage were most susceptible to the serotypes 3, 7, 8 and 9, which corresponded to the main sequence types ST27 (n=12), ST1831 (n=5), ST1831 (n=5) and ST1832 (n=3). The pigs at the growing-finishing stage were susceptible to the serotype 2, which corresponded to the main

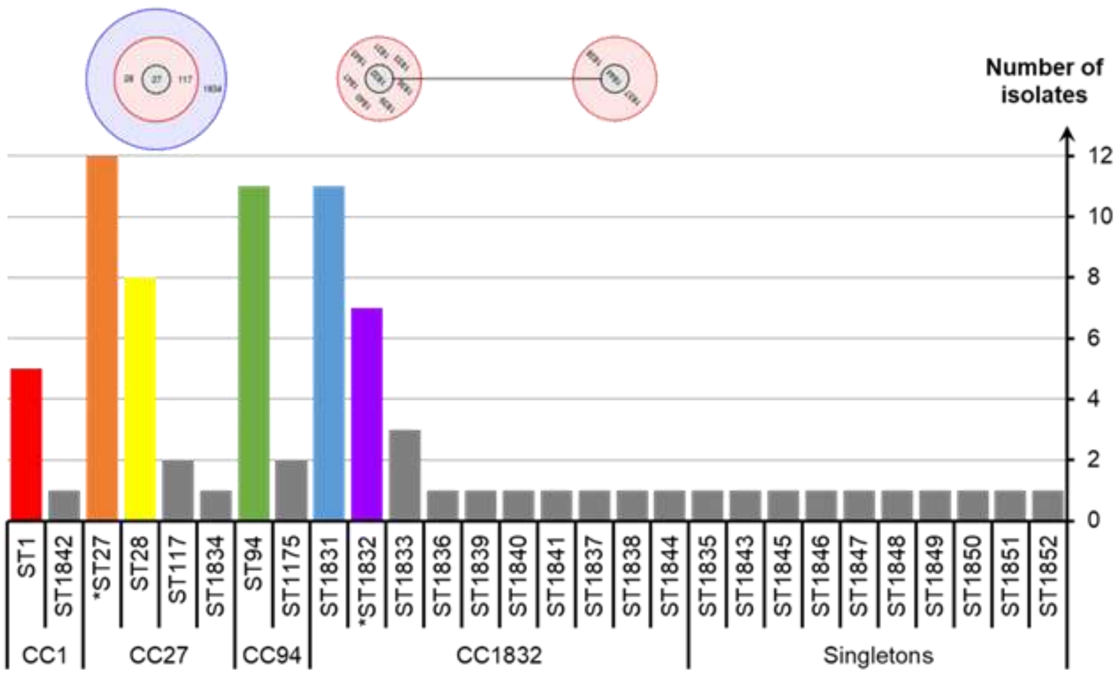
sequence type ST28 (n=6). Taken together, specific STs were significantly correlated with serotypes.

Acknowledgement
This work was supported by the Animal Disease Diagnostic Center of National Chiayi University, and we would like to express our thanks to all the members of the Bacteriology Laboratory in the department of Veterinary Medicine, National Chiayi University, Taiwan.

References

King et al., 2002. J. Clin. Microbiol. 40: 3671-3680.
Rehm et al., 2007. J. Vet. Diagn. Invest. 56: 102-109.
Jolley et al., 2018. Wellcome Open Res. 3: 124.

Graphs or Images 1



P-B-087

STUDY OF SEROTYPES DISTRIBUTION AND ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF STREPTOCOCCUS SUIS ISOLATED FROM DISEASED PIGS IN TAIWAN

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Introduction
Streptococcus suis (SS), a gram-positive coccus with capsule, is one of the most important pathogens in the swine industry. As an opportunistic pathogen, SS colonizes the upper respiratory tract (tonsil and nasal cavity) of the pigs. The infected pigs commonly manifest meningitis, arthritis, pneumonia, septicemia, and may be accompanied by acute death. The capsular polysaccharides (CPSs) antigen of the bacteria is used as the basis of classification and 29 serotypes have been identified by using polymerase chain reaction (PCR). Among the identified SS serotypes, serotype 1 is related to the neurological symptoms accompanied by cerebral microlesion, while serotype 2-8 tend to cause pulmonary lesions. Different serotypes also exert significant differences in drug resistance. In this study, we focus on the epidemiology and laboratory findings of clinical isolates of SS in diseased pigs in Taiwan, investigating the correlations among the strain distribution, serotypes of SS and the antimicrobial drug susceptibility, for better understanding of the epidemiology and development of prevention/treatment strategy.

Materials and Methods
From March 2017 to October 2021, bacteria were isolated from the diseased pigs submitted to the Animal Disease Diagnostic Center (ADDC), Department of Veterinary Medicine of National Chiayi University (Taiwan). All of the specimens, including liver, lung and bronchus, as well as the cerebrospinal fluid and joint fluid, were aseptically sampled during necropsy. Multiplex polymerase chain reaction, restriction fragment length polymorphysim, and broth microdilution test were performed to identify the serotype and minimum inhibitory concentration (MIC). MIC breakpoint was checked following the guideline of Clinical and Laboratory Standard Institute.

Results
The major serotypes were serotype 3 (79/388, 20.4%), 7 (12.9%), 8 (11.6%), 2 and 9 (8.2%), and 1 (7.2%), followed by serotype 4 (2.3%) , 5 (2.3%), 1/2 (1.8%), and 21 (1.5%) (Table 1). The results showed that SS was highly susceptible to ceftiofur (96.4%), cefazolin (91.0%), trimethoprim/ sulfamethoxazole (86.9%), and gentamicin (79.6%). The strains showed moderate susceptibility (40-70%) to florfenicol (68.8%), amoxicillin (61.1%), enrofloxacin (60.1%), tiamulin (60.1%), penicillin G (58.0%), and doxycycline (54.9%). The susceptibility to lincospectin (33.2%), erythromycin (14.7%), tylosin (8.8%), oxytetracycline (8.0%), and lincomycin (5.2%) was lower than 40%. Notably, most SS isolates showed low susceptibilities (<10%) to tylosin, oxytetracycline and lincomycin in this study. (Table 2).

Conclusions and Discussion
According to the results for different pig production stages and serotype distribution, the suckling pigs were prone to be infected with serotype 1 which mainly causes suppurative meningitis or/and arthritis, so amoxicillin, ceftiofur, tiamulin, and enrofloxacin are recommended for treatment. In contrast, the pigs at the nursing stage were especially

susceptible to serotypes 3, 7, 8 and 9, in which the infection in the respiratory system was relatively frequent. In combination with the pharmacokinetic characteristics, cefazolin, ceftiofur, or trimethoprim-sulfamethoxazole were recommended for treatment of these serotypes.

Acknowledgement
Many thanks to the Animal Disease Diagnostic Center of National Chiayi University for providing the bacteria, and professor Kuo and seniors for supporting the MIC test.

References
Athey et al., 2016. BMC Microbiol. 16: 162.
Clinical and Laboratory Standards Institute (CLSI), 2022. CLSI M100.
Kerdsin et al., 2014. J. Med. Microbiol. 63: 824-830.
Matiasovic et al., 2020. J. Clin. Microbiol 58: e00480-20

Table 1

Category / Serotype	1 (n = 28)	2 (n = 32)	3 (n = 79)	7 (n = 50)	8 (n = 45)	9 (n = 32)	N (n = 73)	OT (n = 49)	Total (n = 388)
Pig production stages									
Suckling pigs	**27**		7	7	1	1	10	11	64
Nursery pigs	1	16	52	36	**38**	24	50	28	245
Growing pigs		**8**	13	6	5	5	6	7	50
Fattening pigs		**8**	5			1	3	2	19
Breeding pigs				1		1	2		4
Unknow			2		1		2	1	6
Organs									
Brain/*cerebrospinal fluid*	**10**	1	5	4	1	**12**	2	7	42
Lung/ bronchus	3	**29**	**63**	35	37	12	56	35	270
Liver	4	1	4	5	3	3	11	5	36
Synovial fluid	**9**		5	3	2	2	1	2	24
Other#	2	1	2	3	2	3	3		16

#: include heart, pericardial fluid, pleural fibrin, spleen, stomach, eye, and nasal.
N: unidentified serotype; **OT**: other serotypes included 1/2, 4, 5, 12, 14, 16, 17, 18, 19, 21, 24, 27, 29, 31.

Table 2

Antimicrobial agents	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	Susceptible rate (%)	Intermediate rate (%)	Resistance rate (%)
Penicillin G	0.5	4	49.5	8.5	42.0
Amoxicillin	0.5	32	56.2	4.9	38.9
Cefazolin	0.25	4	71.1	19.8	9.0
Ceftiofur	≤ 0.125	1	95.4	1.0	3.6
Gentamicin	1	256	75.0	4.6	20.4
Oxytetracycline	32	256	6.2	1.8	92.0
Doxycycline	4	16	27.8	27.1	45.1
Erythromycin	1024	> 1024	0.0	14.7	85.3
Tylosin	> 1024	> 1024	8.8	-	91.2
Tylvalosin	256	1024	-	-	-
Lincomycin	512	1024	5.2	-	94.8
Lincospectin	256	512	33.2	-	66.8
Florfenicol	4	32	31.2	37.6	31.2
Tiamulin	16	64	60.1	-	39.9
Enrofloxacin	0.5	16	54.6	5.4	39.9
Trimethoprim/ sulfamethoxazole	0.8 / 4	6.4 / 32	38.4	48.5	13.1

P-B-088

SURVEILLANCE OF FARMS IN VIETNAM AND THEIR ANTICOCCIDIAL USAGE IN ADDRESSING COCCIDIOSIS IN PIGLETS

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Introduction
Coccidiosis is a parasitic disease caused by the protozoan Cystoisospora, it is one of the most common reasons of diarrhea with a high prevalence in piglets (1,2). Coccidiosis should be suspected if there is a diarrhea problem in suckling pigs from 7-24 days of age that does not respond particularly well to antibiotics. Therefore, this survey was carried out with the aim of evaluating the prevalence of coccidiosis in piglets, as well as the effectiveness of anti-coccidial drugs on the market.

Materials and Methods
A total of 62 farms from 3 regions (North - Central - South of Vietnam) at different herd levels (commercial or backyard farm) were included in the survey. 5-10 samples of 4g of feces were collected in pigs between 10-25 days of age with or without diarrhea from farms with or without coccidiosis. These samples were then treated with saturated saline to determine the presence of coccidiosis in feces by the flotation method. Positive coccidiosis samples were analyzed by Mc Master method to determine the average number of oocysts per gram of feces.

Results and Discussion
Percentage of farms positive for coccidiosis
Out of 62 farms surveyed for the presence of coccidiosis in pigs, 50 farms were positive, accounting for 80.6%. This may be due to survival of coccidia for a long time in the environment if the temperature is ideal for their growth (3). Vietnam is a tropical country with high humid and high temperature, while a report of Meyer et al. (1999) also showed the incidence of C. suis in farms often increases with the climate, especially during the hot and humid environment. Increased prevalence of diarrhea caused by C. suis was recorded in summer (66.3%) and autumn (61%) (1).
Percentage of fecal samples positive for coccidiosis
The results showed that 162/409 fecal samples had the presence of coccidiosis and accounted for 39.6% of the total samples examined. These results were in accordance with the findings of Niestrath et al. (2002) who reported prevalence of 42.5% in piglets (2). The positive rate for pigs treated with Baycox 5% was 14.3% (24/168), while 8 out of 12 commercial products had a positive rate greater than 50%.
Average of oocyst/g of feces
In farms that did not use anti-coccidial products, average number of oocysts were 807 oocysts/g of feces. According to the study of Christensen et al. (1994), 100 oocysts per gram of feces resulted in clinical signs and the spreading of coccidiosis, indicating that even a low level of infection can lead to transmission to later herds (4). The result showed that using Baycox decreased positivity rate of coccidiosis and resulted in lower spreading possibility with an average of only 22 oocysts/g of feces. Compared to the farms that did not use any anti-coccidial products, using Baycox resulted in significantly lower prevalence and better control of coccidiosis in fecal samples (P<0.001). In addition, 9 out of 12 anti-coccidial products had the spreading level of coccidiosis more than 200 oocysts/g of feces.

Conclusion

In this survey, the prevalence of coccidiosis in the farms was up to 80.6% and the spreading rate in the fecal samples was 39.6%. However, anti-coccidiosis products such as Baycox, resulted in much lower number of oocysts compared to farms that did not use preventive products or used other commercial products.

References

1. Meyer C et al. 1999. Vet Parasitol 82:277-284.
2. Niestrath M et al. 2002. J Vet Med B Infect Dis Vet Public Health 49(4):176-80.
3. Martineau G. P et al. 2000. Parasitol Res 86:834-837.
4. Christensen J. P et al. 1994. Acta Vet Scand 35(2):165-72.

P-B-089

SURVEILLANCE OF MYCOPLASMA HYORHINIS INFECTION IN TAIWAN

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Introduction
Mycoplasma hyorhinis (Mhr) causes pneumonia, polyserositis, and polyarthritis in nursery and early finishing pigs. Clinical signs of the infection include lameness, inappetence, difficulty in moving, swollen joints, otitis media, and ocular discharge. The growth retardation and decreased feed conversion rate due to Mhr infection has huge economic impact on the porcine industry1,2,3. In Taiwan, the prevalence of Mhr infection is underestimated due to the inability of veterinary microbiology laboratories to isolate Mhr and unavailability of commercial diagnostic Enzyme-linked immunosorbent assay (ELISA) test kit. The aim of this study was to investigate the prevalence of Mhr infection in Taiwan using an ELISA kit developed by Agricultural Technology Research Institute (ATRI) and supplied by TaFoong Vaccines & Biotech Co., Ltd.

Materials and Methods
One thousand blood samples (1000) were obtained from 50 commercial pig farms in northwest (n=2), in central (n=22), in southwest (n=21), and in east (n=5) Taiwan. Blood samples were collected from 10 nursery (6-9 weeks) and 10 finishing (21-24 weeks) pigs in each farm and delivered to ATRI for Mhr antibody detection using ELISA kit supplied by TaFoong Vaccine & Biotech Co., Ltd.

Results
In nursery pigs, the seropositive rate of Mhr ranged from 10 to 70% (mean=40%) in the north, 0 to 100% (mean=63.6%) in the central part, 0 to 100% (mean=44.3%) in the southwest, and 20 to 100% (mean=76%) in the east of Taiwan. The average seropositivity rate was 55.8% and the highest mean seropositivity was found in Hualien region located in eastern Taiwan. In finishing pigs, the seropositivity was 100% in the north and east, 70 to 100% (mean=97.7%) in central part, and 50 to 100% (mean=97.1%) in the southwest region of Taiwan. The average seropositivity rate was 97.8%. The mean seropositivity in finishing pigs was more than 90 % in every county (Table 1, Figure 1).

Conclusions
The seroprevalence of Mhr in nursery and finishing pigs is high in Taiwan. The seropositivity is higher in finishing pigs compared to nursery pigs. The correlation of infection and clinical signs need to be investigated in further studies.

Reference:
1. Lin et al., 1996. Vet Microbiol. 115: 111-116.
2. Pieters MG, and D Maes. 2019. Disease of swine. 863-883.
3. Neto et al., 2012. J Swine Health Prod. 20(2):82–86.

Table 1

Table 1. **The region-wise seroprevalence of *Mhr* in nursery and finishing pigs in Taiwan**

County	No. of farms	*Mhr* seropositivity rate (%)	
		Nursery pigs	Finishing pigs
Taoyuan City	1	70	100
Hsinchu County	1	10	100
Miaoli County	3	63.3	100
Taichung City	1	100	90
Changhua County	9	48.9	96.7
Yunlin County	9	74.4	98.9
Chiayi County	1	0	100
Tainan City	10	47	100
Pingtung City	10	46	94
Hualien County	4	90	100
Taitung County	1	20	100

Graphs or Images 1

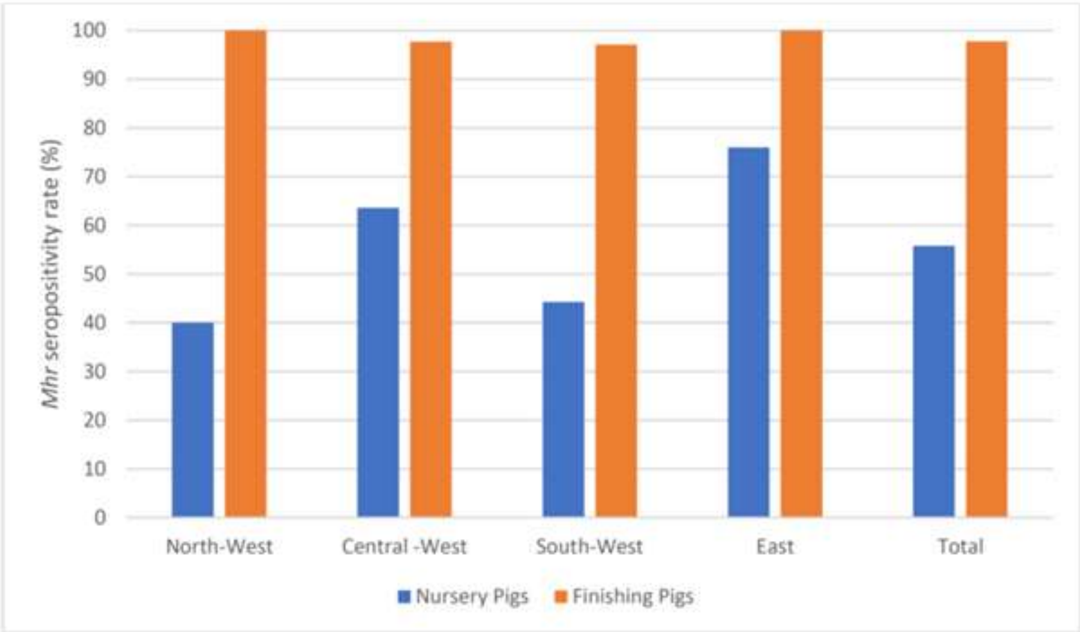

Figure 1. **The region-wise seroprevalence of *Mhr* in nursery and finishing pigs in Taiwan**

P-B-090

SWINE ERYSIPELAS SEROLOGY SURVEY ON JAPANESE SOW FARMS

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Introduction

Humoral immunity plays an important role in host protection against Erysipelothrix rhusiopathiae infection (1). The protective role of specific antibodies against Swine Erysipelas (SE) enhanced via vaccination is the key to controlling reproductive disorder problems in sows (2). In Japan, most farms use live or inactivated vaccines against SE that can be monovalent (SE-monovalent) or combined with Bordetella bronchiseptica and Pasteurella multocida for the prevention of atrophic rhinitis (SE+AR combo-vaccine).
The present study's objective was to survey the serological status of mature gilts and sows on Japanese farms implementing vaccination against SE, considering possible differences by the type of vaccine and vaccination protocol.

Material and Methods

A total of 832 samples (sows from parity 0-9) were collected from 75 farms (sized 35-3,000 sows) located in 17 different provinces in Japan from September 2021 to January 2022. The number of samples collected by each farm was in correlation to the size. Samples were shipped to Spain and analysed at HIPRA Diagnos. Erysipelas titers were determined by an indirect ELISA kit (CIVTEST® SUIS SE/MR). The ELISA results were interpreted as positive when Cut-off IRPC value was > 40. The ability of this kit to detect anti-SE antibodies without bias has been previously reported (3).

Results

The percentage of negative animals was 55%, with higher results coming from gilts with 73% of the animals testing negative opposed to the multiparous sows (49%) (Table 1). The percentage of negative animals was higher using monovalent vaccines than the SE+AR combo - 62% vs 41% respectively (Table 1). According to the type of vaccine antigen, the percentage of negative animals was higher when vaccinated with an inactivated vaccine rather than with a live one (56% vs 42%), but the number of animals was not equally distributed in both groups (Table 3). Regarding the vaccination programme, the percentage of negative animals was higher on a mass vaccination plan (63%) rather than when each cycle was made (48%) (Table 4). Finally, in 70 of the 75 farms (93%) sampled, there was at least one negative animal and only 11 from the 75 farms (14.6%) reached an 80% positivity rate, which is considered herd immunity.

Discussion & Conclusion

Based on the results obtained, only a small proportion of the animals (45%) were positive with enough antibodies to be considered protected, and only 14.6% of the farms reached the herd immunity level of 80%, which indicates a lower risk of suffering SE related problems in the breeding herd (4). These results suggest weaknesses in current implemented vaccination protocols in the Japanese pork industry. The inclusion of novel vaccines containing SE antigens that elicit a stronger immune response should be evaluated for the better prevention of reproductive disorders.

References
1. Tanja Opriessnig et al. Diseases of Swine 11th Edition (page 835-843).
2. Shimoji Y. 2000. Microbes Infect, 965-972
3. Camprodon, A. et al., 2016. Proceedings 24th IPVS 405 (PO-PF3-051)
4. DeGroot M. et al. Banff proceedings 2019-197

Table 1. Antibody response against SE in gilts and sows and by type of SE vaccine (monovalent or combo). Positive results indicate IRPC > 40

Table 1

Antibody response by type of animal		
	Negative	Positive
Gilts (n=207)	73%	27%
Sows (n=625)	49%	51%
Total (n=832)	**55%**	**45%**
Antibody response type of SE vaccine		
	Negative	Positive
SE-Monovalent (n=570)	62%	38%
SE+AR combo (n=252)	41%	59%
Total (n=822)	**55%**	**45%**

Table 2. Antibody response against SE by type of SE vaccine antigen. Positive results indicate IRPC > 40

Table 2

	Negative	Positive
Inactivated (n=714)	56%	44%
Live (n=90)	42%	58%
Total (n=804)	**55%**	**45%**

Table 3. Antibody response against SE by type vaccination plan. Positive results indicate IRPC > 40

Table 3

Table 3. Antibody response against SE by type vaccination plan. Positive results indicate IRPC > 40

	Negative	Positive
Every cycle (n=494)	48%	52%
Mass vaccination (n=319)	63%	37%
Total (n=813)	**55%**	**45%**

P-B-091

THE CONTROL OF THE CLOSTRIDIUM PERFRINGENS TYPE A ASSOCIATED DIARRHEA IN SUCKLING PIGLETS BY VACCINATION

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Introduction
Neonatal diarrhea (ND) is one of the most common disease problems on a pig farms today. Enteroporc coli AC (Ceva Sante Animale, France) contains 7 antigens: adhesins of Escherichia coli (F4ab, F4ac, F5, F6) and toxoids of Clostridium perfringens type A (α and β2 toxin) and C (β1 toxin). The aim of the presented study was to investigate the efficacy of vaccination under the field conditions.

Material and Methods
Randomized, controlled, observer blinded trial was performed on the farm affected by ND caused by C. perfringens type A (α and β2 toxin positive strains), other ND pathogens were ruled out by lab investigation before the start of the study. Two groups of randomly allocated sows of different parity were established: group A (9 litters, average parity 4,1), vaccinated by Enteroporc coli AC 5 and 2 weeks before farrowing and group B (8 litters, average parity 4,25), vaccination according to farm practice (product B: adhesins of E.coli F4, F5, F6, F41 and Rota OSU 6). The presence of diarrhea (yes/no) was observed 1-7 days of life (DOL), 14th and 27-28th DOL. Individual antimicrobial treatment and zootechnical performance at weaning was recorded. Statistical analysis included Mann-Whitney test and Fisher's exact test using GraphPath Software (San Diego, CA 92108, USA).

Results
The frequency of diarrhea was significantly higher in group B on the 2nd and 3rd DOL (P= 0,01 and P= 0,0008). The rest of the observational period, groups did not differ significantly. Number of animals (expressed as %) which were treated by antibiotics at least once, was higher in group B (26,67 vs. 15,60 %) with statistical trend observed (P= 0,06). Weight gain (kg) and ADG (g) were numerically better in group A at the end if the study (7.52 vs. 7,15 kg and 279 vs. 263 g, all expressed as median).

Conclusions
The vaccination of the sows by Enteroporc coli AC, containing both important toxoids of CpA (α and β2 toxoid) reduced the overall frequency of diarrhea in piglets during the first week of age, where the impact of ND is the most significant. Control of CpA by vaccination reduced the need for individual antibiotic treatment. These results underline the importance of α and β2 toxin producing CpA strains in diarrhea in suckling piglets and the effectiveness of the vaccination as tool for reduction of antibiotic treatment in the farrowing house.

Figure 1: Number of animals treated during the study period in both vaccine groups.

Figure 1

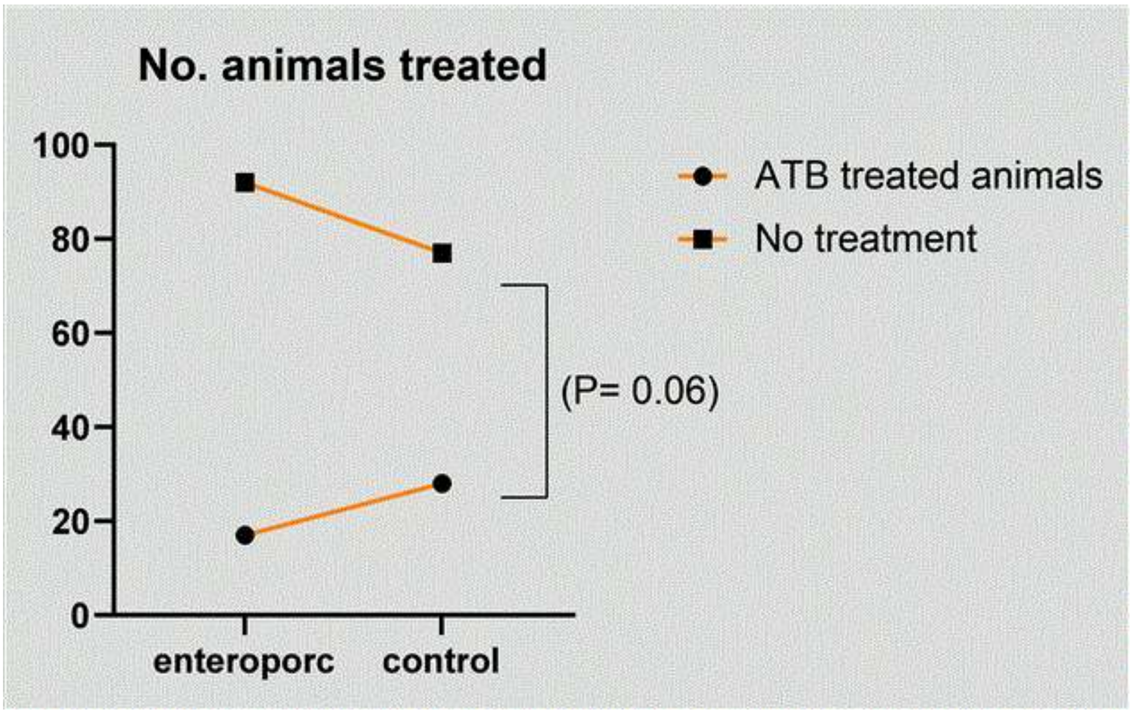
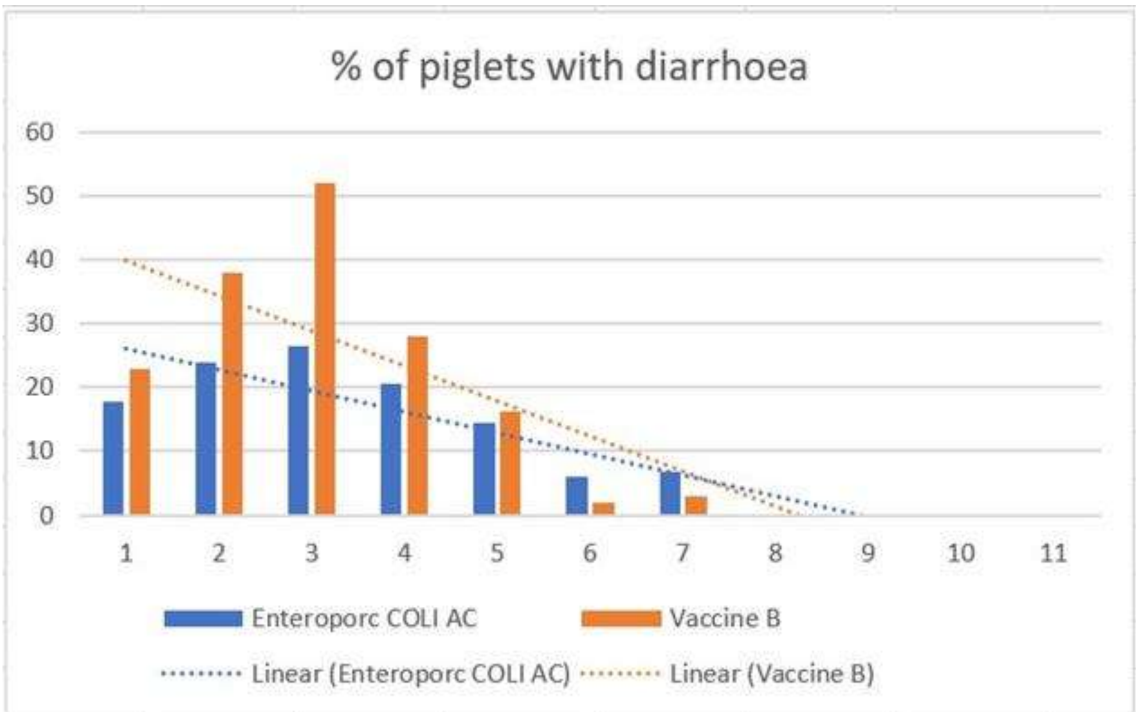


Figure 2: The frequency (%) of diarrhea in the vaccine groups, the difference on 2nd and 3rd DOL was significantly different between study groups.

Figure 2



P-B-092

THE IMPACT OF VEPURED® VACCINATION ON MORTALITY ON A STX2E-POSITIVE FARM IN JAPAN.

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[1]Hipra, [2]Hipra, [3]Katori Livestock Clinic, [4]Jelly Beans Ltd., [5]National Agricultural Insurance Association

Introduction
Oedema disease (OD) is a disease caused by Stx2e-produced STEC. OD is known to cause high mortality and reduced productivity. On the other hand, it has recently been suggested that Stx2e infection may trigger co-infection with other pathogens1.
The purpose of this study was to evaluate the impact of VEPURED® treatment on a Stx2e-positive farm in Japan.

Materials and Methods
This study was conducted on a farm with 2,000 Sows in Chiba Prefecture, Japan. On this farm, pigs are kept using the 2-site system from 6 weeks of age to shipping. On the farm, ZnCO3 and antibiotics were routinely added to drinking water and feed, and so far, no clinical signs of OD have been observed and the overall mortality rate has been low.
In this study, 1532 piglets were divided into two groups: a VEPURED® group (782 piglets) and a control group (750 piglets). The trial period was six months from September 2021 to March 2022. VEPURED® was administered at 2 days of age. In both groups, only the addition of ZnCO3 was discontinued, and antibiotic treatments were continued.
Production parameters (ADG, FCR and age at shipment) and mortality rates of each group were recorded and compared over time while they were in the wean-to-finish barn. In addition, ROI was calculated based on mortality rates and the market prices in Japan. Stx2e was detected by qPCR test using saliva samples 5 times at 4- or 5-week intervals from 8 weeks of age to shipping in both groups.

Results
qPCR test results confirmed that all samples from both groups were positive, and there was a tendency for Stx2e concentrations to be higher in the younger age group. From 8 weeks to 16 weeks of age, 11 out of 12 samples were ++ or higher (of which 5 were +++), but from 16 weeks of age to shipment, only 4 out of 18 samples were ++ (Table 1). There were no significant differences between the two groups in any of the production parameters.
No deaths due to OD were observed in either group during the study period, but deaths mainly due to APP and Streptococcus suis were observed. There was a significant difference (P<0.05) in mortality rates between the two groups from 6 to 13 weeks and from 6 to 26 weeks of age (Figure 1). ROI was 2.31, calculated based on lost of cost opportunity.

Discussion and Conclusion
De Jong, E et al.1 reported that the OD vaccine reduced overall mortality and the proportion of deaths related to Streptococcus suis. This study also suggests that VEPURED® can be expected to significantly reduce mortality due to other infectious diseases on Stx2e-positive farms. Further studies are needed to clarify the mechanism of the reduction.
Although there were no significant differences in production parameters, it is possible that the addition and treatment of antibiotics in both groups might contribute to the result.

Acknowledgments

This work was supported by Hipra Japan.

References
1.De Jong, E et al. Comparison of two E. coli vaccines and their effects on a farm with clinical problems of S. suis. ESPHM proceedings 2022.

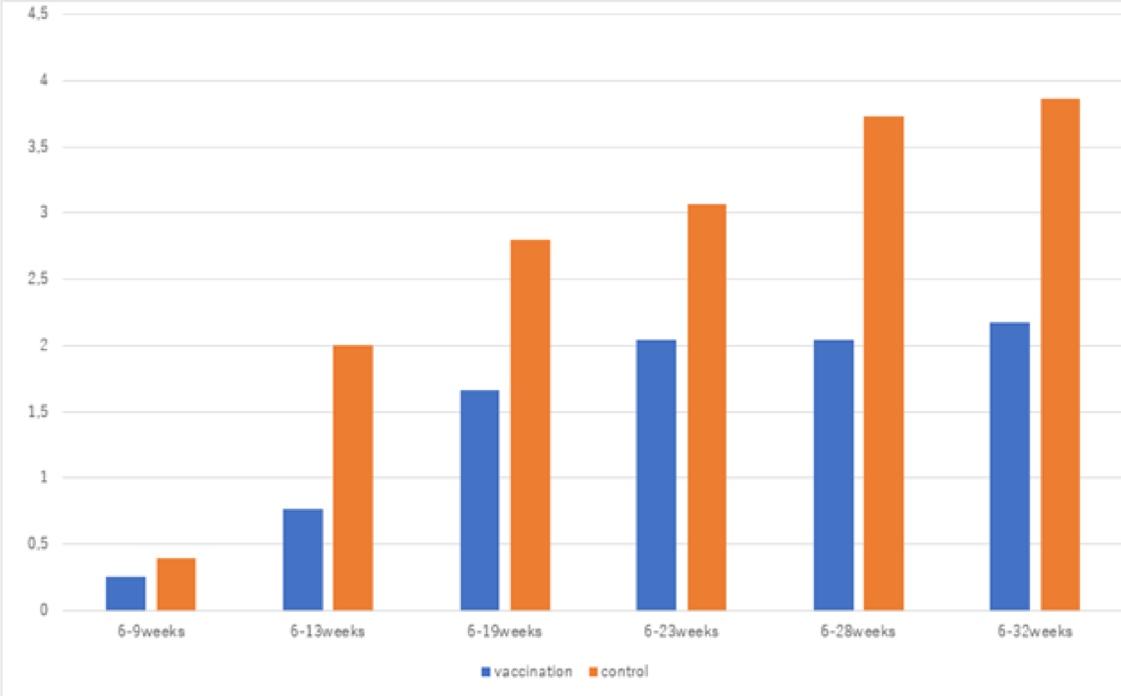
Table1. Results of qPCR test for Stx2e

Table 1

pig's age	vaccination	control
8～9 weeks	+++	++
	+	+++
	++	++
15～16weeks	+++	+++
	++	+++
	++	++
20～21weeks	+	++
	++	+
	+	+
24weeks	+	+
	+	+
	+	+
28weeks (end of fattening)	+	+
	++	+
	++	+

Figure 1. Mortality in both groups
*P=0.038
** P=0.048

Graphs or Images 1


4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
6-9weeks
6-13weeks
6-19weeks
6-23weeks
6-28weeks
6-32weeks
vaccination
control

P-B-093

THE RELATIONSHIP BETWEEN LUNG LESION SCORES OF PIGS MEASURED BY CEVA LUNG PROGRAM (CLP) AT SLAUGHTER AND THEIR AVERAGE DAILY WEIGHT GAIN.

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Introduction
Mycoplasma hyopneumoniae is the primary causative pathogen of swine enzootic pneumonia (EP) and play an important role in porcine respiratory disease complex (PRDC) (1). Pigs affected with EP develop cranioventral pulmonary consolidation (CVPC) characterized by catarrhal broncho-pneumonia with purple to grey regions of lung consolidation (1). EP has negative effect on the health of the pigs and their average daily weight gain (ADG) (1, 2). Several lung scoring methods were developed to evaluate the presence and severity of EP in swine herd (3). Ceva lung program (CLP) is an innovative lung scoring methodology invented to facilitate efficient and hygienic evaluation at slaughterhouse. The aim of this study was to evaluate the relationship between lung lesion scores of pigs measured by CLP and their average daily weight gain.

Materials and Methods
In the CLP, EP lesion is scored based on modified Madec method, which considers the contribution of each lobe to the overall capacity of the lungs and uses the quantification of the affected area by means of four points: (0) no lesion ; (1) lesion affecting < 25% of the lobe surface; (2) lesion affecting 25-49% of the surface; (3) lesion affecting 50-74% of the surface; (4) lesion affecting >75% of the lobe surface (1). The percentage of the total lung surface affected for each animal are determined and then summed to determine the average percent of affected surface out of all lungs evaluated in each batch of slaughter pigs. In this study, 74 batches were selected from the same slaughterhouse in the central region of Thailand. All batches were fed with similar feeding program and formulation. Pigs were sent to slaughter at 133-159 days of age. 50 lungs of each batch were evaluated for the presence and severity of EP by the same auditor using the application. Their growth performance data were recorded, predicted feed conversion ratios (FCR) were calculated to determine the different between predicted and actual FCR (difFCR; predicted FCR – actual FCR). Statistical analysis was performed using RStudio.

Results
From the selected 74 batches, average time to slaughter was 155 days. There was no significant relation between average affected lung surface and time to slaughter. 72 out of 74 batches (97.30%) were affected with EP. The mean value of average affected lung surface was 8.83% (0.00 – 36.93%), and the mean value of ADG was 689.97 g/d (586.22 – 773.93 g/d). The linear regression showed a negative relation between ADG and the average affected lung surface per batch (coefficient = -2.65, intercept = 713.38, r-square = 0.38, p-value <0.001), and the relation between difFCR and average affected lung surface per batch was found (coefficient = 0.0094, intercept = 0.2022, r-square = 0.27, p-value <0.001). The relation is demonstrated in figure 1 and 2.

Conclusions and Discussions

This study confirms the relation between lung score and growth performance of pigs. Their average daily weight gain was decreased by 2.65 g/d for each increased of one percent in average affected lung surface. Feed conversion ratio also affected by the lung score, which directly impact on the production cost. Evaluating lung score and controlling of the EP lesion are important to reduce losses, improve growth performance, and optimize the production cost. CLP is an innovative tool to assist the lung score evaluation.

Acknowledgement and References
1. Garcia-Morante B, et al., Journal of Comparative Pathology (2015)
2. D. Maes, Veterinary Quarterly, 18:3, 104-109
3. R. Krejci, International Animal Health Journal, Vol.9 Issue 2, 2022. 24-28
4. M.E.S. Ferraz, Preventive Veterinary Medicine, Volume 182, 2020

Graphs or Images 1

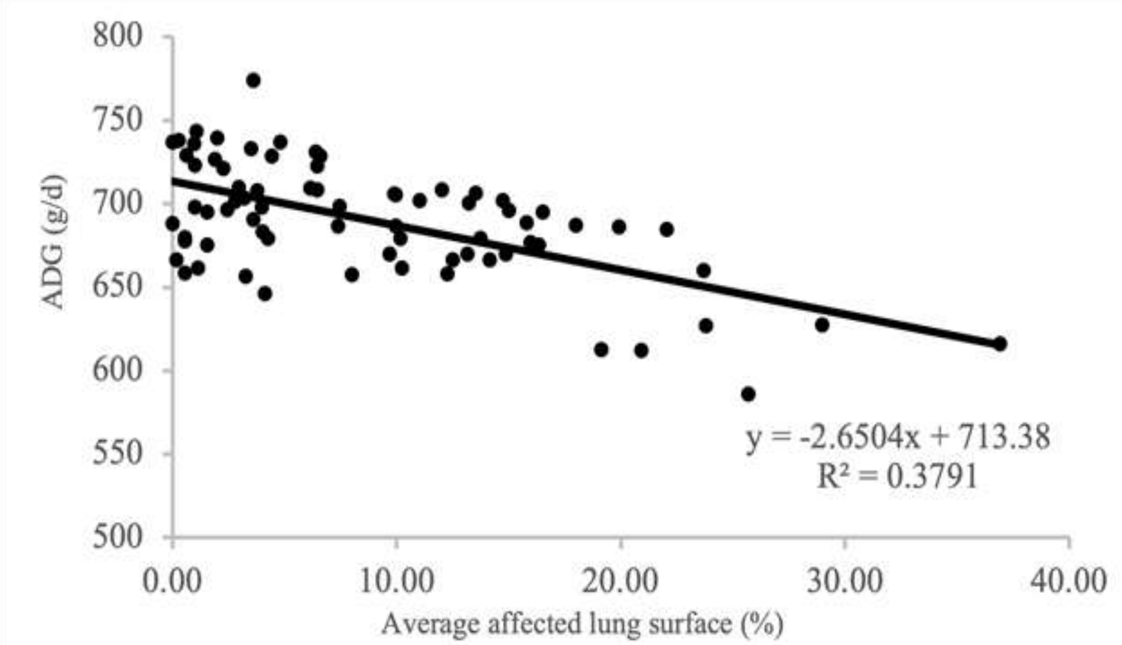

Figure 1. Scatter plot demonstrates the average daily gain (ADG) and the average affected lung surface per batch.

Graphs or Images 2

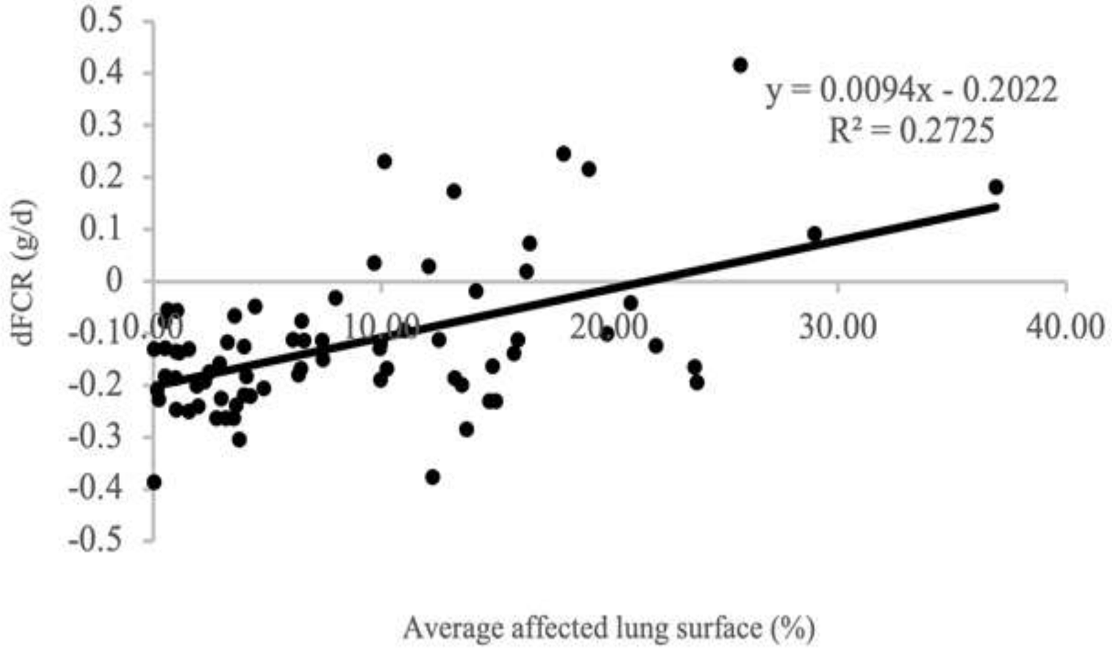

Figure 2. Scatter plot demonstrates the average affected lung surface and the difference between calculated and actual feed conversion ratio (difFCR) per batch.

P-B-094

THE USE OF PROBIOTIC CLOSTRIDIUM BUTYRICUM TO MITIGATE SALMONELLA ON AN ORGANIC SWINE FARM

Mr. Ping-Yi Chen[1], Mr. Wouter Van der Veken[2]
[1]*Huvepharma Taiwan,* [2]*Huvepharma NV*

Introduction
Despite great efforts during production and between sectors, Salmonella remains a massive public health concern. Additionally, the economic impact of an on-farm outbreak cannot be underestimated. Nowadays the understanding about the transfer and proliferation of Salmonella has increased significantly, including the realisation that Salmonella control requires a holistic approach.
From this perspective, certain probiotics have been added to the manager's toolbox to help mitigate Salmonella during production. This is especially important in production systems that have to adhere to additional standards, such as biological or organic production – where the use of some traditional tools is restricted. To evaluate the efficacy of a specific probiotic to help mitigate Salmonella, a program with probiotic Clostridium butyricum was initiated in an organic swine operation.

Material and Methods
An organic pig farm dealing with clinical diarrhoea and a Salmonella level 2 (high seroprevalence in carcasses) was selected for the trial. The herd was diagnosed by PCR to be Brachyspira hyodysenteria positive as well, complicating the challenge further. Probiotic C. butyricum (Top Gut®, $5x10^8$ CFU/g product) was supplemented for the full trial period depending on body weight (BW): 1 kg Top Gut®/ton of feed was used from 10 to 40 kg BW, followed by 0.5 kg/ton of feed from 40 kg BW until slaughter. Salmonella seroprevalence was recorded at the start of the trial (February-May, 45 samples) and at the end (June-September, 31 samples).

Results
Mucous diarrhoea was present at the start of the trial, which was reason to treat all pigs with lincomycin-spectinomycin for 5 days (one batch). Initial Salmonella seroprevalence ratio was 0.69 (31 positives), which dropped significantly to 0.16 (5 positives) at the end of the trial ($P<0.0001$, Fisher's exact test, 2 tailed). Further statistical analysis showed that inclusion of C. butyricum significantly lowered the relative risk of a carcass being classified Salmonella seropositive (RR = 0,23).

Discussion and Conclusion
The herd was reclassified to Salmonella level 1 after the probiotic inclusion, whilst the faecal scores improved as well. As such, probiotic Clostridium butyricum clearly has a place in a larger gut health management plan, including dealing with food safety pathogens such as Salmonella in organic swine production.

P-B-095

TWO ACTINOBACILLUS PLEUROPNEUMONIAE VACCINES ELIMINATING CLINICAL SIGNS, BUT ONLY THE ONE IMPROVING PRODUCTIVITY IN A PARALLEL COMPARISON.

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Background and Objectives
A. pleuropneumoniae (App) is a potentially devasting, primary respiratory pathogen commonly inducing acute mortality, pleuropneumonia, and chronic pleurisies. Vaccination measure is an integral part of on-farm App control [1,2]. Commonly three or more different serotypes are circulating App-endemic farms [3]. Serotype-independent protection, with highly significant and substantial reduction of pleuropneumonia, has been demonstrated previously in a multi-serovar, multi-study analysis for the whole-cell vaccine based on App1 and App2 expressing ApxI, II, and III during production [2]. This vaccine has previously been evaluated together with other ApxI-III containing App-vaccines and found significantly superior in protection against pleuropneumonia and disease losses [4].
The aim of this study was to evaluate zoo-technical, and empirical clinical, parameters following vaccination with one of two whole-cell App-vaccines, based on different serovars, both containing toxoids of Apx I, II and III, in comparison to a non-vaccinated group.

Material and Methods
The farm of this study is a 1,200-finishing farm in Southern Germany. Beginning of 2022 the farmer reported severe coughing, mostly mid- and end-finishing, and poor finishing productivity. 15 tonsillar brushing samples pooled on FTA® cards each beginning (B-F), mid (M-F) and end (E-F) of finishing for App-PCR (App-DNA detection). Ten blood samples each at B-F and E-F were examination by ApxIV-ELISA. Following this test results App vaccinations with one of two different vaccines, twice at 7 and 10 weeks of age, in parallel batches was decided. CV, n=128 (Coglapix®, Ceva Santé Animale; App1 and App2 based) and SV, n=115 (App9-based), and compared to the unvaccinated, previous batch (N-V, n=120).
Finishing pigs were recorded on weight-in and -out, and days-till-slaughter. Group average pen-passes/year are calculated from group average days-to-slaughter added 7 days per batch for total emptying, cleaning, and drying out of the pen, preferably the room, divided into the 365 days/year. Pen-passes/year is an efficacy index on how many batches can be produced per pen (and room).

Results
ApxIV-ELISA positive serum samples: B-F=7/10 and E-F=10/10. App-PCR tonsillar-brushing-pools: B-F=1/3 weakly positive, M-F=2/3 weakly positive and E-F=1/3 weakly and 2/3 highly positive.
The productive performances during finishing in the groups CV, SV, and N-V, respectively, on average daily weight gain (ADG): 973g, 883g, and 918g; average days-till-slaughter (finishing days): 94, 102 days, and 101 days; average pen-passes/year: 3.60, 3.35, and 3.38. In groups CV and SV the occurrence of coughing was drastically reduced (Table 1).

Discussion and Conclusion

Both App-vaccines improved clinical signs of respiratory distress experienced on the farm. However, only pigs vaccinated with CV demonstrated an App control able to positively affect farm productivity and pig growth. This indication of strong protection and superior comparative protective capacity of CV is in line with previous studies [2,3]
The relief of clinical signs, like coughing, was not found to correspond to health and productivity. These findings are in line with previous studies on CV efficacy and comparative efficacy [2,3].

References

1. Taylor D.J., Actinobacillus pleuropneumoniae in: Disease of Swine 11th edition. 2019. 349-354.
2. Mortensen P. et al., Comparative Efficacy in Challenge Dose Models of a Toxin Expressing Whole-Cell Vaccine against Eight Serovars of Actinobacillus pleuropneumoniae in Pigs. Animals, 2022.
3. Renken C. et al. Seroprevalence of Actinobacillus pleuropneumoniae and corresponding serotypes in fattening pigs in three different epidemiological regions in Germany. Proc ESPHM & IPVS, 2016.
4. Hoelzen P. et al., Comparison of Protectivity and Safety of Two Vaccines against Actinobacillus pleuropneumoniae in a Field Study. Agriculture, 2021.

Table 1

Group	ADG *gram growth/day*	Finishing time *days*	Pen-passes/year *# annual batches*
N-V	918	101	3.38
SV	883	102	3.35
CV	973	94	3.60

Table 1: Production parameters: daily growth, days in finishing till slaughter, and pen-efficacy compared between groups.

P-B-096

TWO-LABORATORY RESULTS OF IN VITRO SUSCEPTIBILITY OF BRACHYSPIRA HYODYSENTERIAE TO PLANT-BASED FEED ADDITIVE MIXTURE

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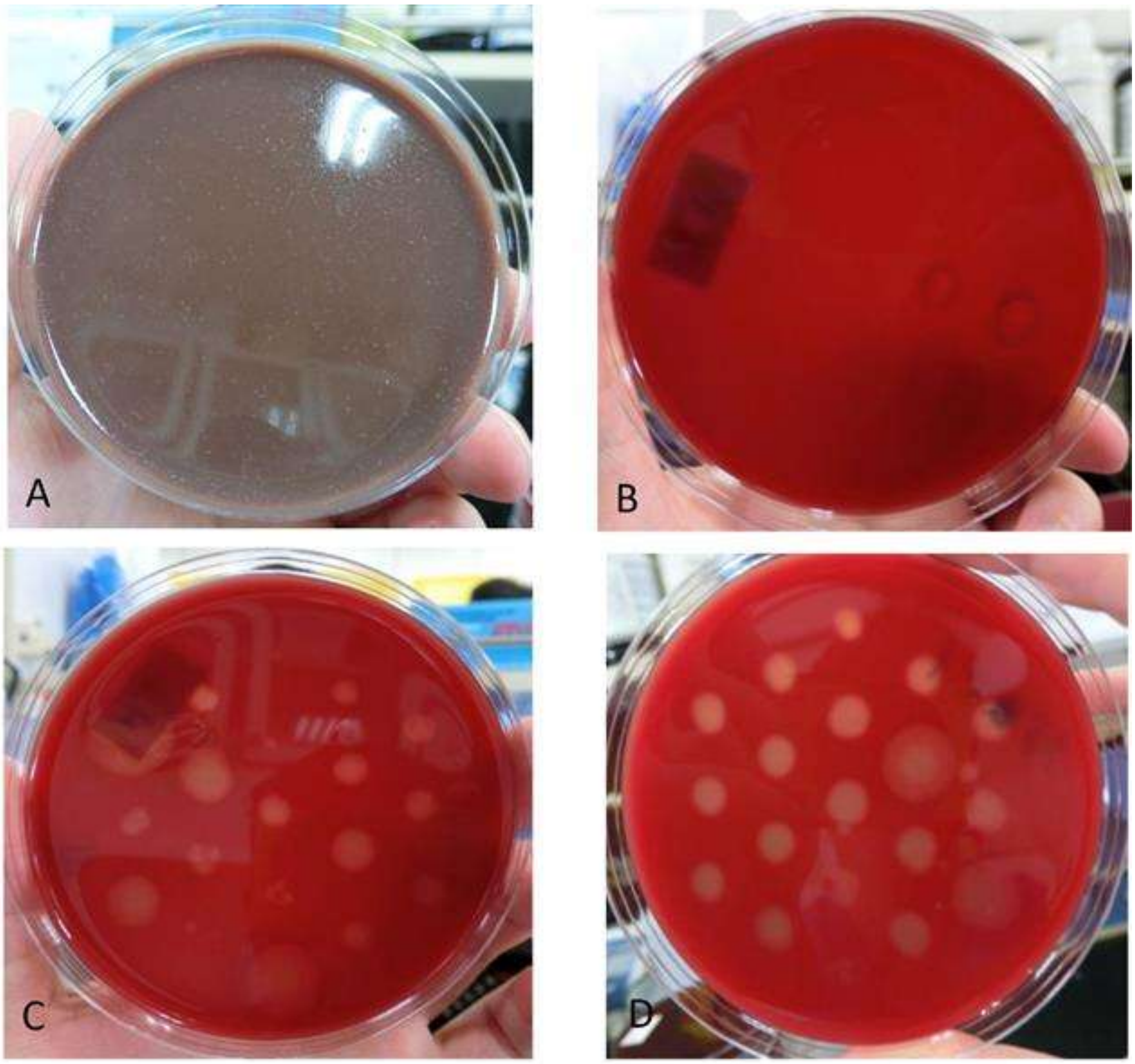
[2]*PATENT CO. DOO.*

The decreased susceptibility of Brachyspira hyodysenteriae, a causative agent of swine dysentery (SD) to antibiotics worldwide (1,2) led to increased scientific and market interest in alternative solutions to control SD.
The aim of this study was to test in vitro antibacterial efficiency of the plant-based feed additives mixture PATENTE HERBA® PLUS (PHP) that already show to control swine dysentery in in vivo conditions (3,4).
Laboratory 1. in Taiwan (Lab1) analyzed the minimal inhibitory concentration (MIC) of the PHP dilutions from 0.001% to 1,6% (2-fold dilutions), expressed in % (v / v) against ATCC 27164 strain and 37 Taiwan isolates of Brachyspira hyodysenteriae following procedures by CLSI document M11-A8. MIC of the PHP dilutions from 0.0125% to 6,4% (2-fold dilutions), expressed in % (v / v), against ATCC 27164 B.hyodysenteriae strain, Brachyspira pilosicoli ATCC 51139 strain and Clostridium perfringens type a-cpb2 were determined using the broth microdilution susceptibility test in laboratory 2 in Italy (Lab2) following the procedures indicated by CLSI M31-A3.
Lab 1. and Lab 2 showed that MIC of PHP against bacterial strain ATCC27164 was 0.2%. Interestingly the MIC of PHP against 35 strains of B.hyodysenteriae isolates from Taiwan was 0.1%. The MIC of the PHP against B. pilosicoli was at 0.2% while the MIC of PHP against Cl. perfringens was 0.1%.
The results of our in vitro testing support our in vivo findings where PHP product showed to be as effective as the combination of Tiamulin/Lyncomicin against SD (4). Our further research will be focused on evaluating PHP efficiency against Cl. perfrigens and B. pilosicoli affected pigs.
Acknowledgment: In some countries, the premixture of feed additives PATENTE HERBA® PLUS is registered as DYSGUARD-S®. The 2. laboratory belongs to Istituto Zooprofilattico Sperimentale Della Lombardia e dell'Emilia Romagna – IZSLER - Italy

Figure 1. The growth of Brachyspira hyodysentery strains under different concentrations of PATENTE HERBA® PLUS (PHP); A) At a concentration of 16000 ppm, PHP has an antibacterial effect against all strains of Brachyspira hyodysenteriae; B) At 1000 ppm of PHP, the standard strain ATCC 27164 and two field strains grew normally; C) At 500 ppm of Patente Herba, all strains grew normally; D) All strains grew normally in the positive control group.

References:
1- Hampson & Burroug, Diseases of swine, 2019.
2- Yeh et al., Microb. Drug Resist. 2017.
3- Delic et al., Acta veterinaria, 2018.
4- Wu et al., IPVS2020

Graphs or Images 1



P-B-097

USE OF TONSILLAR BRUSHING SAMPLES ON FTA® CARDS FOR SCREENING AND DETERMINATION OF INTRA-HERD PREVALENCE OF ACTINOBACILLUS PLEUROPNEUMONIAE IN A GERMAN FARM

Dr. Markus Rahbauer[1], Dr. Michael Faderl[1], Dr. Christina Söckler-Lionetti[2], Dr. Kathrin Lillie-Jaschniski[3], Dr. Preben Mortensen[3], Dr. Wei-Fan Kwan[4]

[1]Tierarztpraxis Scheidegg, [2]Ceva Tiergesundheit GmbH, [3]Ceva Corporate Swine, [4]Ceva Animal Health, Taiwan

Background and Objectives

The use of FTA® cards for the examination of lung samples for A. pleuropneumoniae (App) by capsule specific multiplex PCR (cps-mPCR) typing is already established (Stringer et al. 2021). Especially for countries like Germany, where necropsies on farms are not allowed, this might not be a tool for routine diagnostics. The aim of this study was to examine whether detection and typing of App DNA by cps-mPCR in tonsillar brushings sampled on FTA® cards is possible.

Material and Methods

In a 1200-finishers farm a total of 45 pigs, 15 animals per each age group of: A 40 kg, B 60 kg, and C 80 kg, were sampled by tonsillar brushing. Interdental brushes were used, attached to insemination catheters. Samples of five animals were applied pooled on one FTA® card field and air-dried at room temperature. The cards were shipped to the laboratory (IVD GmbH, Seelze-Letter, Germany) uncooled each inside a clean plastic bag in an envelope and examined by App-PCR (App DNA detection) as a first step, and by cps-mPCR (App typing) as a second step.

Results

In the App-PCR the 3 pooled-sample fields of group A come out: negative, negative, and weakly positive (weak), group B: weak, positive, and weak, group C: weak, positive, positive, respectively (Table 1). All positive samples, one of 60 kg and two of 80 kg pigs were typed as App-serovar 7 in the cps-mPCR.

Discussion and Conclusion

This pilot study demonstrates that detecting and typing of App DNA in tonsillar brushing samples on FTA® cards is possible, indicating a useful non-invasive alternative for screening and determination of intra-herd prevalence of App in farms. Further investigations to fully understand for validating this method need to be done.

Table 1

Field FTA® card	40 kg	60 kg	80 kg
1	negative	weakly positive	weakly positive
2	negative	positive	positive
3	weakly positive	weakly positive	positive

Table 1: Detection of App DNA by PCR from tonsillar brushing samples applied to and transported via FTA cards.

P-N-001

A CASE OF TREATMENT OF TAIL BITING IN NURSERY PIGS UNDER THE BACKGROUND OF AFRICAN SWINE FEVER IN CHINA

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Introduction
Since African swine fever (ASF) was found in China in August 2018, it has caused a great impact on Chinese pig production. If a farm is suspected to be ASF positive, silent management measures will be taken: stop almost all the productive activities including herd transferring, vaccinating, piglets' surgical castration and tail docking, until it is confirmed that all pigs are ASF free without risk in the farm (1). Tail biting is one of pig's vices, resulting in wounds and hemorrhages, infecting and even death. This study was to describe a case of tail biting in nursery pigs under background of ASF in China.

Materials and Methods
In June 2021, incidence of tail biting increased sharply in a large-scale farm with 5,500 sows in Eastern China. Three week batching system was adopted in the farm. The problem of tail biting in batches exceeded 50%, and even reached 70% in some pens (Figure 1). Mortality exceeded early-warning threshold of the farm, wounds could be found on tails and flank of dead pigs (Figure 2). To investigate the root cause, the veterinary team entered the farm and found: in order to prevent African swine fever, the farm had adopted silent management measures for more than one month. Weaned piglets without tail docking and no nursery pigs transferring resulted in high density of nursery unites (>30 pigs/6.48 sq. m) and all pigs with full length tails. The pigs are stressed environmentally and behaviourally. There were no toys or other distractions in the pens. The offending pig hadn't been removed from the group immediately (Table 1).

Results
After the communication between the veterinarian and farm team, a series of measures were taken immediately: providing toys; transfer 50% of the pigs to substitute nursery building, resume piglets' tail docking in the farrowing house. Due to condition limit, no more water tanks or troughs were added, and the feed formula was not changed either. However, after implementation of above veterinary measures, the tail biting problem was solved and did not occur in the later period. The mortality in nursery barns returned to normal also.

Conclusions and Discussion
Tail biting is one of the pig's vices caused by many factors. Farms can significantly reduce tail biting by taking active and effective measures. Under the background of African swine fever, some unconventional procedures that pig farms are forced to take under special circumstances need to be restored to normal as soon as possible to minimize adverse losses.

Acknowledgement and References
(1) Timothy Wier. ASF in China: A Veterinarian's First-Hand Perspective. The Pig Site. 20th October 2021.

Table 1

Table 1: Tail biting investigation and veterinary advice

No.	Check list	Investigation finding	Veterinary advice
1	Tail docking	No	Commence
2	Light turning off at night	No	Commence
3	Distractions in pens	No	Provide
4	Air quality	Good	Continue
5	Water and feed supply	Inadequate	Increase
6	Herd density	$<0.23m^2$/pigs	Halve
7	NaCl concentration in feed	0.3%	Increase to 0.75%

Graphs or Images 1

Fig.1 Tail biting

Graphs or Images 2

Fig.2 Wounds of tail and flank

P-N-002

A CASE REPORT: A CASE OF SUSPECTED GROWTH RETARDATION CAUSED BY ZINC TOXICOSIS

Dr. Yuko Kazuno[1], Dr. Sayoko Ishizeki[1], Dr. Hiromichi Ishikawa[1]
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Introduction:
Supplementing zinc oxide or zinc carbonate at 2000-3000 ppm for the first few weeks after weaning is common practice in the United States and some other parts of the world to control diarrhea and improve growth performance. However, it was also reported that feeding highly bioavailable sources may potentiate toxicity [1].

Materials and Methods:
The case farm is a conventional one-site system and has 300 sows. In October 2019, 75-day-old pigs showing severe growth retardation increased in the nursery barn (Image1). One of them was collected for etiologic diagnosis and histology. The cause of growth retardation could not be identified, but bacterial infection was suspected, and treatment with medication was started. However, since severe growth retardation continued even after that, in January 2020, a 75-day-old pig was collected again and blood was also collected from 3 piglets in the same group and subjected to serum biochemical tests.

Results:
Serum biochemical test results indicated that all three pigs had high lipase levels, suggesting pancreatitis. Chronic pancreatitis and apoptosis of pancreatic acinar cells were also observed in the two pigs subjected to histology. In addition, zinc stain (ZnAF-2) of the pancreas showed accumulation of zinc consistent with necrotic acini (image2). After confirming the usage of zinc carbonate at the farm, it was found that zinc carbonate was added to feed without weighing, and there was a possibility that zinc carbonate was added at least 6000 ppm for two weeks after weaning. After that, when zinc carbonate was weighed and adjusted to 3000 ppm, the occurrence of growth retardation was no longer observed.

Conclusions and Discussion:
It was found from this case that supplementing zinc carbonate at a high concentration may cause growth retardation due to zinc toxicosis, similar to the previous report on zinc toxicosis accompanied by pancreatitis 2. However, based on the other report that apoptosis was observed in pancreatic acinar cells, but no effect on production performance was observed 3, it is inferred that zinc carbonate concentration above a certain level affects production performance. It is important to measure the weight of zinc carbonate correctly and add it to the feed uniformly at the correct concentration as a disease control within the range that does not affect the production results. Since no water quality test was conducted at the time when growth retardation was observed, it is difficult to deny the possibility that there was a problem with water quality.

Acknowledgement and References:

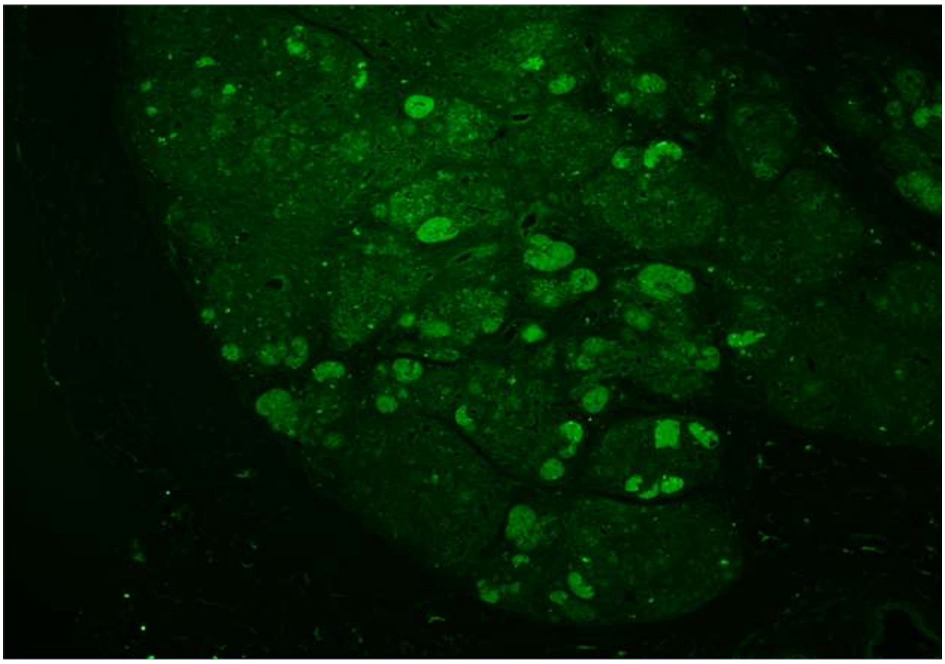
１） Jeffrey J. Zimmerman, et al. Diseases of swine 11th edition, 1052, 1073

２） Tomoyuki Shibahara, et al. Chronic pancreatitis in farmed pigs fed excessive zinc oxide. Journal of Veterinary Diagnostic Investigation 2020, Vol. 32(5) 689–694

3) Eric R. Burrough, et al. Zinc overload in weaned pigs: tissue accumulation, pathology, and growth impacts. Journal of Veterinary Diagnostic Investigation 2019, Vol. 31(4) 537–545

Graphs or Images 1

Graphs or Images 2



P-N-003

A CASE REPORT: EFFECT OF WATER QUALITY IMPROVEMENT BY A WATER PURIFICATION DEVICE

Dr. Keisuke Hane[1], Dr. Risa Shibayama[1], Dr. Megumi Waga[1], Dr. Yuko Kazuno[1], Dr. Sayoko Ishizeki[1], Dr. Yasuo Takeuchi[2], Dr. Hiromichi Ishikawa[1]
[1]Summit Veterinary Services, Ltd., [2]Mitsubishi Chemical Corporation, Advanced Solutions Business Group Strategy & Planning Division Advanced Solutions Planning Division

Introduction
There are many pig farms in Japan using well water as drinking water, and pig producers are required to conduct water quality tests and to carry out disinfection as necessary. It was confirmed that one of the three wells used for drinking water exceeded the standard value (for human) at a pig farm in the Kanto region of Japan, thus we improved the water quality using a water purification device for disasters. Before and after improvement, we investigated the change in water quality and the production performances of pigs. And the heavy metals and trace ions contents in the hairs were measured and compared.

Materials and Methods
The case farm is a conventional one-site farrow-to-finish system and has 800 sows. We used the water purification device for disasters (Mitsubishi Chemical Corporation). 65 pigs (born November 27 - December 3, 2020) that drank water without purification were Group 1 (control group), and 66 pigs (born August 19- August 25, 2021) that drank purified water were Group 2. These two types of water were given from weaning to shipping. We compared postweaning mortality and average daily gain (ADG) between Group 1 and Group 2. Before shipping, hairs were collected from 5 pigs that drank unpurified water and 5 pigs with purified water. The contents of arsenic, manganese, and iron which exceeded the standard value for water quality were measured in them. The water quality tests were conducted by an approved laboratory.

Results
The water quality test results before erecting the water purification device showed that seven items (general bacteria, arsenic, iron, manganese, odor, chromaticity, and turbidity) exceeded the standard value for human use. But after purification, all items became below the standard value (Table 1). Postweaning mortality of Group 1 and Group 2 were 28% and 20%, and ADG were 601g and 639g respectively ($P<0.05$). The hair test results showed that only the arsenic content was significantly decreased in the pigs that drank purified water ($P<0.05$).

Conclusions and Discussion
Under the conditions of this case, postweaning mortality and ADG became better by improving the water quality with the water purification device. Arsenic in the hair was significantly reduced, which may have contributed to the improved production performance. If water quality is not optimal, it may improve production performance by water quality improvement.

Table 1

Table 1 The results of water quality inspection before and after purification

Item	The drinking water used by Group 1 (before purification)	The drinking water used by Group 2 (after purification)	The standard value (human)
General bacteria	Over 300 counts /ml	0 count /ml	≤ 100 counts /ml
Arsenic and its compounds	0.017mg/L	< 0.001mg/L	≤ 0.01mg/L
Iron and its compounds	12mg/L	< 0.03mg/L	≤ 0.3mg/L
Manganese and its compounds	1.0mg/L	0.015 mg/L	≤ 0.05mg/L
Odor	Medicinal odor	Not abnormal	Not abnormal
Chromaticity	64.1 grades	1.3 grades	≤ 5 grades
Turbidity	24.3 grades	< 0.1 grades	≤ 2 grades

P-N-004

A PRELIMINARY: THE EFFECTIVE CLEANSER ON THE NUMBER OF TOTAL BACTERIAL COUNT IN THE SOW FARROWING PENS

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Introduction:
Piglet pre-weaning mortality is a major problem in swine industries, especially in infectious diseases from various pathogens such as E. coli, Clostridium perfringens, Coronavirus and Cryptoisospora suis [1]. The proper cleanser is a crucial role in decreasing piglet pre-weaning mortality by decreasing bacteria, viruses and protozoa. In general, the cleanser was classified into many types by active ingredients. Therefore, the objective of the present study aims to determine the effect of the type of cleanser in a commercial swine farm on the total number of bacteria.

Materials and Methods:
The 3 active ingredients of cleanser were tested in the farrowing pen in a commercial swine farm in Thailand included Linear Alkylbenzene Sulfonate (A), Sodium hydroxide (B) and Sodium Lauryl Ether Sulfate (C). A total of 8 samples of feces were collected in the present study. The feces were collected by sterile cotton swabs and the number of total bacteria before and after using the cleanser were determined. The number of total bacterial were log-transformed before being analyzed. The number of total bacterial count transformation were regarded as a dependent variable and were analyzed by using the general linear models (GLM) procedure of SAS. $P < 0.05$ was regarded to be statistically significant.

Results:
The total bacterial count before and after each type of cleanser is presented in Figure 1.
Figure 1 The number of total bacterial count (log cfu/ml) before and after using cleanser.
The Figure 1 demonstrated that cleanser B has shown a significant decrease in the total bacterial count ($P = 0.044$).
Figure 2 The percentage of reduction of the number of total bacteria count of each cleanser.

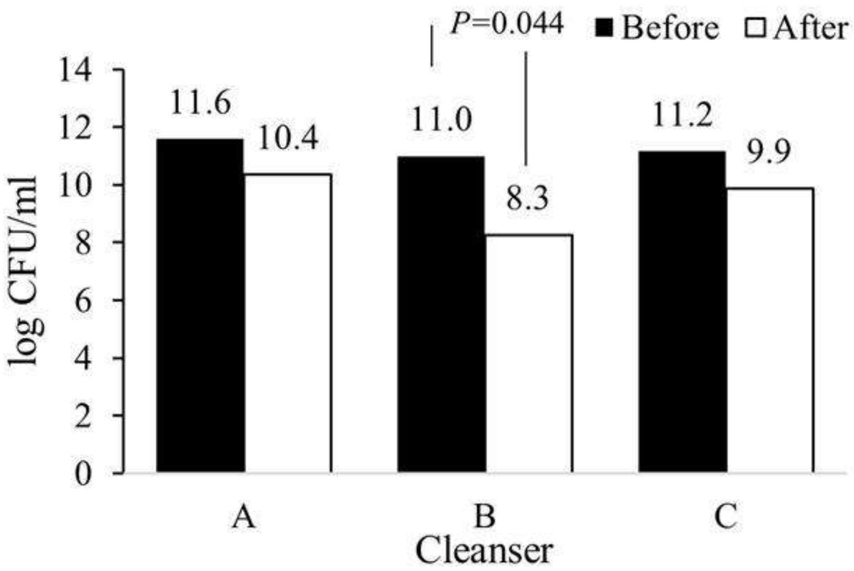
The percentage of reduction of the number of total bacteria count of each cleanser is presented in Figure 2. This figure indicates that cleanser B has highest the percentage of reduction of the number of the total bacteria count ($P > 0.05$).

Conclusions and Discussion:
The most effective cleanser on the total bacterial count was found to be sodium hydroxide, which was usually used for cleaning and sanitizing. The anionic surfactant, Linear Alkylbenzene Sulfonate and Sodium Lauryl Ether Sulfate, has a low effective cleanser on the total bacterial count. However, sodium hydroxide is corrosive, using the sodium hydroxide should be carefully used without animals during the cleaning.

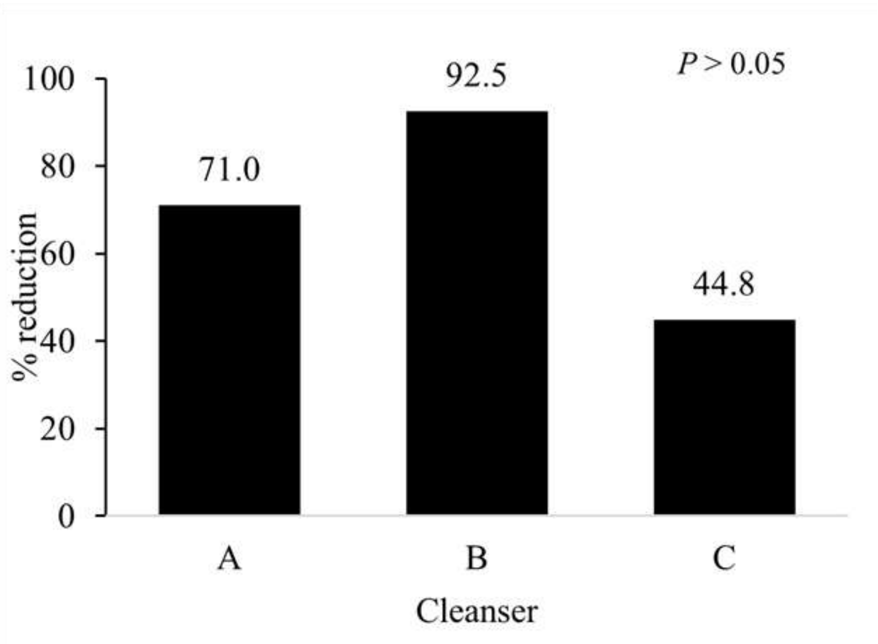
Acknowledgment:
Financial support for the present study was provided by The Faculty of Veterinary Science, Chulalongkorn University.

References:
[1] Zimmerman et al., 2013. Diseases of Swine, 11th Ed. Wiley-Blackwell, New Jersey.

Graphs or Images 1



Graphs or Images 2



P-N-005

A SYNERGISTIC BLEND OF ORGANIC ACIDS IS EFFECTIVE IN ENHANCING THE GROWTH PERFORMANCE OF WEANED PIGS

Dr La Van Kinh[1], Dr. Lane Pineda[2], Dr Yanming Han[2]
[1]Center for Tropical Animal Science, [2]Trouw Nutrition

Introduction
Weaning is a stressful event in pigs' lives with a cumulative effect on gut health, immunity, and post-weaning growth performance. Traditionally, antibiotics (ATB) have been used as dietary supplements to prevent weaning-associated stress and stimulate piglet growth. However, public concerns about the emergence of antimicrobial resistance have led to the assessment of alternative approaches. In this context, alternatives like the synergistic blend of feed additives (FA) have been regarded as a novel antibiotic replacement because of their health-protecting and growth-promoting properties [1, 2]. A synergistic FA, like antibiotics, has a broad antimicrobial effect and supports a functional gut necessary to enhance animal performance [1, 2, 3]. This study was designed to investigate the effects of a synergistic blend of FA based on free and buffered short and medium-chain fatty acids (SGG) (Selacid GG MP, Selko, The Netherlands) as a replacement for antibiotics on the growth performance and economics of weaned piglets under commercial farm conditions.

Materials and Methods
A total of 1200 (600 males and 600 females) weaned pigs with an average initial weight of 7.8 kg were allocated to one of three dietary treatments with 10 replicates of 40 pigs each. The treatments tested included (1) NC, a basal diet without antibiotics, (2) ATB, a basal diet with 250 ppm BMD + 300 ppm Halquinol/ton feeds and (3) SGG, a basal diet with 3 kg SGG/ton feeds. The study was conducted on a commercial farm and the pigs were reared in a closed housing system. The pigs were given a two-phase diet based on corn-soya over a 35-day feeding period. The zootechnical performance was measured at the end of each feeding phase and over the entire experiment. On the last day of the experiment, the cost to produce a kilogram live weight (LWG) was calculated. Data were analyzed using the MIXED procedure in SAS.

Results
Over the entire nursery period, the growth performance of pigs supplemented with a synergistic blend of FA (SGG) was significantly better compared with pigs given an antibiotic (ATB) and basal diets (NC) ($P<0.05$, Table 1). Pigs supplemented with SGG had greater ADFI (+2.1%) and ADG (+4.4%) than those pigs given an ATB diet. In addition, pigs were more efficient in converting feed into meat, with lower FCR when supplemented with SGG ($P<0.05$). Relative to ATB treatment, SGG reduced the FCR by 3.6 points (2.3%, Figure 1). The feed cost per kilogram LWG was lower (USD 1.08 vs 1.10) in pigs supplemented with SGG compared with the ATB group, indicating the economic viability of the supplement.

Conclusion
Dietary supplementation of a synergistic blend of FA (SGG) is a cost-effective replacement for in-feed antibiotics that can enhance feed utilization and support the growth performance of weaned pigs under commercial farm conditions. The growth-promoting efficacy of ATB was not observed in this study and may imply possible antibiotic resistance at the farm.

References:

1. Long et al. 2018. Anim Feed Sci. 235: 23–32.
2. Han et al. 2018. Livestock Sci. 216:210–218.
3. Liu et al. 2018. Anim Nutrition 4:113-125.

Table 1

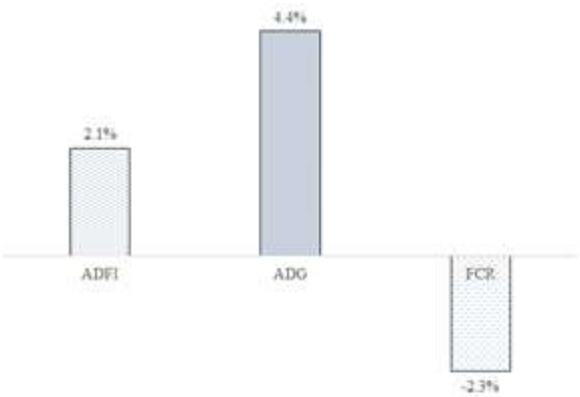
Table 1. Growth performance and economics of pigs supplemented with SGG or ATB during the entire nursery period (d1-35)

Parameters	NC	ATB	SGG
ADG, g/d	387.6^{b}	383.0^{c}	399.8^{a}
ADFI, g/d	607.3^{b}	609.6^{b}	622.3^{a}
FCR, kg/kg	1.568^{ab}	1.594^{a}	1.558^{b}
USD/LWG	1.07	1.10	1.08

^{a,b}Means in a row not having the same superscript are significantly different ($P < 0.05$).

Graphs or Images 1

Figure 1. Performance improvement in pigs given a synergistic blend of organic acids (SGG) in relation to ATB.



P-N-007

COMPARATION OF ALTRENOGST USAGE IN GILTS WITH OR WITHOUT PUBERTY

Dr Di Gao[2], Dr Ying Wang[2], Dr. Hong-Yao Lin[1]
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Introduction
In modern farm management there is a high need for standardization of processes to improve productivity and labor efficiency. Estrus synchronization in gilts commonly improves farm productivity. Altrenogest is a synthetic progesterone analogue which can stop terminal follicular growth and maturation through reduction of LH concentrations and therefore extends the luteal phase. When treatment is stopped after 18 days, LH secretion resumes promoting a synchronized estrus in all treated gilts. Responses amongst gilts may vary depending on their puberty status. The aim of this study was to understand the best types of animals to use Altrenogest in.

Materials and methods
Regumate (MSD Animal Health) and another local Altrenogest product were used. 332 gilts (approximately 210 – 230 days age) in two farms were divided into 4 groups as per Table 1.

Results
Results are reproduced in Table format (Table 2)

Conclusion
Gilts with established prior heat have a higher estrus rate after the treatment of altrenogest. It is probable that gilts without a prior heat by 210 days may have reproductive issues or a lack of boar exposure. That would lead to low estrus rate after the treatment of altrenogest as the hypothalamic pituitary gonadal axis may not be fully developed[1]. The results suggest that the most effective use of altrenogest is to treat sexually mature gilts with at least one confirmed heat. The results also suggest that beside synchronization of estrus, altrenogest also may improve farrowing rate and litter size. This result is in accord with the study of Martinat-Botté.[2]

References
1. Plant TM. 60 YEARS OF NEUROENDOCRINOLOGY: The hypothalamo-pituitary-gonadal axis. J Endocrinol. 2015 Aug;226(2):T41-54.
2. Martinat-Botté F., Bariteau F., Forgerit Y., Macar C., Poirier P. and Terqui, M. (1995a), Synchronization of oestrus in gilts with altrenogest: effects on ovulation rate and foetal survival. Animal Reproduction Science 39, 267-274

Table 1

TABLE 1

Group	Gilt (No & heat record)	Treatment
A	130, with prior heat	Heat check and mate, no Altrenogest
B	63, with prior heat	Regumate® 18 days
C	65, no prior heat	Regumate® 18 days
D	74, no prior heat	Another Altrenogest product 18 days

Table 2

TABLE 2

Group	Estrus% after treatment	35 d conception rate	Total born	Born alive
A	100%	80.8%	12.9	11.8
B	100%	90.3%	13.1	12.4
C	69.2%	100%	13.8	13.5
D	74.3%	75.6%	12.7	12.0

P-N-009

COPING WITH NEONATAL DIARRHEA BY USING TANNIFIT PLUS: ITS EFFECT ON PIGLET DIARRHEA AND MORTALITY RATE ON DAY 5

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Introduction

Colibacillosis is one of the most important pathogens causing diarrheal disease in neonatal and weaned piglets (1, 2). The E. coli is particularly problematic in pig farms. Using antibiotics to control disease led to increasing selection pressure and resulted in the selection of antibiotic-resistant bacteria (2). There are many alternative products for preventing diarrhea caused by E. coli. TanniFit Plus, dietary supplementary feed for stabilization of fluid and electrolyte balance of the piglet to support the physiological digestion, is consisted of botanical extract, Boswellia serrata extract with its effect on anti-inflammatory in the intestine and chestnut tannins which are known for its astringent, decongestant effect in the intestine, alternative to antibiotic and can increase the appetite of sick piglets (4). Thus, we performed oral supplementation the piglets with TanniFit Plus and comparing with using antibiotic, and investigate for the number of piglet diarrhea and mortality rate on day 5 of age.

Material and Methods

In this study, we selected the 3,600-breeding sow herd with 1-2 parity that had incident of Colibacillosis in the farrowing house. The piglets showed yellowish diarrhea during 1-3 days of age and found in poor condition later on. The animal care keeper and Veterinarian tried to provide the piglets with Colistin orally for preventing them from diarrhea. However, the recovery rate of the piglet is very slow. As a result, we performed the following experiment. Altogether 19 sows were divided into 2 groups: the piglets of 10 sows (group 1, control) were fed oral antibiotic (1 ml of colistin) with a dose of 36.00 mg/pig on day 1 of age; the piglets of 9 sows (group 2, TanniFit Plus) were supplemented once daily with 2 ml of TanniFit plus (XVET GmbH, Hamburg, Germany) during days 2-4 of age. The total number of piglets born, number of piglets born alive, stillborn piglet, mummified piglet and litter birth weight were recorded. The average number of piglet diarrhea and average number of piglet death per sows were also recorded. The student T-Test was used to compare the difference of means between control and TanniFit Plus groups. The P value ≤ 0.05 was considered statistically significant.

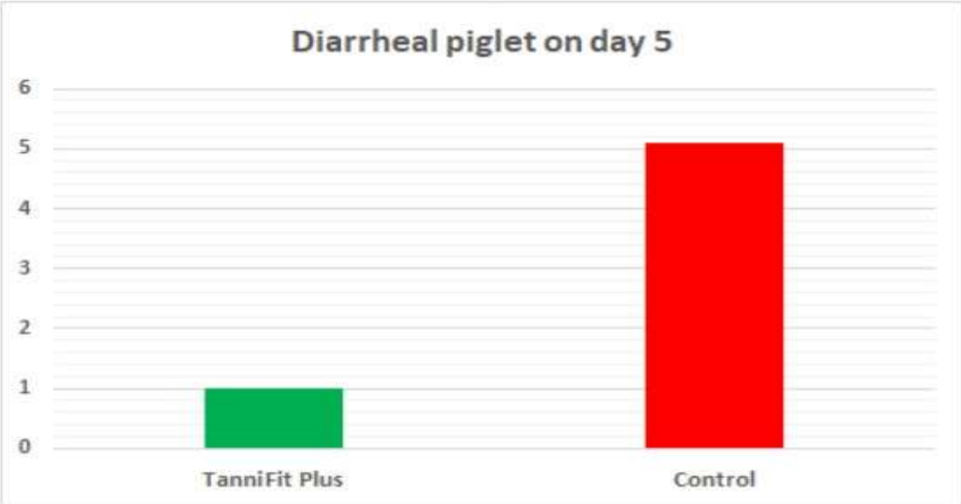
Results and Discussion

The average total number of piglets born, number of piglets born alive, stillborn piglet, mummified piglet and litter birth weight in control and TanniFit Plus are as follows: 14.2 vs 14.0; 11.20 vs 11.11; 1.50 vs 0.89; 1.50 vs 2.50; 1.50 vs 2.00 and 17.40 vs 17.22, respectively. There was a statistically significant lower in the average number of piglet diarrhea on day 5 of age in TanniFit Plus group (1.0 piglet/sow) than control group (5.1 piglets/sow) ($P \leq 0.01$) (Figure 1). A tendency of a lower average number of piglet death on day 5 of age in TanniFit Plus group (0.33 piglet/sow) was found when compared with control group (0.99 piglet/sow) ($P = 0.2$) (Figure 2). From the present results, it is clearly showed that piglet received TanniFit Plus, containing chestnut tannins and Boswellia serrata extract, not only can stabilized fluid and electrolyte balance but also decreased an inflammatory of the intestine, resulted in improving intestinal barrier integrity and Function (3, 4), which lead to benefit a physiological digestion in the early life of suckling piglet, particularly during the occurring of Colibacillosis in the farrowing house.

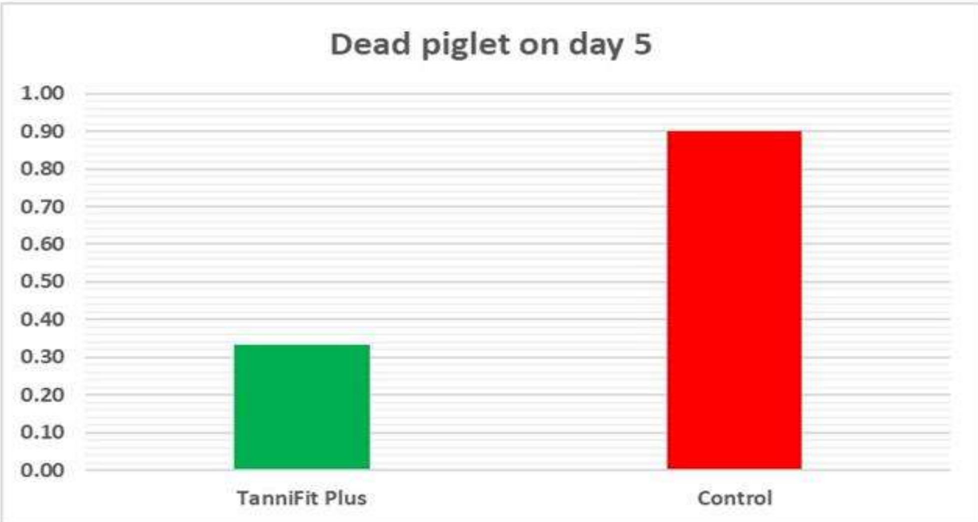
References

1. Hartadi et al. (2020) Sys Rev Pharm 11, 687-698.
2. Nguyet et al. (2022) Scientific Reports 12, 9038.
3. Yu et al. (2020) J Anim Sci Biotech. 11:8
4. Girad and Bee (2020), Animal 14 (1), 95–107.

Graphs or Images 1



Graphs or Images 2



P-N-010

CORRELATION BETWEEN PLACENTA WEIGHT AND SOW REPRODUCTIVE PERFORMANCE IN HYPER-PROLIFIC SOWS

Mr. Jakavat Ruampatana[1], Mr. Ponlatorn Rukklang[2], Mr. Worapatch Kongthong[3], Mr. Nattapon Luangprasert[3], Dr. Morakot Nuntapaitoon[1,4]

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Introduction:
Placenta contributes the fetal development in all animals. The placenta contains more vessels and tissue for the fetus. In sow, the number of piglets in each sow was higher than other species. The available uterine space and surface in sows were limited, especially in hyper-prolific sows [1]. This event enhanced the incidence of retarded and inhomogeneous offspring [2]. Therefore, the purpose of this study was to investigate the correlation between placenta weight and sow reproductive performance in hyper-prolific sows.

Materials and Methods:
The study was carried out in a commercial swine herd in Thailand, located in the western region of Thailand. The data from 50 Landrace x Yorkshire crossbred sows and 1382 piglets were recorded. The data of sow performances included the total number of piglets born per litter (TB), number of piglets born alive per litter (BA), litter weight at birth (LW) and piglet weight at day 1 (PW1). Pearson's correlation was performed by SAS 9.4 program. In addition, multiple ANOVA was used to evaluate the effect of BA (regression) on placental weight.

Results:
Descriptive statistics on sow reproductive performance was presented in Table 1.

The correlation between placenta weight and sow reproductive performance was shown in Table 2.

The placenta weight positively correlated with TB ($r = 0.281$, $P = 0.016$), BA ($r = 0.355$, $P = 0.002$) and LW ($r = 0.291$, $P = 0.040$). No correlation between placenta weight and PW1 was found.

The number of piglets born alive influenced placenta weight (Figure 1), with placenta weight increasing with higher the number of piglets born alive per litter.

Conclusions and Discussion:
The placenta weight was positively related to litter size and litter weight. The present study confirmed that the uterus was developed to support the fetus by increasing placental tissue development, blood supply and nutrient transportation [3, 4].

Acknowledgments:
Financial support for the present study was providing by the Faculty of Veterinary Science and a grant for Agricultural Research Development Agency (Public Organization) (M. Nuntapaitoon).

References:
[1] Bruessow and Waehner, 2008. Biotechnol. Anim. Husb. 80: 370-377.
[2] Zhang et al., 2016. J. Intergr. Agric. 15: 848-854.
[3] Pere and Etienne, 2000. Reprod. Nutr. Dev. 40: 369-382.
[4] Mesa et al., 2012. J. Anim. Sci. 90: 4217-4222.

Table 1. Sow reproductive performance (n = 73)

Table 1

	MEAN±SD	Min	Max
TB	18.9 ± 4.5	7.0	28.0
BA	16.7 ± 3.4	7.0	23.0
Placenta Wt.	2.4 ± 0.9	1.0	5.6
LW	19.9 ± 3.8	9.8	29.0
PW1	1.2 ± 0.2	0.8	1.7

Table 2. Correlation between placenta weight and sow reproductive performance

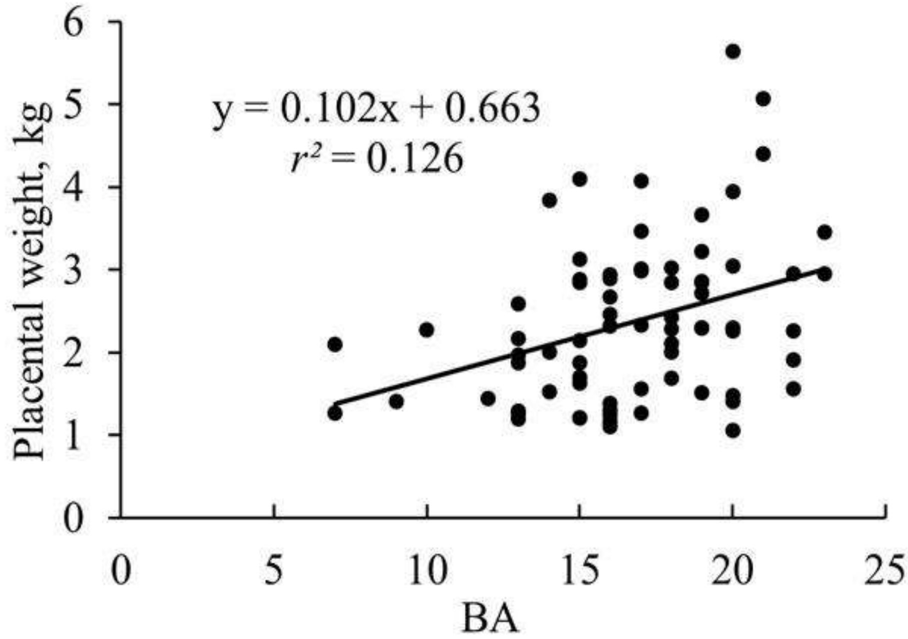
Table 2

	Placenta Wt.	TB	BA	LW
TB	0.281*			
BA	0.355**	0.779***		
LW	0.291*	0.405**	0.627***	
PW1	NS	-0.510***	-0.580***	NS

*P<0.05, **P<0.01, ***P<0.001
NS-Not significance

Figure 1. Relationship between a number of born alive piglets and placental weight.

Graphs or Images 1



P-N-011

DORSAL FAT REDUCTION IN PIGS WITH NATURAL CHOLINE SUPPLEMENT

Dr. Shivi Maini[1]
[1]Indian Herbs

INTRODUCTION
Pig farming is constantly changing. Since its technification in the 1970s, new knowledge and technologies are constantly being added to the farm routine. Indices such as feed conversion ratio (FC), daily weight gain (DWG), carcass yield (CR), absence of diseases and low mortality in all phases of production have always been considered critical in modern pig farming. The quality of the meat and the way the animals are reared (and fed) became decisive factors for consumers when choosing a product in the market.

MEAT
The consumer considers the following as indicators of pork quality: appearance, color, smell, taste, tenderness, amount of fat, etc .Pigs accumulate fat under the skin in the dorsal region. This deposition expressed in millimeters of thickness is a criterion to differentiate carcasses as lean or fatty. The market demands leaner carcasses with less back fat. To achieve this, Ractopamine, a β-adrenergic that reverses the rate of fat and muscle deposition in pigs close to slaughter, was used for a long time, resulting in less fat deposition at this stage and greater availability of nutrients for muscle formation. However, the use of this molecule is currently banned in global market and the industry is looking for alternatives to its use.

CHOLINE METABOLITES
Choline is commonly supplemented in monogastric diets because it is an essential component of the cell membrane phospholipids, participates in liver metabolism by preventing fat accumulation in the carcass, is a precursor of acetylcholine and is essential for a balanced and functional methylation cycle. Choline not only provides the choline moiety for phospholipid and lipoprotein synthesis, but also assists in the regeneration of methionine, thus saving methionine for protein synthesis. As such, choline supplementation ultimately supports the optimal functioning of fat and protein metabolism. The suggested minimum level of choline in pig diets is around 300mg/kg. When choline is ingested, it is metabolized to phosphatidylcholine, the molecule responsible for choline’s known effects. Phosphatidylcholine also plays an emulsifying role and activates cell receptors to mobilize fat.It has been shown in research that BioCholine (Natural choline supplement) is more effective than choline chloride in achieving levels of phosphatidylcholine capable of performing the functions attributed to choline. One of the reasons for BioCholine’sability to completely replace the presence of choline chloride, and at much lower doses, is that its phospholipid constituents bind to peroxisome

proliferator-activated receptors PPARα. PPARα are nuclear receptors, the more PPARα receptor activation, higher is adiponectin production. Adiponectin is an adipokine protein involved in lipid and glucose metabolism, glucose utilization and lipogenesis. To be activated, this adiponectin must bind to AdipoR1 and AdipoR2 receptors, expressed in insulin-sensitive tissues such as skeletal muscle, liver, pancreas and adipose tissue, where it increases AMP-dependent protein kinase (AMPK) activity, promoting fatty acid oxidation and the entry of glucose into tissues. It functions to remove fat from areas of fat accumulation (mainly liver in poultry and subcutaneous dorsum in pigs), to catabolize fat and to make nutrients available for other tissue formation. Animals supplemented with BioCholine ® from a young age activate PPARα receptors earlier and as adults the fat reduction effect is greater), the greater the effect of adiponectin on the animals. The mechanisms of action described have been verified by HPLC analysis and metagenomic studies (University of Georgia USA) and in Studies carried out in European and Latin American countries. It is validated that use of BioCholine ® not only improves production indices such as weight gain,FCR, mortality, but also reduces backfat in pigs . Recent studies validate BioCholine usage to reduce backfat and a complete replacement of choline chloride and rectopamine in pigs.

P-N-012

ECONOMIC BENEFIT OF COMBINING AN IMMUNOSTIMULANT AND A FMD VACCINE IN COMMERCIAL SWINE FARMS IN SOUTH KOREA

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[1]*Pig Management and Clinic*

Introduction
Foot and Mouth Disease(FMD) is one of the diseases with the highest economic losses to the pig industry (1). FMD pig vaccination is a government mandatory regulation in South Korea. Since this policy is performed, there are some side-effect like an abnormal meat loss at the vaccine injection site (e.g. granuloma).
An ultrasonicated injectable lysate of Corynebacterium(Ultra-corn®, Virbac, France) is an immune booster that is mainly focused on the nonspecific immune system. It could possibly help to increase FMD antibodies by combination with a FMD vaccine. And there is maybe a chance to minimize the impact of economic loss (abnormal meat). This could be assessed by a preliminary field trial comparing the combination of Ultra-corn® and FMD vaccine with FMD vaccine only in nursery pigs. It was the objective of this trial in Korean field conditions.

Materials and Methods
Two pig-farms were selected. In each farm, 2 nursery groups of 70 days old pigs received either the FMD vaccine alone or the combination between FMD vaccine and Ultra-corn® at the ratio of 50 ml + 6 ml (Farm A) or 50 ml + 4 ml (Farm B). Each pig received an intramuscular injection of 2 ml.
After vaccination, day post 1 inspection(DPI 1) was performed. A FMD antibody analysis was performed by random blood sampling of 25 pigs in each group at finisher stage. Economic effect analysis was also performed by comparing abnormal meat weight loss between groups. Farm B had two finisher farms.

Results
Day post 1 inspection criteria are shown in Table 1. The level of post vaccination clinical signs is low and similar between test group and control group.
A good FMD antibody status is achieved in all tested groups, without difference between test and control groups (Table 2).
For economical impact (abnormal meat weight loss), the effect of the combination is better in Farm A.

Conclusion and Discussion
There were limited and transient side-effects in DPI 1 whatever the vaccination group. For FMD antibody titers, the immunostimulant and vaccine combination had no negative effect compared with vaccine injection only. For abnormal meat weight loss, the combination of 6 ml immunostimulant + 50 ml of FMD vaccine gave better results. Reduction of abnormal meat weight loss means that the farm has a higher profit. Though interpretation of this trial should be cautious and pending confirmation by other trials, such combination could be beneficial for swine farms profitability.

References
(1) (Zimmerman, et al., 2019) Disease of swine, 11th

Keywords: Ultra-corn®, swine, FMD vaccine, field trial

Table 1

Table 1. Day post 1 inspection (DPI 1)[1] (unit : %)

Group[2]	vomiting	lethargy	anorexia	allergy	death	edema	erythema
A Test	**0.0**	**0.0**	**0.8**	**0.2**	**0.2**	**0.7**	**2.8**
A Control	**0.0**	**0.0**	**1.1**	**0.3**	**0.1**	**0.9**	**3.4**
B Test	**0.0**	**0.0**	**0.8**	**0.8**	**0.2**	**0.7**	**1.5**
B Control	**0.0**	**0.0**	**0.3**	**0.8**	**0.1**	**1.2**	**2.5**

[1] No clinical signs were observed in all groups on DPI 7(data not shown)

[2] Number of nursery pig in each group : A test(1,000), A Control (980), B Test(600), B Control (730).

Table 2

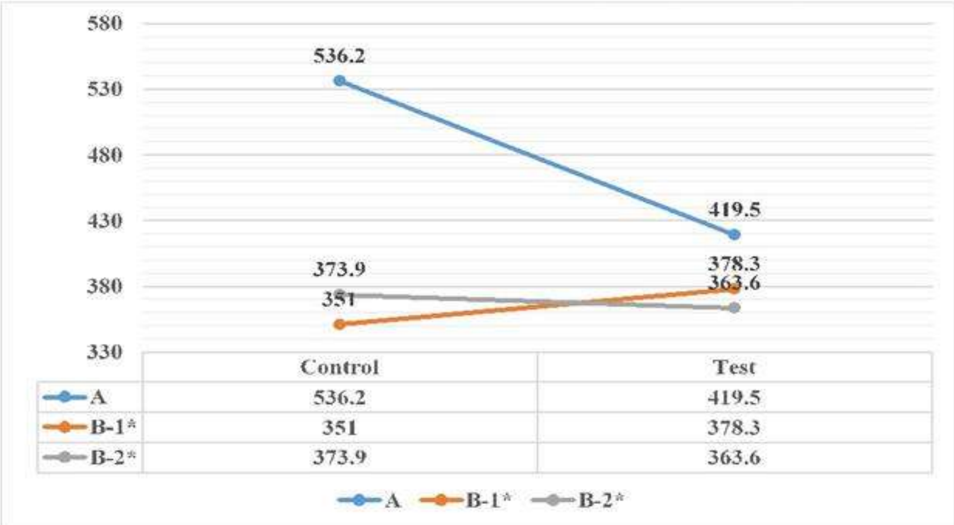
Table 2. FMD antibody status

Group	P[1]	N[2]	Sum[3]	%
A Test	**22**	**3**	**25**	**88**
A Control	**23**	**2**	**25**	**92**
B Test	**25**	**0**	**25**	**100**
B Control	**25**	**0**	**25**	**100**

[1][2] Percentage Inhibition above 50% is positive, below 50% is negative, [3] Random sampling of 25 finishers in each group.

Graphs or Images 1

Figure 1. Economical impact (abnormal meat weight cumulated loss per group, unit: kg)



***Farm B has two finisher farms.**

P-N-013

EFFECT OF AN ANTI-GONADOTROPIN RELEASING FACTOR VACCINE ON GROWTH PERFORMANCE OF FEMALE FINISHING PIGS IN THAILAND

Mr. Alongkot Boonsoongnern[1], Ms. Chonnatcha Muangpaisarn[2], Mr. Narut Thanantong[1], Mr. Pichai Jirawattanapong[1], Mr. Sahathat Phuttapatimok[1], Ms. Sunaree Nantakhruea[2], Ms. Tanyanant Kaminsonsakul[1], Mr. Wichian Navasakuljinda[2], Ms. Wilaiwan Pinitmontree[2], Ms. Yonlayong Woonwong[1], Ms. Pichanun Wongchanapai[2], Mr. Nattavut Rattanavanichroj[1]
[1]Department of farm resources and production medicine, Faculty of Veterinary Medicine, Kasetsart University, Kamphaeng Saen Campus, [2]Zoetis (Thailand) Limited

Introduction
Improvac® is an anti-Gonadotropin releasing factor (anti-GnRF) vaccine, recently indicated for temporary estrus suppression in female pigs (1). Several studies have shown that Improvac® impaired ovarian activities with positive effects on growth performance, and a decreased aggressive behavior that was commonly found in intact gilts during a late finishing period. Sexually maturing gilts in fattening phase which ovarian functions have begun were found to have a reduced feed consumption when compared to that of their male counterparts (2, 3).
The objectives of this study were to investigate zootechnical performance of crossbred finishing gilts vaccinated with Improvac® compared to non-vaccinated gilts, with or without 10% lower lysine and energy level of feed formulations.

Materials and Methods
196 crossbred finishing gilts (Large White x Landrace x Duroc) from a PRRS negative herd were individually identified and weighed at 9 weeks of age. The animals were randomly allocated into 4 groups. Forty-nine pigs in each group were randomly distributed into seven pens (7 pigs in each pen). Gilts in the two vaccinated groups (Group 3 and 4) were injected with two doses of Improvac® subcutaneously at 11 and 15 weeks of age. Pigs in groups 1 and 3 received 10% lower diet specification of lysine and energy level. The study design is shown in table 1.

Gilts were weighed before the changes of diets and at the end of the study (27 weeks of age), every gilt was individually weighed and analyzed for average daily gain (ADG).

Results
The growth performance outcome in each group is shown in table 3 ($\bar{x}$ + SD).

Conclusions and Discussion
The vaccinated groups, either with 10% low spec diet or standard diet possessed better growth performance parameters in ADG and a final market weight, than that of both non-vaccinated groups. Group 4 (vaccinated with standard diet) had the highest ADG at 933.91 gram/day and market weight at 140.10 kg. This study suggests that the best program for optimizing a growth performance in finishing gilts is Improvac® administration combined with a standard diet formula.

References
1. Bohrer BM et al. 2014, J. Anim. Sci. 4719-4724.

2. Castillo-Sagbay KA et al. 2016, Proc. 853, 24th IPVS, Ireland.
3. Rodrigues LA et al., 2019, Animal. 13:6: 1326-1331.

Table 1. Study design

Table 1

Group	N	Vaccine	Dose	Diet
1	49	na	Na	10% low spec diet
2	49	na	Na	Standard diet
3	49	Improvac®	2 ml/SC	10% low spec diet
4	49	Improvac®	2 ml/SC	Standard diet

Table 2. Experimental diet specification in finishing phase and feeding program

Table 2

Diet phases/ Age (week)	Dietary SD lysine level		Energy level (ME kcal/kg)	
	90%	100%		
Starter/9-15	1.25	1.25	3,300	3,300
Grower/16-21	1.035	1.15	3,050	3,150
Finisher/22-27	0.945	1.05	2,900	3,000

SC = subcutaneous

Table 3. Growth performance

Table 3

Group	ADG (gram/day)	Market weight (kg)
1	889.84 (68.09)^{a}	134.56 (9.81)
2	892.03 (73.04)^{ab}	134.76 (10.71)
3	902.30 (65.35)^{ab}	136.10 (9.69)
4	933.91 (70.90)^{b}	140.10 (8.96)
p-value	**0.05**	**0.21**

a,b Means with different superscripts were significantly different ($P < 0.05$).

P-N-014

EFFECT OF TWO CONVENTIONAL NSAIDS ON POSTPARTUM SOW BEHAVIORAL

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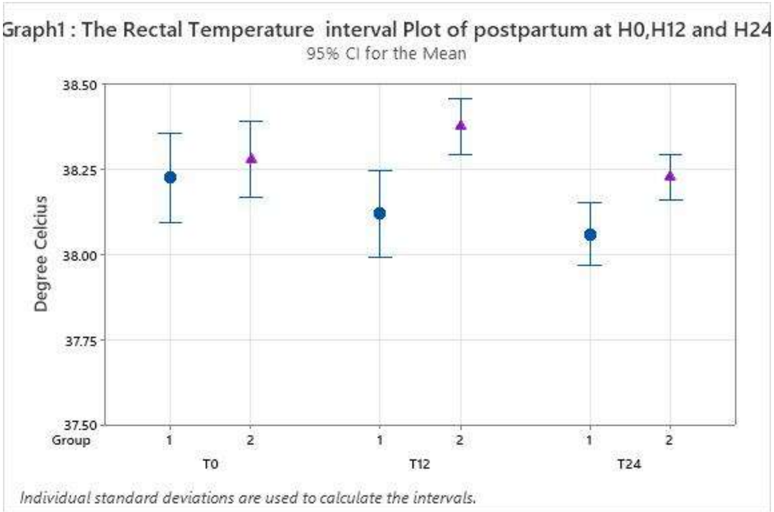
Introduction
Parturition is a crucial time for the sow, since pain and inflammation, as well as systemic events, contribute to postpartum stress. Use of NSAIDs during the post-partum sow is common practice to mitigates clinical Postpartum Dysgalactia Syndrome (PPDS) and has been documented by numerous studies1.
The objective of this study was to compare the efficacy and administration method of a single administration of Oral Meloxicam 15 mg / ml oral suspension for pigs versus a generic injectable Tolfenamic acid 4 mg / ml administered to sows.

Materials and Methods
The study was conducted on a 620 sow heads, farrow-to-finish with batch farrowing system, located in the Eastern part of Thailand. The total of 171 sows were randomly assigned to 2 groups.
Group T1 (81 sows) received an intramuscular injection of Tolfenamic acid 10 ml, while group T2 (90 sows) received 7 ml of meloxicam orally. Rectal temperature was monitored at H0, H12, and H24 during the first 24 hours after delivery. Feed consumption was measured throughout the initial 6 days. The statistical analysis of rectal temperatures and daily feed intake results performed using version17 of Minitab software.

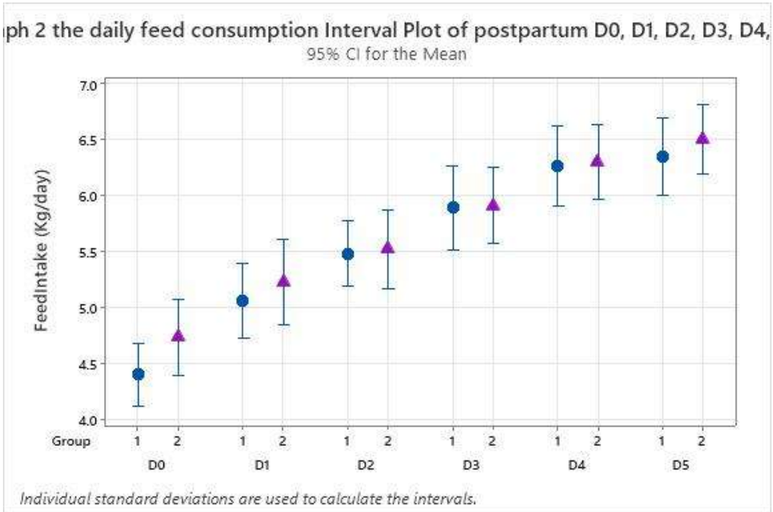
Results
There was no difference of the reduction in rectal temperature between the groups (T1&T2) at H0 and H24 as show in graph1. Graph2 shows the daily feed intake during observation first 6 days. There was the slightly differences overall of feed intake (T1:5.6 kg vs T2:5.7 kg)

Discussion
In comparison to injectable Tolfenamic acid injection (T1) and orally Meloxicam (T2), the results indicate that numerically difference effect on the behavior of sows, Tolfenamic acid can reduce rectal temperature during the first 12 hours faster than oral orally Meloxicam, which may not be related to relieve pain. These resulted in feed intake at first 24 hours of T2 sows higher than T1 sows. Therefore, an alternative to orally Meloxicam for pain and inflammation management in future pig farming, a needle-free treatment may be the best option. These are advantageous for animal welfare and disease transmission via needles.
Reference
1. C Gutierrez, D. Guinez, V.Grosse-Liesner, A. Ruiz, C Roudergue. Farrowing liveweight and ADGW in litters from sows treated with Metacam. IPVS. 2016 : p639

Graphs or Images 1



Graphs or Images 2



P-N-015

EFFECTS OF CATOSAL® INJECTION IN PIGLETS ON DAY 3 POST-PARTUM TO IMPROVE PIGLET PRODUCTIVITY IN SWINE FARMS IN NORTHERN REGION OF THAILAND

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Introduction
Worldwide, the piglet-production industry faces many metabolic abnormalities impairing performance parameters, including weaning weight (WW), average daily gain (ADG), and pre-weaning mortality. Catosal® is a source of phosphorus and vitamin B12 to minimize metabolic moment effects and improve piglet productivity.

Materials and Methods
The study was conducted in swine farms with respect to piglet productivity as determined by weaning weight, piglet uniformity, and pre-weaning mortality. The pig farm with 4,000 sows in production between September to October 2022 in northern Thailand was selected for the study.
On day 3 post-partum, a total of 16 sows with 200 piglets were selected and divided into two groups according to the farrowing and piglet characteristics, general health status, body condition score (BCS) between 3-4 (1-5 scale; Young et al.; 2001, parity between 2 and 3, average born-alive number of 12 piglet/sow, and average piglet birthweight of 1.34 kg. On day 3 post-partum, piglets in the control and treatment groups were treated with normal saline (0.5 cc) and Catosal® (0.5 cc), respectively. Individual piglet weights were recorded on days 0, 3, and 18. Total pre-weaning mortality, total creep feed, and commercial replacement milk were recorded on day 18. The percent pre-weaning mortality, average weaning weight (Day 18), ADG from day 3 to weaning, and ADG from birth to weaning were calculated, and statistical analysis was performed by JMP software. The quality of weaning piglets was classified into three grades; A >6 kg, B 4.5-6.0 kg, and C <4.5 kg, and the piglets with WW of <3.0 kg with skinny condition were classified into the pre-weaning mortality group.

Results and Discussion
There was a statistically significant difference in average WW (day 18), ADG (day 3-18), and ADG (day 0-18) between control and treatment groups (p<0.05). The average weight on day 18 in the Catosal® treated group (5.39 kg with %CV of 18.6%) was higher than the control (5.01 kg with %CV of 17%), ADG from birth to weaning of Catosal® (220 g/day) group was higher as compared to the control group (200 g/day), the ADG (day 3-8) in Catosal® group (270 g/day) was higher than the control group (210 g/day). There was no significant difference in the average pre-weaning mortality rate between control (16%) and Catosol® group (14%); however, the mortality in skinny group with < 3.0 kg BW piglets (classified as the mortality group) was lower in the Catosol® group (0.29%) than the control group (1.83%). Furthermore, more piglets were graded as A and B and a smaller number of piglets were graded as C in the Catosal® group (A=35%, B=55%, C 10%) compared to the control group (A=25%, B=50%, C=25%).

Conclusion
The data from the study confirm that Catosal® minimizes metabolic moment effects and improves piglet productivity, especially the piglet qualities and significantly improves

weaning weight, weaning piglets' quality and uniformity, and reduces low piglet quality. In conclusion, Catosal® injection (0.5 cc) at day 3 post-partum improves performance parameters at the farrowing unit and can provide long-term beneficial effects because of good quality weaning piglets.

Acknowledgment
This work was supported by Elanco Co. Ltd.

Reference

1. Lanfranchi E et al., 2008, Proc IPVS, P 479.
2. A Nuntaprasert et al., 2006, Proc IPVS.
3. Young et al., 2001, The relation between body condition score and backfat in gestation sows, Swine Day 2001

Table 1

Table 1. Effect of Catosal® on Production Performance

Group	Pre-weaning mortality (amount/%)	Average weight(kg)			ADG(g/day)	
		D0	D3	D18/%CV	BW-D18	D3-D18
Control (n=100)	16/16%	1.34	1.90	5.01^{a}/18.16	200^{a}	210^{a}
Catosal® (n=100)	14/14%	1.34	1.90	5.39^{b}/17.00	220^{b}	270^{b}

P-N-016

EFFICACY OF A COMBINED INJECTABLE TOLTRAZURIL AND GLEPTOFERRON (FORCERIS®) COMPARE WITH INJECTABLE IRON DEXTRAN AND ORAL TOLTRAZURIL UNDER A FIELD CONDITION IN THAILAND

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Introduction
Suckling piglet coccidiosis is an important cause of pre-weaning diarrhea (1). The parasite invades and destroys epithelial of the gut lead to diarrhea and significant reduction of weight gain (1). At the same time, iron deficiency anemia (IDA) is the most common nutritional deficiency. Piglets that did not receive an iron injection shortly after birth develop IDA resulting poor growth (2). It is common practice to prevent newborn piglets from IDA (2). Forceris® is a combined injectable toltrazuril and gleptoferron product which prevent both coccidiosis and IDA as a single intramuscular injection. Previous study demonstrated that treatment with toltrazuril together with iron by injection was safe, effective, and reduced handling of animal for medication (3). The aim of this study is to compare the efficacy to prevent coccidiosis and IDA in suckling piglets compare with traditional iron and toltrazuril administration under a field condition.

Materials and Methods
This study was performed in 4,400 sow size integrated farm. 234 piglets from 20 litters were divided in to 2 group as control and treatment group. Control group (123 piglets, 10 litters) were treated with injectable 200 mg iron dextran intramuscularly on the second day of life and were treated with toltrazuril 5% orally on the third day of life. Treatment group (111 piglets, 10 litters) were treated with 1.5 ml of Forceris® intramuscularly once on the second day of life. Three piglets (small, medium, large) from each litter (30 piglets each group) were randomly selected and measured for blood hemoglobin level using Hemocue® Hb 201+ (Hemocue AB, Sweden) on day 2, and 18 days of age (weaning age). Fecal samples were collected from 5 randomly selected piglets in each litter on day 14, and 18 days of age to detect oocyst excretion per gram using McMaster technique. Performance data were compared, and statistical analysis were performed using RStudio.

Results
Hemoglobin level of day 2 piglets were not different between control and treatment group (9.5 vs 9.8 g/dl, $p>0.05$) while treatment group had significantly higher when measured at weaning (10.2 vs 12.8 g/dl, $p<0.001$). Furthermore, treatment group had significantly higher percentage of optimal piglets (Hb level > 11.0 g/dl (2)) at weaning (43.3% vs 100.0%, $p<0.001$). Hemoglobin levels are demonstrated in figure 1. No oocyst detection observed from both control and treatment group on both 14 and 18 days of age. Average weaning weight was significantly higher in treatment group (5.2 vs 6.0 kg, $p<0.001$) and average daily gain (ADG) was also significantly higher in treatment group (222 vs 264 g/d, $p<0.001$). Performance data are demonstrated in table 1.
Conclusions and Discussions
Gleptoferron allows 4.6 times higher total iron absorption by the piglet than iron dextran (4). In this study, Forceris® provides higher hemoglobin levels and more optimal piglets at

weaning (Hb level > 11.0 g/dl). Even though there is no oocyst excretion observed in both group due to the effective treatment and probably low pressure under the good hygiene management of the farm, better growth performance of piglets from treatment group (Forceris®) as result of effective control of IDA and better hematologic status was observed under the condition of our study.

Acknowledgement and References

1. Niestrath M, Takla M, Joachim A, Daugschies A., J Vet Med B Infect Dis Vet Public Health. 2002 May;49(4):176-80.
2. Chevalier TB, Moneque HJ, Lindemann MD., J Swine Health Prod. 2021;29(4):189-199.
3. Joachim A, Shrestha A, Freudenschuss B et al., Parasites Vectors 11, 206 (2018).
4. Morales J, Manso A, Martín-Jiménez T, et al., J Swine Health Prod. 2018;26(4):200-207.

Table 1

Index	C	T	Diff	p-value
Parity	1.2	1.2	0	1
Birth weight	1.23	1.24	0.01	0.6368
Weaning weight	5.23	6.00	0.77	5.692E-09
ADG	222	264	42	1.05E-14
Preweaning mortality rate	5.7%	3.6%	-2.1%	0.5451

Table 1. Comparison of sow's performance data between control (C) and treatment (T) group.

Graphs or Images 1

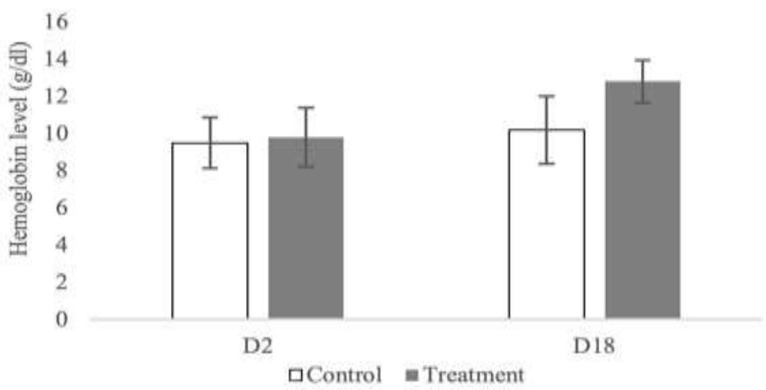

Figure 1. Comparison of hemoglobin levels of piglets between control and treatment group.

P-N-017

EFFICACY OF A EUBIOTIC PLANT-BASED PREMIXTURE OF FEED ADDITIVES ON THE INTESTINAL MICROBIOME OF FATTENING PIGS

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Introduction
There are various challenges affecting intestinal homeostasis during all stages of pig production. The gut microbiome (intestinal microbial ecosystem) of pigs is a major component of intestinal defense and nutrition processes. Several infectious or other agents can induce dysbiosis phenomena in the gut microbiome which in turn will affect the health status and performance of the animals (Duarte and Kim, 2022). The aim of this study was to document the effects of a eubiotic plant-based premixture of feed additives (PATENTE HERBA® PLUS, Patent Co, Misicevo, Serbia - TP) on the intestinal microbiome and performance of fattening pigs.

- Materials and Methods

A total of 176 growers at the age of 70 days allocated in 16 pens were included in the study. The total study period lasted 84 days. Animals were divided equally into two treatment groups as follows: Group A: received feed without the test product; Group B: received feed with product dosed 2 kg/ton. Fecal samples were collected rectally from 5 designated pigs per pen at the beginning, the middle (129. days of age), and the end of the study at 157 days of age. Samples were pooled per pen and analyzed with 16S ribosomal RNA (rRNA) gene sequencing to assess the characteristics of the intestinal microbiome. Performance data, such as body weight (BW), feed conversion ratio (FCR), feed consumption per feeding day (FCFD), and average daily gain (ADWG) were also recorded and calculated.

- Results

Performance parameters showed the absence of statistically significant differences between trial groups during the study. However, in the last 50 days of the trial, group B had a numerically lower FCR (2.449) and a lower FCFD (2.824kg) in comparison to group A (FCR 2.566; FCFD 2.971kg), while ADWG in the group A was 1.158kg and in the group B 1.153kg. Differences were observed in alpha diversity parameters of the intestinal microbial populations i.e. relative abundance, Shannon’s and Simpson’s diversity indexes, due to time effect The families Prevotellaceae Lachnospiraceae, Clostridiaceae, Ruminococcaceae, S24/Muribaculaceae, Lactobacillaceae, Streptococcaceae, Veillonellaceae were present in all samples and groups of the study. The Prevotellaceae family was the most abundant one at the 1st sampling point, whereas Clostridiaceae was the most abundant at the 2nd and 3rd sampling points. Throughout the study, the Shannon index of diversity was improved in the treatment group as regards species observations and analysis (Fig. 1). Additionally, numerically increased relative abundance of Lactobacillus spp observations was also observed in the treatment group (Fig. 2).

- Conclusions and Discussion

Alterations in Families diversity could be attributed to age progression and adaptation processes of the intestinal microbiota. Findings support the absence of a negative effect of the test product on gut microbial populations, whereas the mild increase of beneficial microbial populations and diversity indexes was present in the group that received the TP.

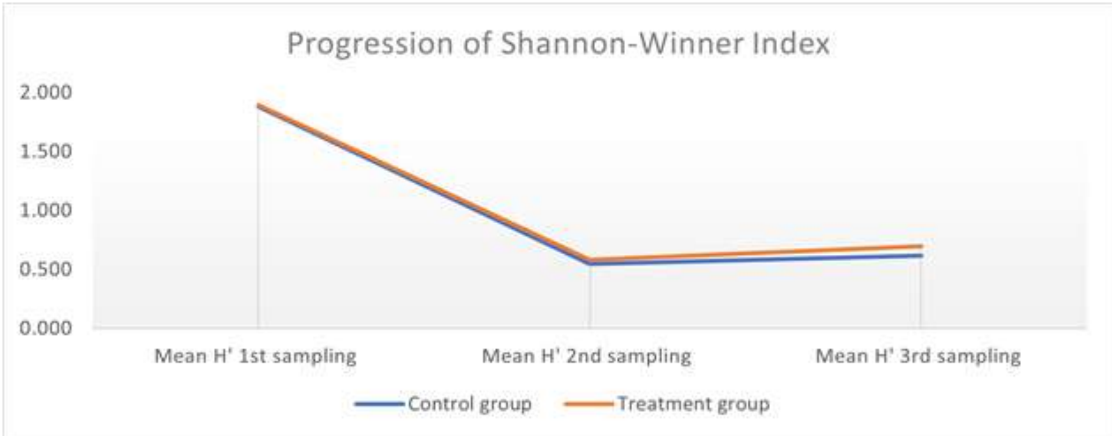
- Acknowledgement In some countries, the premixture of feed additives PATENTE HERBA® PLUS is registered as DYSGUARD-S®. The 16S ribosomal RNA (rRNA) gene sequencing was performed at Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa.

References

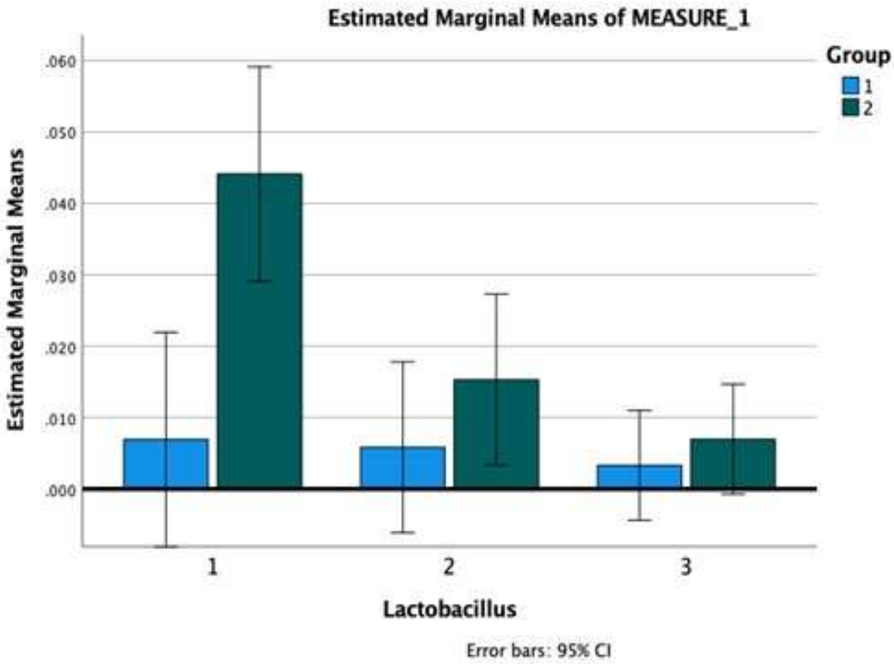
Duarte ME, Kim SW. Anim Nutr. 2022

Figure 1 Progression of Shannon-Winner Index
Figure 2 Lactobacillus spp relative abundance

Graphs or Images 1



Graphs or Images 2



P-N-018

EFFICACY OF SODIUM BUTYRATE AND HUMIC ACID SUPPLEMENT FOR WEANING PIGS

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Introduction
Butyric acid is a member of short chain fatty acid (SCFA) with four carbons in molecule. Sodium butyrate (SB) supplement plays several roles to animal health including protection and healing of mucosa, modulating immune response, and virulent reduction of pathogenic bacteria (2, 3). Humic acids (HA) are three dimentional macrocolloidal molecules resulting from decomposition of organic substance. HA and their sodium salt can be supplement for the control of diarrhoea, dyspepsia, and acute intoxication. HA has anti-inflammatory, antitoxic, and antimicrobial properties when supplement to animals as well (1, 4, 5). This study aimed to evaluate the efficacy of SB and HA supplement in piglet feed having pre-weaning and post-weaning diarrhoea in a Thai pig farm.

Materials and Methods
4 WOA piglets were moved to wean to finish unit. 184 pigs were randomly selected and set into 4 groups. Piglets with pre-weaning diarrhoea were in group 1 & 2 while normal pigs were in group 3&4. Group 1&2 were supplemented with SB 1.5g/kg feed and HA 1.2g/kg feed for 6 wks. Group 2 was also treated with S/TMP 25mg/kg bw for 7 days. Group 3 was supplement with SB 1.5g/kg feed for 6 wks while group 4 was treated with S/TMP 25mg/kg bw 7 days, as the conventional practice in this farm. Feces samples from all groups were collected before and after the supplement at D1 and D43 after weaning. Fecal scores (1=normal; 2=semisolid; 3=watery) were observed every 3 days from D1-D43 (Figure 1). Statistical analysis by t-test and chi-square at $P<0.05$, were calculated for fecal scores.

Results
Results of study are illustrated in table 1-3. Feces become normal from D13, D10, D10, and D19 for pigs from group 1-4, respectively. Fecal bacteria are including E.coli, HEC, α-hemolytic streptococci, Actinomyces spp., and Enterococcus spp.
Conclusions and discussion
According to this study, the combination of SB and HA in feed supplement for pre-weaning diarrhoea and feed supplement with SB for post-weaning diarrhoea show the promising result within 2 wks. In conclusions, SB and HA supplemented can reduce the clinical sign of pre-weaning and post-weaning piglet diarrhoea.

References
1. EMEA/MRL/554/99-FINAL, February 1999
2. Guilloteau et al., 2010, Nutr Res Rev, 23:366-384.
3. Liu et al., 2018, Adv Nutr, 9:21-29
4. Makhanon et.al., 2020, Thai J Vet Med, 50 (Suppli.):245-246
5. Visscher et.al., 2019, Front. Vet. Sci., 6:29

Table 1

Table 1. Fecal score of pigs with pre-weaning diarrhoea

Group*	Description**	D1	D4	D7	D10	Others****
1	Fecal score 1	27(58.7%)	27(58.7%)	35(76.1%)	43(93.5%)	46(100%)
	2	10(21.7%)	10(21.7%)	10(21.7%)	3(6.5%)	0
	3	9(19.6%)	9(19.6%)	1(2.2%)	0	0
	Mean score***	1.61 ± 0.802^{a}	1.61 ± 0.802^{a}	1.26 ± 0.491^{a}	1.07 ± 0.25^{a}	1.00^{a}
2	Fecal score 1	30(65.2%)	30(65.2%)	40(87.0%)	46(100%)	46(100%)
	2	11(23.9%)	11(23.9%)	5(10.9%)	0	0
	3	5(10.9%)	5(10.9%)	1(2.2%)	0	0
	Mean score***	1.46 ± 0.690^{a}	1.46 ± 0.690^{a}	1.15 ± 0.420^{a}	1.00^{a}	1.00^{a}

* Group 1: HA 1.2g/kg+SB 1.5g/kg for 6wk; Group 2:STMP 7d+HA 1.2g/kg+SB 1.5g/kg for 6wk

**Fecal score: 1=normal; 2=semisolid; 3=watery

***Mean score between group $^{a, b}$: $P<0.05$

****D13, D16, D19, D22, D25, D28, D31, D34, D37, D40, and D43

Table 2

Table 2. Fecal score of pigs with post- weaning diarrhoea

Group*	Description**	D1	D4	D7	D19	Others****
3	Fecal score 1	39(84.8%)	40(87.0%)	40(87.0%)	46(100%)	46(100%)
	2	5(10.9%)	4(8.7%)	5(10.9%)	0	0
	3	2(4.3%)	2(4.3%)	1(2.2%)	0	0
	Mean score***	1.20 ± 0.500^{a}	1.17 ± 0.486^{a}	1.15 ± 0.420^{a}	1.00^{a}	1.00^{a}
4	Fecal score 1	42(91.3%)	43(93.5%)	46(100%)	45(97.8%)	46(100%)
	2	3(6.5%)	3(6.5%)	0	1(2.2%)	0
	3	1(2.2%)	0	0	0	0
	Mean score***	1.11 ± 0.379^{a}	1.07 ± 0.250^{a}	1.00^{b}	1.02 ± 0.147^{a}	1.00^{a}

* Group 3: SB 1.5g/kg for 6wk; Group 4:STMP 7d

**Fecal score: 1=normal; 2=semisolid; 3=watery

***Mean score between group $^{a, b}$: $P<0.05$

****D10, D13, D16, D22, D25, D28, D31, D34, D37, D40, and D43

Graphs or Images 1

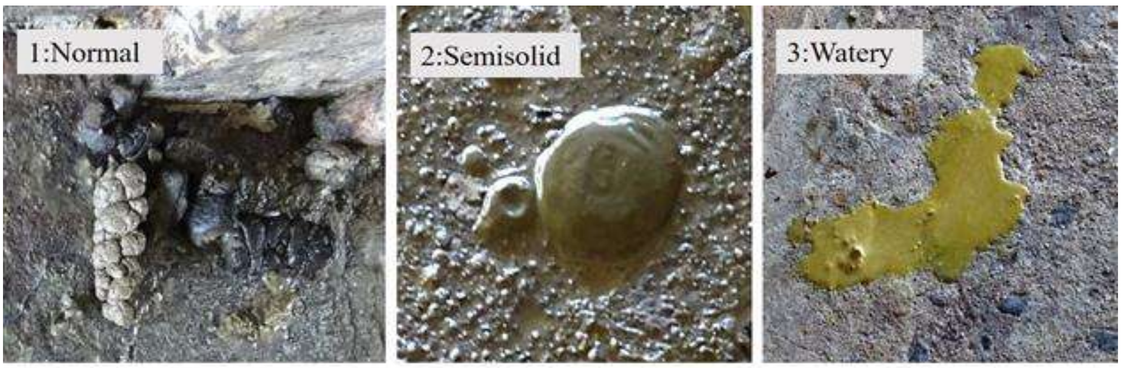

Figure 1. Fecal scores

P-N-019

EVALUATION OF THE EFFICACY OF A NUTRITIONAL ORAL GEL TO IMPROVE PIGLET STATUS AND REDUCE POST WEANING LOSSES.

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Introduction
Hyper prolific sow is one success of genetic development but every coin has two sides. The hyper prolific sow usually comes with low quality weaning piglet which may lead to poor performance in post-weaning period. This may due to nursing ability not enough to support all of their offspring to survive or reach their best quality status. To overcome this problem the supplement to fill the gap of sow ability should be used (Boulot et al., 2008). However, such supplementation should fit with piglet's gut physiology, so that they can utilize the nutrients in those supplements for their growth without negative effect on their health. Porcistart (Virbac) is a nutritional oral gel which contains more than 32 ingredients designed to match with piglet needs during a very early stage of life. Its ingredients are processed and formulated to be easy to digest and absorb by piglet's digestive tract. This product can reduce pre-weaning mortality and increase weaning weight as reported in previous studies (Voisin et al 2014, Bousquet et al 2019) but there was no report of its effect to piglet after weaning. The aim of this study is to prove its efficacy to reducing post-weaning loss especially during the first week after weaning.

Materials and Methods
Ninety-nine sows with mixed parities in commercial pig farm were divided into 2 groups; control (n=49) and treatment (n=50) before farrowing. The piglets of sows in control group (n= 564) were handled with farm routine protocol (milk replacer for 14 days) while the treatment group (n=571) were fed with Porcistart from D0 to D10 in average 140 gram/piglet/10 days. The piglets were cross-fostered within the group only. The pre-weaning performance and post-weaning mortality rate were recorded. The average born alive, birth weight, weaned piglet/litter, wean weight were analyzed by one-way ANOVA test and multiple comparison test with Tukey's HSD test. The loss at birth, pre-weaning mortality and post-weaning mortality were analyzed by Chi-square or Fisher's Exact test. All analysis were performed in R-studio version 4.2.2.

Results
The result was illustrated in Table 1.

Conclusions and Discussion
The results demonstrated that the tested nutritional oral gel does not only significantly improve weaning weight but also reduces mortality rate in the first week after weaning which is the most challenging period for piglets which face many factors that compromise their health and growth capacity (Lalles et al., 2007). As a pre-weaning supplement, Porcistart has many ingredients that can promote gut development, maintain acidity of gut microenvironment and reduce inflammation. It results in smoothly feed transition (from milk to creep feed or solid content) around weaning (Blavi et al., 2021). From this study, this nutritional oral gel can be one of the tools for pig farmers or veterinarians to improve health status of the weaning piglets as well as increase their survival rate after weaning.

References
1. Blavi L et al 2021. Animals. 11(2): 302.
2. Boulot S et al 2008. Advances in Pork Production. 19: 213-220.
3. Bousquet E et al 2019. Proc. APVS 2019.
4. Lalles J-P et al, 2007. Proceedings of the Nutrition Society. 66(2): 260-268.
5. Voisin F et al, 2014. Proc. IPVS 2014: 423.

Table 1

Table 1: Mean parameters and percentages per group

Parameters	Control	Porcistart	p-value
Pre-weaning			
Born alive (piglets)	11.51	11.42	>0.05
Loss at birth (%)	2.12	2.58	>0.05
Birth weight (Kg)	1.72	1.83	>0.05
Weaned/Litter (Piglets)	11.04	10.64	
PWM (%)	4.34	7.02	>0.05
Wean weight (Kg)	4.93	6.20	< 0.0001
Lactation length (Days)	21.00	21.00	
Post-weaning (7 days)			
Start (piglets)	545.00	566.00	
Finish (piglets)	521.00	564.00	
Loss (%)	4.40	0.35	< 0.0001

P-N-020

FIELD KOREAN STUDY OF AN INJECTABLE MINERALS FORMULATION ON REPRODUCTIVE PERFORMANCES OF SOWS

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[1]*Virbac,* [2]*XP BIO INC,* [3]*Get In Touch,* [4]*DARBY Genetics Inc*

Introduction

An increased systemic oxidative stress is described during late gestation and lactation, due to high energy demand, particularly in highly prolific sows, which can impair fertility (1). Among trace minerals, selenium and copper are known as antioxidants (1, 2). This study was done to assess the effects of an injectable mineral formulation containing phosphorus, potassium, magnesium, copper and selenium (Fosfosan®, Virbac) on sows and issued piglets performances in Korean field conditions.

Materials and methods

A total of 124 weaned sows from a breeding farm following one week batch management were randomly allocated to a tested (T) or control (C) group according to parity and back fat thickness measured by standardized ultrasonography. The randomization occurred on 5 successive batches. In the T group, each sow received an intramuscular injection of the tested mineral formulation at weaning and 2 weeks before farrowing (5 mL per injection). The C group received a saline serum according to the same posology. Sows oestrus detection and insemination were performed according to farm procedures. Back fat thickness was measured at farrowing and next weaning. Colostrum was sampled per sow at farrowing and stored frozen pending immunoglobulins assay by Elisa methods (IgG, IgM, IgA). Two male and two female piglets per litter were individually selected at birth (based on median litter body weight) and identified for individual weighing till finishing. Piglets cross fostering was managed within each group. Categorical data were compared between groups by the Fisher's exact test and numerical data by the t test and the F test to compare variances. A general linear model was eventually selected by using relevant covariates.

Results

Homogeneity of the groups at inclusion (weaning) was confirmed on sows parity and back fat thickness. No local or general side effect was reported after treatment. Subsequent oestrus rate was not different between groups, but weaning to oestrus interval tended to be lower in T group ($p=0.066$) and variance was significantly lower in T group ($p=0.002$). Farrowing rates were not different between groups. Back fat thickness was numerically higher in T group at farrowing and next weaning. The number of weaned piglets per litter was not different between groups. The variance of finishing weight was significantly lower in T group ($p<0.001$, Table 1). Colostrum IgG concentrations were significantly higher in T group ($p=0.035$), IgM and IgA being only numerically higher in T group, with a tendency for IgA ($p=0.08$, Table 2).

Conclusion

The mineral formulation tested in sows was beneficial regarding the return to oestrus (delay and variability), body condition at farrowing and weaning, and colostrum immunoglobulins concentrations. Finishing weight of issued piglets was less variable in T group. These data complete the previous ones reported in Brazilian conditions (3).

References

1. Berchieri-Ronchi CB et al. 2011. Animal 5: 1774-1779.
2. Weiss G et al. 2015. Immunol Rev 264: 182-203.
3. Vasconcelos DMS et al. IPVS 2022.

Table 1

Table 1. Performances of sows and issued piglets

Group	Control	Tested
Parity	3.08 ± 2.0	3.24 ± 2.0
Back fat thickness at inclusion	17.65 ± 3.90 mm	17.95 ± 3.11 mm
Oestrus rate	90.3 %	91.9 %
Weaning to oestrus interval	5.43 ± 1.63* d	4.95 ± 1.08* d
Farrowing rate	82.3 %	83.9 %
Back fat thickness at farrowing	19.94 ± 3.83 mm	20.79 ± 3.11 mm
Back fat thickness at weaning	15.77 ± 3.30 mm	16.57 ± 2.93 mm
Weaned piglet per litter	12.3 ± 0.6	12.5 ± 0.5
Issued pigs finishing weight	99.4 ± 9.1* kg	100.1 ± 6.3* kg

*: Variances significantly different between groups ($p < 0.005$)

Table 2

Table 2. Colostrum immunoglobulins concentrations

Group	Control	Tested
IgG	44.6* ± 32.2 mg/mL	57.7* ± 30.3 mg/mL
IgM	7.37 ± 3.01 mg/mL	8.12 ± 2.89 mg/mL
IgA	14.1 ± 5.1 mg/mL	16.4 ± 7.9 mg/mL

*: Means significantly different between groups ($p < 0.05$)

P-N-021

IMPACT OF IMMUNIZING MALE AND FEMALE FATTENING PIGS WITH IMPROVAC® AGAINST GONADOTROPIN-RELEASING FACTOR ON PROFITABILITY FOR PORK PACKERS IN CANADA, THAILAND, BRAZIL, AND FRANCE

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Introduction

Pig production is facing societal pressure regarding animal welfare and the impact on the environment. Increasing animal welfare requirements such as stopping physical castration, from the producer's point of view, is primarily associated with rising production costs and therefore a competitive disadvantage. Improvac®, an anti-GnRF (gonadotropin-releasing factor) vaccine which was originally developed as an alternative to physical castration of male pigs, has recently emerged as tool for use in gilts to reduce their sexual behavior during fattening, and the injuries such as mounting and aggression [1]. Immunization against gonadotropin-releasing factor in male and female pigs intended for market has shown to provide benefits not only for swine producers but also for pork packers. The aim of this study was to estimate the key drivers of profitability for pork packers in four countries: Canada, Thailand, Brazil, and France.

Material and Methods

A health economic model was built to estimate the differences in performance and economic parameters between immunized (IM) male pigs and physically castrated (PC) barrows as well as between IM and untreated gilts, as valid from the perspective of the pork packers. Parameters relevant for pork packers were derived from the dataset of two meta-analyses [2], [3]. Only statistically significant differences (P<0.05) in meta-analyses were applied to IM pigs, otherwise the same baseline value was used for IM and non-IM pigs [Table 1]. Data collected for these 4 countries included carcass weight and yield of primal cuts (ham, shoulder, loin, and belly), as well as price per kg carcass weight, potential premiums or penalties applied to the carcasses, economic value of ham, shoulder, loin, and belly. From the perspective of the pork packers, expenditures were pig purchase costs based on live or carcass weight. Income was generated from yield of primal cuts multiplied by the price per kg of the respective cutout. Results were expressed as difference between IM and non-IM pigs and net return for the pork packers was calculated by subtracting the incremental expenditures from incremental income. In one-way sensitivity analyses (OWSA) all parameters were changed over a range of ±20% to identify key drivers of profitability.

Results

Results as calculated by the model in local currencies were converted to US dollar ($) using contemporary conversion rates. The incremental net return per pig between non-IM and IM carcasses were calculated for Canada, Thailand, Brazil, and France as follows: $12.57, $23.24, $23.57, and $10.61 per male carcass, respectively, and $3.94, $ 8.44, $8.61, and $3.42 per female carcass, respectively [Table 2]. Results of OWSA did not change the higher profitability of IM male and female carcasses in all 4 countries. In base case analyses between IM and non-IM pigs, the most influencing parameter on the incremental net return was the live or carcass weight followed by the yield of shoulder for male carcasses or the value of belly for female carcasses [Figures 1 and 2].

Discussion and Conclusion

It is worth mentioning that the ranking of most influencing parameters to pork packers was not relevantly different among the four countries. Therefore, it can be rationally concluded that the key drivers of profitability are similar, at least within a range of final live weights between 121 and 135 kg. Carcasses of IM male and female pigs intended for market were consistently more profitable for pork packers in all four countries with results being robust against changes of carcass traits or prices in most analyses.

References:

1. Rodrigues et al., 2019. Animal 13, 1326-1331
2. Poulsen Nautrup, B., et al, 2018. Res. Vet. Sci. 119, 182–195
3. Poulsen Nautrup, B., et al, 2020. Res. Vet. Sci. 131, 159–172

Table 1

Table 1: Overview of the default data considered in the model as valid from the perspective of the packers. Carcass parameters were defined for pigs not immunized with Improvac® (non-IM), i.e., physically castrated male pigs or untreated gilts. Efficacy data represent the differences between IM and non-IM pigs as reported in meta-analyses (Poulsen Nautrup B., et al., 2020, 2018). All parameters were subjected to changes in one-way sensitivity analyses (OWSA).

	Default male pigs	Default female pigs
Production and carcass parameters		
Final live weight in non-IM pigs (kg)	117	115
Carcass weight in non-IM pigs (kg)	92	92
Yield of ham in non-IM pigs (kg)	27.2	28.3
Yield of shoulder in non-IM pigs (kg)	16.2	15.5
Yield of loin in non-IM pigs (kg)	18.6	15.8
Yield of belly in non-IM pigs (kg)	17.6	17.5
Difference in live weight between IM and non-IM pigs (kg)	+ 2.1	+ 3.8
Difference in carcass weight between IM and non-IM pigs (kg)	± 0.00	+ 3.1
Percentage difference in yield of ham between IM and non-IM pigs (% points)	+ 1.14	± 0.00
Percentage difference in yield of shoulder between IM and non-IM pigs (% points)	+ 2.99	± 0.00
Percentage difference in yield of loin between IM and non-IM pigs (%)	± 0.00	± 0.00
Percentage difference in yield of belly between IM and non-IM pigs (%)	± 0.00	+ 2.26

Table 2

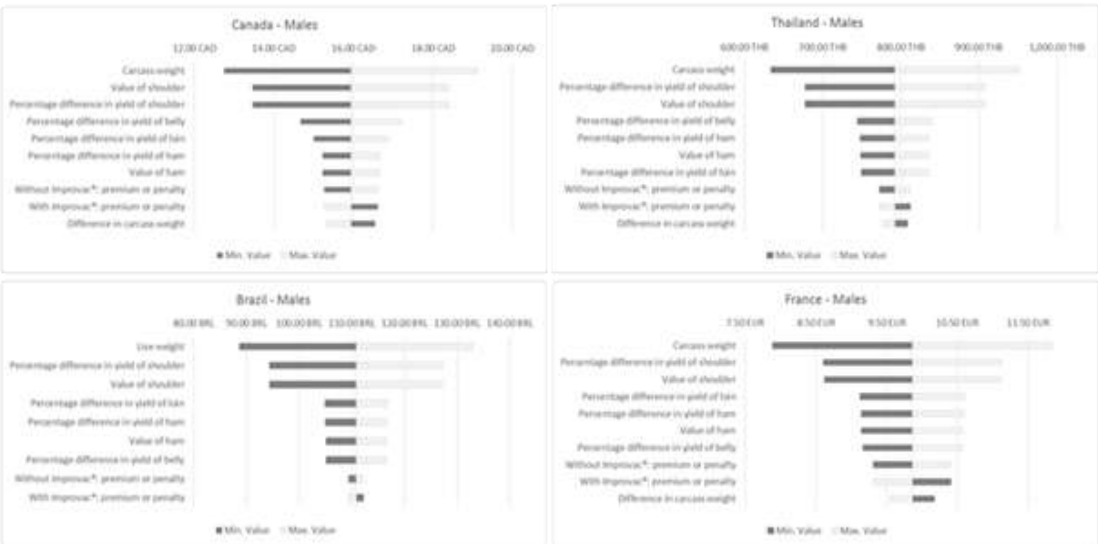
Table 2: Results of base case analyses calculated for the 4 countries in local currencies and converted to USD. Outcome was the incremental net return per pig, i.e., the difference in net return between pigs immunized with Improvac (IM) and non-IM pigs, i.e., physically castrated male pigs or untreated gilts.

		Incremental net return (per pig) between IM and non-IM pigs: Base case results	
		Perspective of the packers	
		Male Pigs	Female Pigs
Country	**Final live weight in non-IM pigs**	**Local Currency / USD***	**Local Currency / USD***
Canada	135 kg	CAD 15.94 / USD 12.57	CAD 5.00 / USD 3.94
Thailand	135 kg	THB 792.31 / USD 23.24	THB 287.75 / USD 8.44
Brazil	125 kg	BRL 110.85 / USD 23.57	BRL 40.49 / USD 8.61
France	121.1 kg	EUR 9.86 / USD 10.61	EUR 3.18 / USD 3.42

*Source of conversion rates: European Commission's official monthly conversion rates for June 2022 (access: June 23, 2022)

Graphs or Images 1

Fig. 1. Tornado diagrams for one-way sensitivity analyses run for the outcome of incremental net return between **male pigs** immunized with Improvac® (IM) and physically castrated pigs as calculated for 4 countries from the perspective of the packers. Each parameter was changed ±20% of base case values and results depicted as horizontal bars. Positive figures indicate higher net return with IM pigs.



Graphs or Images 2

Fig. 2. Tornado diagrams for one-way sensitivity analyses run for the outcome of incremental net return between **female pigs** immunized with Improvac® (IM) and untreated gilts as calculated for 4 countries from the perspective of the packers. Each parameter was changed ±20% of base case values and results depicted as horizontal bars. Positive figures indicate higher net return with IM pigs.

P-N-022

MATERNAL SUPPLEMENTATION WITH NATURAL ANTIOXIDANTS (HERBAL C AND HERBAL E) DURING PREGNANCY IN SOWS

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Modern swine production is at the head of worldwide meat production, based on large farms implementing efficient genetic selection, health assessment, and nutritional and reproductive strategies. Improvement of prolificacy has been mainly achieved by the selection of genotypes with a high ovulatory rate and consequently a high number of developing fetuses. However, the space into the uterus for the adequate development of the fetuses and their placentae is limited, so large litters are affected by deficiencies of placental development affecting the functionality of the organ and, therefore, the supply of oxygen and nutrients to the fetuses .

The consequence is the impairment of fetal growth in a process known as intrauterine growth restriction (IUGR), leading to low birth-weight (LBW) neonates. The reduced supply of nutrients and oxygen to the IUGR fetuses causes malnutrition and hypoxia which, in turn, increases their oxidative stress . Hence, a possible strategy for counteracting IUGR may be based on the administration of antioxidant agents which ameliorate the antioxidant/oxidative status, improve the placental function, and increase the birth weight and viability of the offspring . Specifically, previous studies in sheep with twin pregnancies have shown that maternal oral supplementation with antioxidant vitamins C and E during pregnancy increases these vitamins in the cord blood of near-to-term fetuses, diminishing their oxidative stress and increasing their body weight.

Previous studies under experimental conditions have shown that such combination improves the antioxidant/oxidative status of the sow and favors the transfer of vitamin E to the piglets and their postnatal growth patterns.

These results suggest that supplementation with antioxidant agents is a promising strategy for diminishing incidence and consequences of IUGR in swine. Combination of two herbal antioxidants natural vitamin C & E (Herbal C and Herbal E) were evalauted for its efficacy during pregnancy on reproductive traits and piglet performance (number of live, dead, and mummified newborns and litter weight at birth and individual body weight at both birth and weaning) in a total of 1027 sows (504 treated and 523 control females) kept under commercial breeding conditions at commercial farm in Chile (Agrosuper, Rancagua, Chile) under standard commercial swine breeding conditions. The supplementation increased the number of live-born piglets (13.64 ± 0.11 vs. 12.96 ± 0.13 in the controls; $p = 0.001$) and the total litter weight, decreasing the incidence of low-weight piglets without affecting the number of stillbirths and mummified newborns.

Such an effect was modulated by the number of parity and the supplementation, with supplementation increasing significantly the number of living newborns in the first, second, sixth, and seventh parities (0.87, 1.10, 1.49, and 2.51 additional piglets, respectively; $p < 0.05$). The evaluation of plasma vitamin concentration and biomarkers of oxidative stress (total antioxidant capacity, TAC, and malondialdehyde concentration, MDA) performed in a subset of farrowing sows and their lighter and heavier piglets showed that plasma levels of both vitamins were significantly higher in the piglets than in their mothers ($p < 0.05$ for vitamin C and $p < 0.005$ for vitamin E), with antioxidant supplementation increasing significantly such concentrations. Supplementation of herbal antioxidants decreased plasma MDA levels both in the sows and their piglets ($p < 0.05$). Finally, the piglets from supplemented mothers showed a trend for a higher weaning weight ($p = 0.066$) and, specifically, piglets with birth weights above 1 kg showed a 7.4% higher weaning weight ($p = 0.024$). Hence, the results of the present study, with high robustness and translational value by offering data from more than 1000 pregnancies under standard breeding conditions, supports that maternal supplementation with herbal antioxidants during pregnancy significantly improves reproductive efficiency, litter traits, and piglet performance.

P-N-023

NATURAL PROSTAGLANDIN F2ALPHA INJECTION IMPROVES SOW AND PIGLET PERFORMANCES

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Introduction:
Natural prostaglandin F2α was used for inducing farrowing in pregnancy sow. It is widely accepted in commercial swine farms. Using natural prostaglandin F2α was applied after parturition for reducing postpartum discharge and incidence of metritis [1]. The reduced discharge after farrowing may affect sow performance both during and after lactation. The objective of the present study was to determine the effect of natural prostaglandin F2α before and after farrowing on sow reproductive performances and piglet performances.

Materials and Methods:
In total, 183 gestating sows were randomly allocated into four groups. On day 115 of gestation, all sows were administrated the intramuscular with normal saline (Control, n=35), a double administration of 1 ml natural PGF2α (Enzaprost®, Ceva Santé Animale, Libourne, France) and 24 h after first piglet born (E11, n=45), a single administration of natural PGF2α (E20, n=58) and a double administration of 2 ml natural PGF2α and 24 h after first piglet born (E22, n=45). The piglets (n= 2,150) were counted and weighed at weaning. The wean-to-service interval (WSI) was compared between groups. The all statistical analyses were performed using SAS 9.0 (SAS, 2002). All data were analyzed by using general linear model. The parity was classified into 2 groups (primiparous and multiparous). The statistical model included group, parity and their interaction. Values with $P < 0.05$ were regarded as statistically significant.

Results:
The sow in all treatment groups has higher litter weaned weight and a number of weaned piglets/litter than in control group (Table 1).

The Figure 1 demonstrated that sow in the control group has significantly higher WSI than all treatment group sows.
a, b Significant difference within parity group ($P < 0.05$)

Conclusions and Discussion:
The previous studies demonstrated that farrowing induction improved milk production and piglet mortality [2, 3]. The increasing of litter weaned weight and number of weaned piglets/litter in treatment sows related with improving milk production [4]. The progesterone was decreased by injected natural PGF2α before/after farrowing which is related lactogenesis [5]. Therefore, injected natural PGF2α in sow improved WSI, litter weaned weight and number of weaned piglets/litter, especially in multiparous sows.

Acknowledgment:
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References:
[1] De Rensis et al. 2012. Theriogenology. 77: 1-11
[2] Nuntapaitoon et al. 2020. CUVC2020.
[3] Morrow et al. 1996. Swine Health Prod. 4: 73-8.
[4] Maneethong et al. 2021. Anim Biosci. 34: 833-843.
[5] De Passillé et al. 1993. J Anim Sci.71: 179-84.

Table 1 The litter weaned weight and number of weaned piglets/litter in each group by parity.

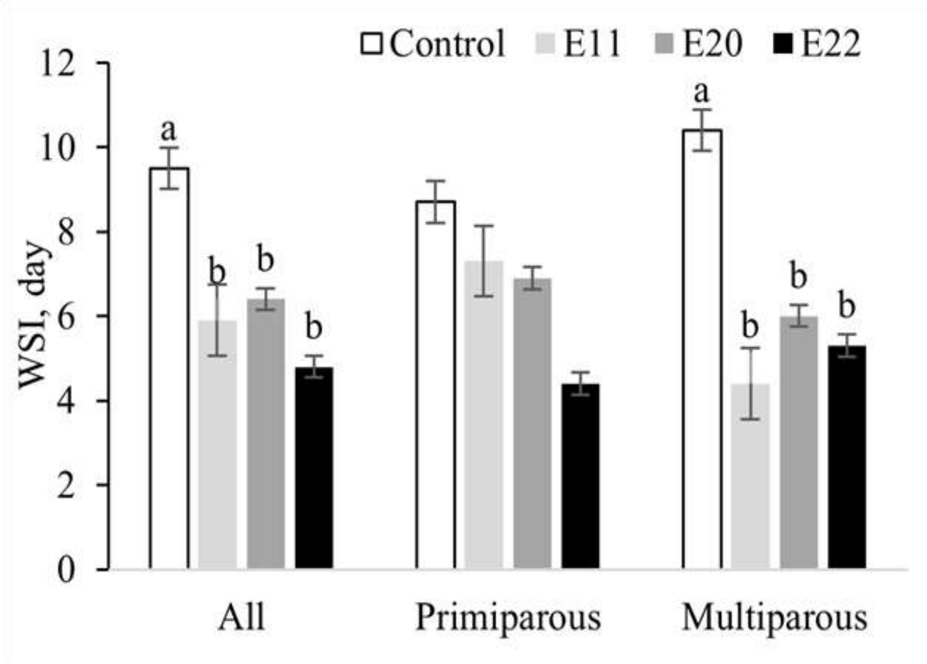
Table 1

Parameter	Control	E11	E20	E22
Litter weaned weight, kg				
All	45.9[b]	62.4[a]	58.2[a]	59.8[a]
Primiparous	41.3	56.5	53.1	58.3
Multiparous	50.4[b]	68.3[a]	63.4[a]	61.3[ab]
Number of weaned piglets/litter				
All	6.8[b]	8.8[a]	8.1[a]	8.3[a]
Primiparous	6.3	7.8	7.1	8.0
Multiparous	7.4[b]	9.8[a]	9.1[a]	8.6[ab]

a, b Significant difference within row (P < 0.05)

Figure 1 The wean-to-service interval of each group by parity group.

Graphs or Images 1



P-N-025

PALM KERNEL MEAL PROTEIN HYDROLYSATES ENHANCE POST-THAWED BOAR SPERM QUALITY

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Introduction
Semen cryopreservation is associated with producing reactive oxygen species, which leads to lipid peroxidation of sperm membranes, resulting in the reduction of sperm motility and decreased fertilizing ability [1]. Palm kernel meal contains bioactive peptides that have antioxidant and antimicrobial activity [2,3]. There is no information on the use of palm kernel meal-derived bioactive peptides for boar semen cryopreservation. This study aimed to examine the effects of bioactive peptides from Palm kernel meal protein hydrolysates (PKMPH) on post-thawed boar sperm quality.

Materials and Methods
Seventeen boar ejaculates were collected and divided into six equal aliquots based on PKMPH concentrations (0, 1.25, 2.5, 5, 10, and 15 µg/ml) in lactose egg yolk extender and freezing extender. Semen samples were processed and cryopreserved by the liquid nitrogen vapor method. After that, frozen semen samples were thawed at 50 °C for 12 seconds [4] and evaluated for sperm motility using a computer-assisted sperm analyzer (CASA, Androvision®, Minitube, Germany), sperm viability using SYBR-14/EthD-1 staining and acrosome integrity using FITC-PNA/EthD-1 staining.

Results and Discussion
The result demonstrated that the supplementation of PKMPH with 2.5 showed a superior post-thaw sperm quality compared to other concentrations (Figure 1). Semen samples supplemented with 2.5 µg/ml improved motility by 10.7%, progressive motility by 7.7%, viability by 12.3%, and acrosomal integrity by 18.2% respectively, when compared with the control group. However, the high concentration of PKMPH higher than 5 µg/ml showed slightly cytotoxic effect. The reason might be that an excessive of antioxidants may disturb the balance between free radicals and antioxidants in mammalian cells [5]. In conclusion, adding 2.5 µg/ml in the freezing extender with PKMPH peptides showed a higher post-thawed sperm quality and effectively reduced the oxidative damage associated with cryopreservation.

References:
[1] Chianese, R. and Pierantoni, R. 2021. Antioxidants, 10(1), 1–19.
[2] Zarei M, et al. 2014. Food Research International,62:726-34.
[3] Tan, Y. N., et al. 2013. Food Chemistry, 136(1), 279-284.
[4] Chanapiwat, P. and Kaeoket, K. 2020., Thai J Vet Med. 50(3): 283-295.
[5] Rahal et al. 2014. Biomed Res Int. 2014:19.

Graphs or Images 1

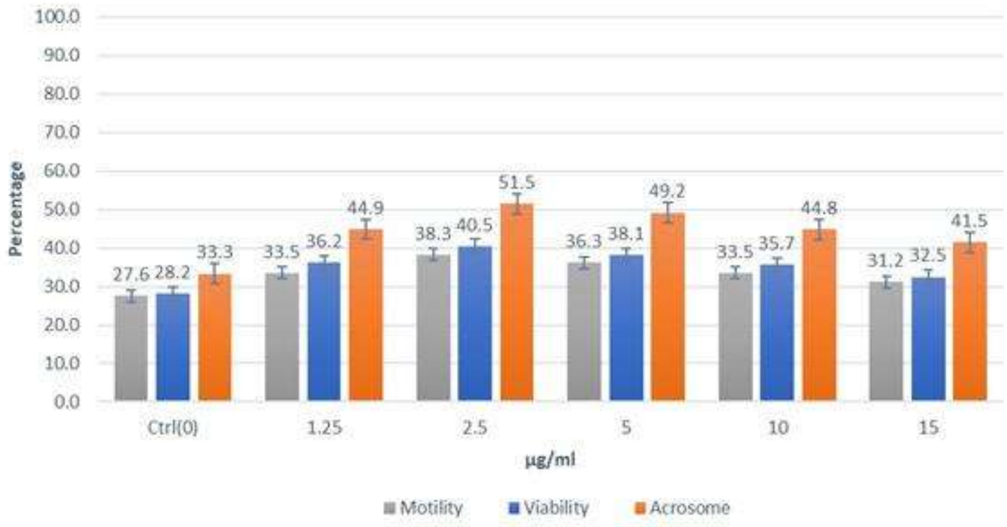

Figure 1. Post-thaw sperm parameter at difference concentrations of PKMPH

P-N-026

PIG FARM'S BIOSECURITY ASSESSMENT MODEL STANDARDIZED AND APPLICATION

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Introduction
In 2021, the Animal Disease Control Center of Yunlin County build the biosecurity level assessment for livestock farms to define and subdivide different items and levels to evaluate the biosecurity level more clearly. The results can be used as the basis for the biosecurity management rank of pig farms, and provide important information for the policies of epidemic prevention in the future.

Methods
To eliminate the subjective data, the biosafety team was composed of particular trained persons, and used the biosecurity level assessment form(table 1) to evaluate pig farms in Yunlin county. Data was analyzed by Chi-square test, and the p-value less than 0.05 was considered significant.

Result
There were total 1,231 pig farms in Yunlin county but 58 pig farms were without feeding when the biosecurity team visited, so only 1,173 pig farms were evaluated. The biosecurity level assessment form divided the total score into three grades: excellent (> 80 points), good (62-79 points) and fair (< 61 points). The survey results showed that 44% of the excellent and 53% are good of pig farms, and 3% fair of pig farms need to be strengthened their biosecurity. The average overall biosecurity level score is about 80 points, with a standard deviation of 9.6. The individual score results (Figure 1) showed that the most pig breeders have a clear concept of biosecurity for some items, such as visitors and vehicles should stay away from the farm. The concept of biosecurity in some projects is still needed to be improved, such as access control, parking location of livestock carriers and fodder trucks, frequency of disinfection, and location of dead pig's disposal; the poor of implementation items are the distance between the surrounding farms and the physical barriers. This analysis also found that the more animals kept, the higher biosecurity score they get ($p < 0.01$) (Figure 2), indicating that the breeders have a higher concept of biosecurity.

Conclusion
According to the results of the pigs number survey made by the Council of Agriculture in 2021 and 2022, the overall pig farm decreased by 54.1%, and the total number of pigs decreased by 23.6%, but the average feeding number per farm has increased from 520 to 867. It shows that the pig farm’s scale is increasing. The analysis also found that the more pigs to raise, the higher concept of biosecurity the breeders have. Therefore, it's a priority to improve the biosecurity level of pig farms and give guidance on small livestock farms. In high-risk small farms, the economic problems and the lack of manpower are the difficulties of improving biosecurity. It caused disease transmission and economic losses. If the infectious disease outbreak, it'll be a loophole in epidemic prevention. The suggestion is that pig farms can adjust their feeding mode, such as segmented, off-site breeding ,or integrate,

cooperatives with agricultural enterprises, it'll become easier to prevent the epidemic disease and strengthen biosecurity equipment and concepts together. This biosafety assessment research method can be used as an epidemic prevention rating model in countrywide livestock farms. The results can not only improve biosecurity and epidemic prevention levels in countrywide livestock farms but also provide important epidemic prevention references for the government's formulation of policy.

Table 1

Table1. The form about biosecurity level assessment for livestock farms

Biosecurity level assessment for livestock farms

Environment					
		Excellent(10 pts)	Good(5 pts)	Fair(1 pts)	Pts
1	Distance between the surrounding farm	□ Above 50 meters	□ Below 50 meters	□ Adjacent	
2	Access control	□ Gate and each barn have access control	□ No gate control but each barn have access control	□ None	
3	Fence setting	□ Already setting	□ Only on the side of the road	□ None	
Visitor and vehicle control					
4	Visitor/Driver	□ Meet in the off-site reception room	□ Meet in the reception room	□ None	
5	Livestock carriers	□ No enter/Enter after disinfection	□ Fixed area operations	□ Pass through each barn	
6	Fodder trucks	□ No enter/Enter after disinfection	□ Fixed area operations	□ Pass through each barn	
7	Rendering car	□ No enter/Enter after disinfection	□ Fixed area operations	□ Pass through each barn	
Prevention					
8	Disinfection frequency	□ More than once a week	□ Once in 2 weeks	□ None	
9	Location of dead pig's disposal	□ Put inside and cover	□ Put off-site and cover or put inside cover	□ Put off-site and uncovering	
Other					
10	Other item (2 pts each)	□ Breeding record detail	□ Disinfection tank per each barn	□ Rain boots per each barn	
		□ Isolation barn	□ Sanitizer spray on the driveway	□ Mousing regularly	
Total		pts/total pts:90+12=102 pts			

Biosecurity level assessment:□Excellent(> 82 pts) □Good(62~81 pts) □Fair(< 61 pts)

Graphs or Images 1

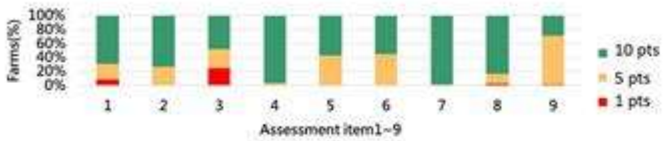

Figure1. Score distribution of assessment items 1~9

Graphs or Images 2

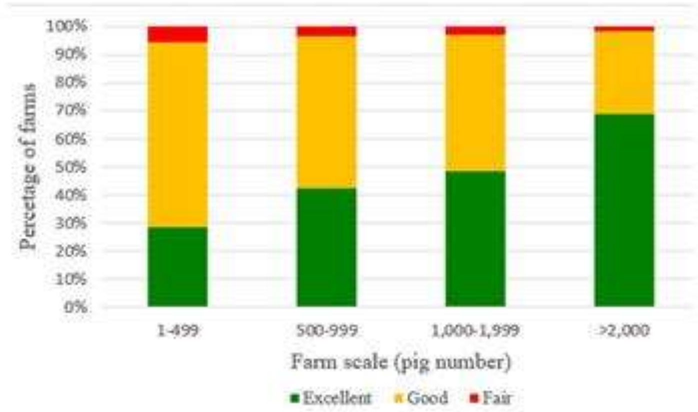

Figure2. The correlation between the number of breeding and the total score of biosecurity($p < 0.01$)

P-N-027

Production Applications of AI Based Audio Monitoring in the Diagnosis of Swine Respiratory Diseases

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Introduction
The Swine Respiratory Disease (SRD) is still one of the most common problems in pig production. It is also a difficult and complex problem to solve. The main clinical symptom of the SRD is coughing. It impacts feed intake, growth rate, and feed conversion. All these will affect producer's economic return [1].
Nowadays veterinarians use coughs to assess the severity of SRDs and intervention effectiveness. This requires regular and continuous observation by professionals with rich clinical experiences. Continuous and real-time monitoring at site is not possible, especially at night. It is also difficult to find abnormalities and be precise [2]. For scale farms and large integrators with few staff, it is difficult to keep up with the disease development and may then miss the best intervention window.
As coughs can be used to screen and diagnose early respiratory diseases [3-4], the Berckmans team conducted in-depth research on swine cough recognition and developed a set of AI powered system - SoundTalks®. It has already been used in swine production. By monitoring the barn 24/7, it detects changes in real-time. These changes reflect health status change and are indicated by different coloured lights. Green means healthy; yellow and red indicate possible respiratory problems; red is a high alert [Fig 1]. The ReHS (Respiratory Health Status) curve tells the respiratory health status of the farms visually [Fig 2]. Based on degrees of severity, intervention measures are given in time.

Materials and Methods
Analyze on early warning and diagnose respiratory problems when ReHS indexes changes. Summarize appropriate intervention guidance from different batch data, create guidance for the following batch. Provide feedback on the intervention measures effectiveness through analyses.

Results
Through data analyses and real-time feedback, Wean-to-Finish production management was done with greater emphasis no details. For example, a Hubei fattening farm used SoundTalks which showed below-expectation intervention results initially [Fig 3]. With revised intervention guidance improving the barn environment, the performance became drastically better at a later stage.

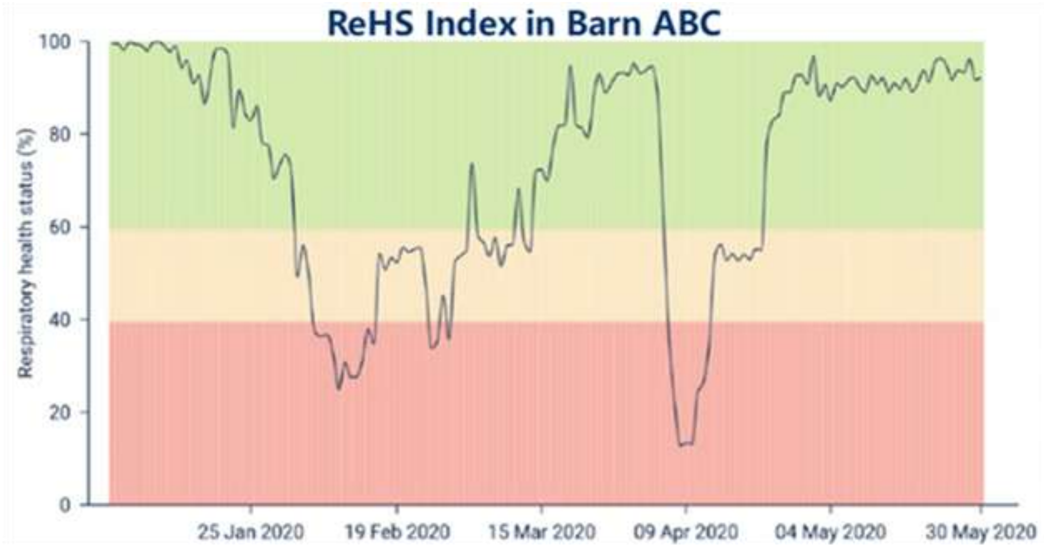
Fig 3 Respiratory Health Status (ReHS) Graph
Making visualized batch-by-batch comparison, ReHS helps guide production management. For example, a Shaanxi producer drew on past performance data [Fig 4] to guide intervention implementation when similar problems occurred and achieved better performance results. Even for the second batch that had an APP outbreak the batch performance was better, too. Management team paid extra attention when placing the third batch, despite a cough alert in May 2022, the overall interventions led to good performance results.

References :

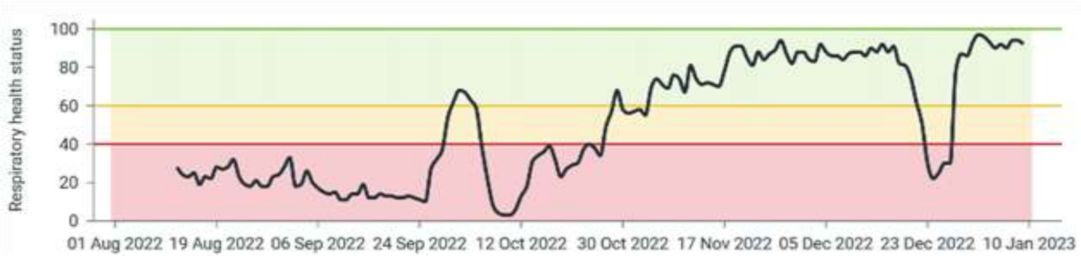
[1] Opriessnig T, Gimenez-Lirola LG, Halbur PG . Polymicrobial respiratory disease in pigs [J]. Anim Health Res Rev, 2011, 12(2):133-148.
[2] Lai Xuan , Zhao Jian, et al. Recognition of continuous cough sound of pigs based on continuous speech recognition technology [J]. Journal of Agricultural Engineering, 2019, 35(16): 174.
[3] Silva M, Ferrari S, Costa A, et al. Cough localization for the detection of respiratory diseases in pig houses[J]. Computers and Electronics in Agriculture, 2008, 64(2): 286 - 292.
[4] Mitchell S, Vasileios E, Sara F, et al. The influence of respiratory disease on the energy envelope dynamics of pig cough sounds[J]. Computers and Electronics in Agriculture, 2009, 69(1): 80 - 85.

Table 2

Table 3



Graphs or Images 1



Graphs or Images 2

P-N-028

REDUCING STRESS SUSCEPTIBILITY IN PIGS WITH NATURAL HEAT STABLE UNIVERSAL ANTIOXIDANT AND ADAPTOGEN 'HERBAL C'

Dr. Shivi Maini[1]
[1]Indian Herbs

Stress in pigs:
In recent decades there has been a growing concern about animal stress on intensive pig farms. Stress produces alterations in the normal physiology of pigs and affects their welfare and productive performance. Stress in pigs can be caused by many factors related to social, environmental, metabolic, immunological, production, nutritional, transportation, gestation, lactation, slaughter and other factors.
Heat stress above 32C depresses feed intake in pigs. Heat stress may have negative effect on sperm production in boars, poor reproductive efficiency, low litter size and higher mortality in pre-weaned piglets. Oxidative stress is downstream of all these stressors. It causes redox imbalance between the pro- and anti-oxidants in favor of prooxidants and leads to lipid peroxidation, proteolysis, DNA damage, and apoptosis. Both reactive oxygen species (ROS) and reactive nitrogen species (RNS) at certain levels are signaling molecules involved in homeostasis.Thus, it is essential to mitigate stress. Dietary modifications are among the most preferred and practical ways to alleviate the effect of stressors in pigs including heat stress (Surai et al., 2017).

Antioxidant supplementation for stress mitigation:
Antioxidants are widely used to maintain physiological functions & for stress management in swine industry (Konca et al., 2009). Most production species are able to endogenously synthesize sufficient vitamin C. However, this is not the case for the younger animals (e.g. newborn piglets) and for those under challenging conditions, such as multitude of stress exposure. Exogenous vitamin C and other antioxidant supplementation is reported to improve productive performance, reduce blood cortisol levels and potentiate immune response. Several other antioxidants such as synthetic vitamin E, selenium, prebiotics, natural antioxidants are also given to pigs to mitigate stress (Lagana et al., 2007).
The usual vitamin C supplements (synthetic ascorbic acid or coated vitamin C) are very unstable to heat, humidity and light. Some of them however, are more stable to these factors but have low bioactivity. A number of natural phytobioactives are well known for their antioxidant activity viz. gallotannoids (Surai, 2002). Herbal C is a unique combination of phytogenic components containing highly stable and bioactive vitamin C analogues that guarantee up to 6 times more antioxidant capacity than the normally used sources of Vitamin C. This composition guarantees sustained efficacy of Herbal C in combating oxidative stress and improving animal performance. Herbal C is phytogenic animal feed supplement & highly heat stable antioxidant and adaptogen. Herbal C contains natural vitamin C in free state and in conjugation with other phytobioactive molecules of high antioxidant potential that acts synergistically to provide high bioactivity, stability and bioavailability of vitamin C (Goshal et al., 1996).

The unique array of phyto-ingredients and composition Herbal C is liable for self-replicating, sustained antioxidant activity and higher bioavailability (Chatterjee et al., 2006). Active moiety of synthetic vitamin C is thermo-labile & gets quickly dissipated in aqueous solution. Retention and activity of synthetic vitamin C is reduced by 10% every month during storage at room temperature and by more than 80% during pelletization. In contrast, Herbal C is naturally conjugated with other phyto-molecules, it remains stable in aqueous medium, during storage at room temperature and also under pelleting temperature with sustained bio-activity.

Summary :
The recommended amount of vitamin C in pigs is (150 to 250 IU/kg). Herbal C supplementation ensures optimal physiological functions, increased immunocompetence, attainment of homeostasis through its adaptogen activity, improvement in production & performance in pigs, restoration of fertility in sow and boars, control of morbidity and mortality in pre-weaned pigs. Several unique advantages of Herbal C over synthetic vitamin C makes it the perfect choice for alleviation and management of oxidative stress in pigs.

P-N-029

SURVEILLANCE OF IRON DEFICIENCY ANEMIA IN SUCKLING PIGLETS TREATED WITH IRON DEXTRAN IN THAILAND

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Introduction
Iron deficiency anemia (IDA) is the most common nutritional deficiency in pig. Piglets are born with approximately 50 mg of iron, which is very low, and sow milk can provide only 1 mg of iron per day. Piglets that did not receive an iron supplement shortly after birth develop IDA resulted in poor health and growth performance 1. In Thailand, it is common practice to prevent newborn piglets from IDA by using iron dextran injection within the first 3 days of postnatal life. This procedure has been implemented in swine industry for several decades. Previous studies reported that piglets born from the litter with large litter size have relatively low hemoglobin level and have higher risk of anemia 2. Meanwhile, the number of hyperprolific sow population and the average number of total piglets born per litter have been increased continuously during the last two decades in the Thai swine industry 3. The present study aims to determine the prevalence of anemic piglets treated by iron dextran injection in swine commercial herds in Thailand.

Materials and Methods
Eight swine commercial herds from all regions of Thailand were selected to be include in the present study. All of the piglets were treated with 200 mg injectable iron dextran intramuscularly at 2 - 4 days of age. In each herd, the piglets at 2, 7 and 21 (weaning) days of age were randomly selected from the litters with different parity of sows (parity 1 to 9). In each litter, three piglets (small, medium, large) were measured for blood hemoglobin level (g/dl) by using Hemocue® Hb 201+ (Hemocue AB, Ängelholm, Sweden). Normal iron status was defined as hemoglobin level of > 11.0 g/dl, iron deficiency (sub-anemic) was defined as hemoglobin level of > 9.0 g/dl but ≤ 11.0 g/dl, and anemia was defined as hemoglobin level of < 9.0 g/dl 4. Sows' parameters were recorded, and the statistical analyses were performed by using SAS software version 9.4 (SAS Inst. Inc, Cary NC, USA.).

Results
In total, sample from 1,276 piglets were collected. Of these piglets, 405 piglets were 2 days old (D2), 415 piglets were 7 days old (D7), and 456 piglets were 21 days old (D21; weaning). The blood samples were tested for blood hemoglobin level immediately after collection. The distribution of hemoglobin level is presented in Fig. 1. Weaned (D21) piglets had significantly higher hemoglobin level and lower prevalence of anemia compares with the D2 and D7 piglets. The hemoglobin level and the percentage of anemic piglet are presented in Table 1.

Conclusions and Discussions
Although the prevalence of anemia is reduced at weaning, due to the expected replenishment of the iron after the injection, 22.6% of piglets remain anemia even though they have been treated with 200 mg of iron dextran. This condition can impair piglet health and their growth performances 1. Previous studies indicate that single injection of 200 mg iron dextran may not be enough to resolve anemia problems in piglets until weaning and

differences between the products and actives exist 5. The use of hyperprolific sows' genetics in the modern swine industry maybe, partially, increase the risk of anemia in the suckling piglets due to inadequate colostrum intake 6. Alternative product or protocol of IDA treatment need to be reconsidered to reduce piglet anemia, optimize growth performance, and improve the quality of piglet at weaning.

References
1. Chevalier TB, J Swine Health Prod. 2021;29(4):189-199.
2. Vongsariyavanich, S., Theriogenology 172: 150 – 159.
3. Perri AM, J Swine Health Prod. 2016;24 (1):10-20.
4. Heidbüchel K, Animals (Basel). 2019 Sep 4;9(9):651.
5. Juthamanee, P., Livestock Science 251: 104630.

Table 1

Table 1. Average hemoglobin level and prevalence of anemic piglets

Day of age	Avg. Hb level	% Anemia
D2	8.7 g/dl [a]	48.9%
D7	9.0 g/dl [a]	44.6%
D21	11.4 g/dl [b]	22.6%

Graphs or Images 1

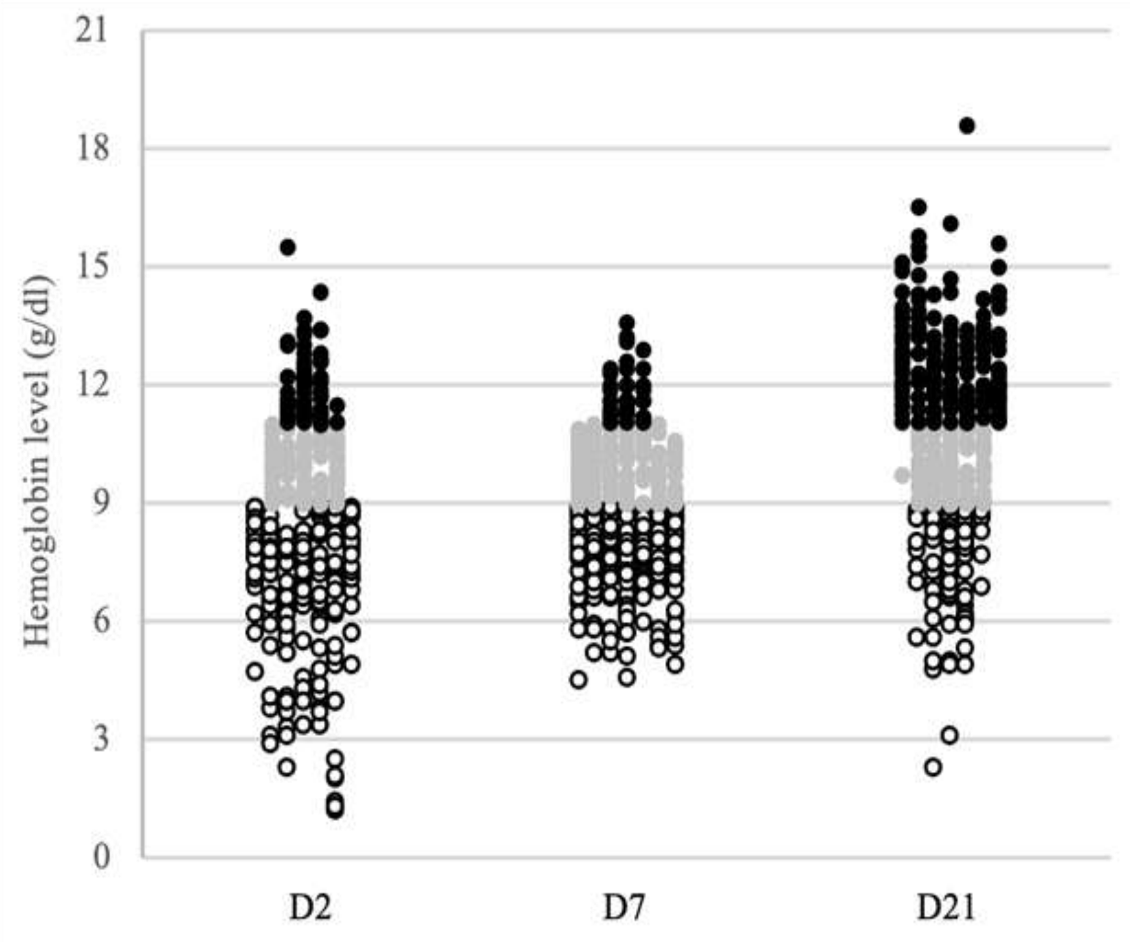

Figure 1. Distribution of hemoglobin level (g/dl), normal; > 11.0g/dl, sub-anemic; > 9.0 g/dl but ≤ 11.0 g/dl, anemia; < 9.0 g/dl

P-N-030

SURVEY ON HEMOGLOBIN CONTENT IN WEANING PIGS

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Introduction
Iron is an essential mineral and an important component of hemoglobin (Hb). Insufficient supply or poor absorption of iron will lead to iron deficiency anemia. Insufficient red blood cells and insufficient hemoglobin count result in decreasing appetite, lethargy, increasing respiratory rate, and mortality. Furthermore, it was demonstrated that piglets with higher Hb concentration at weaning had higher average daily gain during the subsequent post-weaning period [1]. However, the rate of iron deficiency in piglets studied was still very high [2]. Therefore, it is necessary to evaluate the Hb content in the blood of weaned pigs. And this study was conducted to estimate the Hb content in the blood of weaned pigs, then evaluate the iron deficiency and anemia situation of piglets in Vietnam.

Materials and methods
Weaned pigs were distributed into 3 sampling groups. Group 1 was the heaviest pigs, group 2 was medium weight pigs and group 3 was the lightest pigs. Around 20 animals from each weight group were randomly grabbed for blood collection to determine the concentration of Hb in the blood using the HemoCue® Hb 201+ System.

Results and discussion
A total of 235 blood samples of weaned piglets at 5 different sows farms were surveyed to measure the Hb content. The results were showed in Table 1 with classifying Hb levels according to NRC (1979), 25.5% (60/235) of the surveyed piglets had Hb levels in the blood lower than 90g/l and at levels risk of anemia to severe anemia. This data was quite similar to a previous survey, with a prevalence of Hb deficiency in piglets of 28% [2]. Moreover, up to 60% of surveyed farms (3/5 farms) had piglets with Hb deficiency below the minimum level of 90g/l. This showed that the percentage of piglets with Hb deficiency or iron deficiency anemia was quite high. It could be because of the current industrial pig production system, piglets are not exposed to soil (natural source of iron) [3]. Although piglets had been injected iron supplement, the low Hb might be due to the injection technique, inadequate dosage, or poorly absorbed iron in the products injected.

Conclusion
Based on the results of this survey, a high percentage of piglets were considered to be iron deficient and classified as anemic even though the piglets received iron supplementation in the first days of life. Therefore, it is imperative to continuously improve on iron supplementation measures for piglets.

References
1. Bhattarai S, Nielsen JP. Association between hematological status at weaning and weight gain post-weaning in piglets. Livest Sci. 2015;182:64–68.
2. Perri AM, Friendship RM, Harding JSC, O'Sullivan TL. An investigation of iron deficiency and anemia in piglets and the effect of iron status at weaning on post-weaning performance. J Swine Health Prod. 2015;24(1):10–20.
3. Venn JAJ, McCance RA, Widdowson EM. Iron metabolism in piglet anemia. J Comp Pathol Therap. 1947;57:314–325.

4. National Research Council. 1979. The National Research Council in 1979: Current Issues and Studies. Washington, DC: The National Academies Press.

Table 1

Table 1: Percentage of hemoglobin levels in the blood of piglets [4]

Hb level	Status	Number of piglets	Percentage (%)
60-<70	Severe anemia	2	0.85
70-<80	Anemia, growth retardation	15	6.38
80-<90	Risk of anemia	43	18.30
90-<100	Minimum for optimum yield	18	7.66
=100	Normal	157	66.81

P-N-031

THE APPLICATION OF ALTRENOGEST IN THE BATCH MANAGEMENT OF YOUNG FEMALE

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[1]*Ceva Sante Animale,* [2]*Ceva Sante Animale*

Introduction
Dongliao Black Pigs are precious local breeding in China, mainly located in Jilin Province. Dongliao Black Pigs have black whole body fur, high fertility and early sexual maturity, exhibiting strong adaptability, cold resistance, and rough feeding tolerance. Altrenogest is a synthetic progestogen that allows for synchronization of estrus in gilts with positive effects on reproductive performance (1). Altrenogest has been widely used in L×Y binary and purebred gilts, however, there is no report on Dongliao Black Pigs. The aim of this trial was to study the effect of altrenogest on the young female Dongliao Black Pigs, to provide a reference for synchronization of estrus in Chinese local breeding with altrenogest.

Materials and Methods
A total of 233 young female Dongliao Black gilts were divided into 2 batches. These animals were 70kg average body weight and the records of first estrus were unknown. The gilts were fed individually altrenogest (Altresyn, Ceva Sante Animale) for 18 consecutive days by 20 mg (5 ml) per animal per day at the fixed period of time. From the 2nd day after drug withdrawal, the animals received light stimulation, flush feeding and boar exposure. The females were checked for heat twice daily in the presence of boar and serviced immediately with AI when showing heat signs.

Results
The estrus period of Dongliao Black gilts was 5 days (4-8 days after end of synchronization). The estrus within 72 hours and total estrus rate was 89.3% and 93.8% respectively in first batch. The parameter of the second batch was 82.0% and 84.7% respectively. The average parameter of 2 consecutive batches was 88.3% and 89.2%, showing positive results after altrenogest treatment (Table 1).

Conclusions and Discussion
Batch management of L×Y binary or purebred herds can be achieved by feeding altrenogest orally on gilts at 20 mg/day for 18 days and the gilts usually return to estrus 5-8 days after the last feeding (2). The trial results showed, that this method could be successfully applied to Dongliao Black Pigs even these gilts were of different body frame and sexually mature earlier than white pigs. The parameters of the two batches were a little different, which may be related to the nature variability and state of the animals. The Farm should ensure that young female Dongliao Black Pigs fed with altrenogest have a record of puberty.

Acknowledgement and References
[1] Wang Z, et al. Effects of altrenogest on reproductive performance of gilts and sows: A meta-analysis[J]. Animal reproduction science, 2018, 197: 10-21.
[2] Thitachot, et al. "Influence of Backfat Thickness and the Interval from Altrenogest Withdrawal to Estrus on Reproductive Performance of Gilts." Animals 11.5 (2021): 1348.

Table 1

Table1：Estrus rate of young Dongliao Black gilts

	1st batch	2nd batch	Average
Estrus synchronization	89.3%	82.0%	88.3%
Total estrus	93.8%	84.7%	89.2%

Graphs or Images 1

Fig.1 Boar exposure

Graphs or Images 2

Fig.2 Altrenogest feeding

P-N-032

THE EFFECT OF ADDING BETA-GLUCAN TO NURSERY PIG FEED IN FIELD PIG FARM

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Introduction:
Beta-glucan is a natural immune enhancer that can provide non-specific and specific immune functions in animals. It has been added to animal feed as a substitute for antibacterial agents to enhance animal immunity. Porcine reproductive and respiratory syndrome virus (PRRSV) is widespread among the swine population, causing reproductive failure in sows and respiratory disorder in piglets. Since the first outbreak of PRRSV in Taiwan in 1991, it has still been the main problem of the swine industry in Taiwan. However, few studies analyzed PRRSV infection in pigs after administering beta-glucan to nursery pig feed. In this study, the condition of PRRSV infection in nursery pigs will be evaluated after adding beta-glucan to nursery pig feed.

Materials and Methods:
We selected weaned piglets from a PRRSV-contaminated pig farm, and divided them into two group randomly. The treated group added beta-glucan (1000 grams per ton) to the feed between 4 weeks and 10 weeks old, while the control group did not add it. Pigs were divided into two groups 3 days before weaning, with 300 pigs in the treated group and 550 pigs in the control group. There were 15 pigs randomly selected in each group for blood collection at 4, 7, and 10 weeks old. Blood samples were sent to the Animal Disease Diagnostic Center of National Pingtung University of Science and Technology for serum separation and subsequent testing. Before weaning, about 150 piglets in each group were tagged with ear tags of different colors for identification, and both groups weighed at 4 and 10 weeks of age. In this study, the weight, survival rate, PRRS viremia positive rate and viral load were compared between the two groups.

Results:
The nursery survival rates of the control group and the treated group were 74.6% and 73.2%, respectively, and there was no significant difference between the two groups. The average weight of the control group (22.36 kg) at 10 weeks was significantly higher than that of the treated group (19.44 kg). The positive rate of PRRSV in the treated group (20%) was higher than that in the control group (6.7%) at the age of 4 weeks. At 7 weeks of age, the positive rate of PRRSV in the treated group (26.7%) was lower than that in the control group (53.3%). At the age of 10 weeks, the positive rate of PRRSV in the treated group (6.7%) was also lower than that in the control group (26.7%). Comparing the total PRRSV positive rate in the nursery stage, the treated group (16.7%, 5/30) was significantly lower than the control group (40%, 12/30).

Conclusions:
The results of this study showed that the PRRSV detection rate of pigs in the treated group was significantly lower than that in the control group during the nursery stage. beta-glucan might be reduce the pressure of PRRSV infection in nursery stage.

Acknowledgement: We gratefully acknowledge the technician and veterinarian in ADDC of NPUST for their contributions to this study.

References:
[1] Chethan GE et al., 2017. Vet. Immunol. Immunopathol. 191, 36–43.
[2] Lin CN et al., 2013. BMC Vet. Res. 9(1), 1-6.

Table 1

Table 1. Comparison of PRRSV antibody and antigen in different group.

Age (weeks)	PRRSV antibody (S/P ratio)		PRRSV load ($\log_{10}$ copies/ul)	
	Control	Treated	Control	Treated
4	0.91 (4/15, 26.7%)	0.66 (6/15, 40%)	1.76 (1/15, 6.7%)	3.04 (3/15, 20%)
7	1.62 (12/15, 80%)	1.93 (14/15, 93.3%)	2.37 (8/15, 53.3%)	2.65 (4/15, 26.7%)
10	2.22 (15/15, 100%)	2.29 (15/15, 100%)	2.76 (4/15, 26.7%)	1.83 (1/15, 6.7%)
The nursery stage	1.91 (27/30, 90%)	2.11 (29/30, 96.7%)	2.50 (12/30, 40%)	2.48 (5/30, 16.7%)*

* Significant difference between the control group and the treated group ($P<0.05$).

P-N-033

THE EFFECTS OF SUPPLEMENTING HERBAL/ PHYTOGENIC CHOLINE (BIOCHOLINE) IN GROWING SWINE DIETS ON GROWTH AND CARCASS CHARACTERISTICS

Dr. Shivi Maini[1]
[1]*Indian Herbs*

Introduction
Researchers have supported claims of a reduction in fatness of slaughter pigs, through the use of choline and betaine. Choline has its direct role in methylation reactions, it has a pronounced lipotropic effect. It stimulates both liver lipid mobilisation, affecting the blood lipoprotein profile, and carnitine synthesis which thus improves fatty acid oxidation in mitochondria. In number of studies, choline has been reported to decrease and/or redistribute carcass fat in several animal species, including chickens, fish and pigs. Australian pork production company, Bunge Meat Industries, which markets more than 800,000 pigs per year, reported that it had observed almost a 15% decrease in the backfat thickness of animals given choline and betaine in their grower and finishing diet. (Bunge's studies, Australian Pig Science Association). A trial was undertaken to evaluate efficacy of natural choline supplement BioCholine on growth, carcass characterstics, fack fat and lean gain in growing pigs.

Materials and methods
An experiment was conducted at FBF Technology Centre, Facons Bottom Farm, Iken, Woodbridge, Suffolk, UK in total of 600 grower pigs. Pigs were selected from a medium – health, single source BQP commercial finishing unit for the trial and 20 pigs were housed in each pen. Feed was supplied as pellets by BOCM PAULS which did not contain any prophylactics or antibiotic growth promoters. Pigs were divided into 3 groups i.e., Group A kept as untreated control while Group B was fed herbal supplement BioCholine @ 500 gm/ton and Group C @ 250 gm/ton of feed to assess the effect of BioCholine on growth rate, feed intake, FCR and slaughter characteristics of pigs.

Results and Discussion
During Grower Phase (39 kg – 62 kg), there was significant improvement in FCR in the groups supplemented with BioCholine @ 500 gm/ton of feed (2.10) and BioCholine @ 250 gm/ton of feed (2.08) in comparison to control group (2.17). The Daily Live Weight Gain was similar and statistically non-significant in the 3 groups though the group with BioCholine @ 250 gm/ton showed highest growth (892.99 g/pig/day). During Finisher Phase (62 kg – 103 kg): In this phase also FCR in BioCholine @ 500 gm/ton of feed was better (2.58) in comparison to control group (2.64) with no significant difference in Daily Live Weight Gain (1044 g/pig/day). Overall Growth Rate: The highest growth rate during the whole trial period was 980.94 g/pig/day in the BioCholine @ 250 gm/ton group though there was no significant difference between all the groups. Slaughter Characteristics: Lean Meat% With supplementation of BioCholine @ 500 gm/ton of feed, the lean meat% was higher (61.36%) with an improvement (+2%) though it was not significantly different from Control (61.23%) and the group which was given BioCholine @ 250 gm/ton of feed had significantly lower lean meat percentage (60.80%) than other groups. Backfat P2 With supplementation of BioCholine @ 500 gm/ton of feed, the backfat P2 was lower (10.93 mm) with an improvement (-1.26%) though it was not significantly different from Control (11.07 mm) and the group which was given BioCholine @ 250 g/ton of feed had higher P2 (11.62 mm) than other groups. There

was no significant difference in hot weight, probe/weight, shoulder fat and leg fat in the groups.

Conclusion

We can conclude that using BioCholine as the only source of functional choline metabolites in the pig's diet can be considered an alternative in order to achieve the backfat thickness reducing effect, increase the daily weight gain, increase the carcass yield, reduce the feed conversion rate, with the additional advantage of not presenting any kind of risk or prohibition due to it being a 100% natural product.

P-N-034

THE SUPPLEMENTATION EFFECT OF THE COMBINATION OF 4 BACILLUS PROBIOTIC SPECIES ON THE PERFORMANCE OF PIGLETS DURING CLOSTRIDIUM PERFRINGENS OUTBREAK.

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[2]K.M.P. Biotech Co., Ltd.

Introduction
Clostridium perfringens (C. perfringens) causes intestinal diseases in domestic animals, such as pigs and chickens through overgrowth and the secretion of their multiple toxins. In pigs, its symptoms include severe diarrhea, necrotic of mucosa, intestinal villi atrophy, hemorrhagic small intestines and the lesions are typically in jejunum and ileum. Antibiotics play a role in the prevention and clinical treatment of diseases caused by C. perfringens. With regard to antimicrobial resistance, antibiotics are prohibited for growth promoters and therapeutic propose in feed. C. perfringens infection has become an important problem in the pig industry. Probiotic has been used in animal production to prevent pathogenic bacteria infections. They prevent the overgrowth of C. perfringens by producing antimicrobial peptides, inhibiting the growth of pathogenic bacteria such as C. perfringens and improving performance of pigs. Therefore, the objective of this study was to evaluate the effects of supplementation of multi-species of Bacillus probiotic on the performance of piglets under C. perfringens outbreak.

Materials and Methods
Bacillus probiotic product (B-Live™, K.M.P. Biotech Co., Ltd., Thailand) in this study is composed of 4 species of Bacillus subtilis, Bacillus licheniformis, Bacillus pumilus, and Bacillus amyloliquefaciens. Each species contains at least 1.0×10^{10} cfu/liter. This experiment was performed in Clostridium infected breeding herd. Piglets were infected with C. perfringens during the first weeks of age with a mortality rate as high as 40%, the infection was confirmed by gram staining and bacterial isolation. Piglets from 140 litters were divided equally into 2 groups (i.e. control and treatment), treatment group was fed daily with B-Live 1 ml./piglet on 1st week of age. After that, 0.5 ml./piglet of B-Live was well mixed with their creep feed for feeding piglets until weaning. The performance parameters of both groups, including sow parity, total number of piglets born, born alive, litter birth weight, number of pigs weaned, piglet losses per litter and pre-weaning mortality rate were recorded. All data was presented as mean ± SEM and the differences between the results of treatment and control group were considered statistically significant at the 95% confidence when P-value ≤ 0.05.

Result
The piglet performance was showed in Table 1. On farrowing day, there was no significant different in term of parity of sow, total number of piglets born, born alive, and litter birth weight. Clinical finding, the piglets started showing clinical signs (i.e. convulsion) of C. perfringens infection on the first week of age. Comparing the percentage of piglet mortality, a lower percentage of pre-weaning mortality rate of piglet in treatment group of piglets that received B-Live than control group was found (4.38% vs 22.80%).
Discussion

The present results showed that the piglets fed with the multi-species Bacillus showed significantly lower pre-weaning morality rate than those in the control group. The reason for a higher protection found in treatment group might be that probiotic Bacillus may provide the benefits to the host through various mechanisms, such as (1) producing antimicrobial substances, such as bacteriocin that inhibit the overgrowth of various pathogenic bacteria, (2) competing with pathogenic microorganisms for enterocyte binding and (3) maintaining intestinal barrier integrity. In conclusion, feeding multi-species Bacillus, B-Live by orally on the first week of age and continue feeding by mixing with creep feed to the piglets until weaning has beneficial effect on the piglet performances, particularly reducing pre-weaning mortality rate in the C. perfringens infection breeding herd.

References
1. Songer, J. G. and Glock, R. D. (1998). J. Swine Health Prod, 6(5), 223-225.
2. Posthaus, H., et al. (2020). J. Vet Diag Invest, 32(2), 203-212.
3. Schoster, A. et al. (2013). Anaerobe, 20, 36-41.

Table 1

Table 1: Production performance parameters in control and treatment groups (Mean ± SEM)

Groups	No. Sow	Parity	Total number of piglets born	Born alive	Litter birth weight	Weaned pigs per sow	Piglet losses per litter	%Pre-weaning mortality
Control	69	4.9 ± 0.1	14.5 ± 0.4	12.3 ± 0.3	17.5 ± 0.5	9.3 ± 0.4	3.0 ± 0.3	22.8 ± 2.9
Treatment	69	5.2 ± 0.1	14.0 ± 0.3	12.1 ± 0.2	18.0 ± 0.3	11.5 ± 0.2	0.6 ± 0.1	4.3 ± 1.1
P-value	NA	0.159	0.410	0.732	0.489	0.000	0.000	0.000

P-N-035

USING A HEALTH ECONOMIC MODEL TO ESTIMATE THE PROFITABILITY AND ENVIRONMENTAL SUSTAINABILITY IMPACTS OF USING IMPROVAC® IN MALE AND FEMALE FATTENING PIGS FOR PRODUCERS IN CANADA, THAILAND, BRAZIL, AND FRANCE

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Introduction
A commercial vaccine against endogenous gonadotrophin releasing factor (GnRF), Improvac®, has shown to provide important sustainability benefits for pig producers: improved animal welfare, efficient production, and reduction of environmental burden [1]. Feed consumption and associated manure production is regarded as the main contributor to the environmental impact of pork production and the feed conversion ratio (FCR) has the largest impact on global warming [2]. This study estimates and compares the profitability and environmental sustainability impacts of using Improvac® in male and female pigs intended for market in four countries.

Material and Methods
A health economic model was built using baseline parameters for non-immunized (non-IM) fattening pigs, i.e., physically castrated males and intact market gilts, to compare their performance and economic parameters relevant for producers with Improvac® immunized (IM) male and female counterparts, based on datasets of two meta-analyses [3], [4]. Only statistically significant differences (P<0.05) were considered, otherwise the same baseline value was used for IM pigs [Table 1]. Data collected from Canada, Thailand, Brazil, and France included initial and final live weights, carcass weights, FCR, and cost data: Improvac® vaccination cost per IM pig, physical castration cost per non-IM male pig, average feed cost per kg for IM and non-IM pigs, price per kg carcass or live weight (depending on the country), and premiums/penalties for non-IM and non-IM carcasses. Parameters were changed over a range of ±20% in one way sensitivity analyses (OWSA) to define key drivers of profitability. The model also allowed two-way sensitivity analyses (TWSA).

Results
Final weights reported: Canada 135 kg, Thailand 135 kg, Brazil 125 kg, and France 121 kg. For comparative reasons, results as calculated by the model in local currencies were converted to US dollar ($) using contemporary conversion rates. The base case analysis showed a higher net return with IM male pigs compared to non-IM pigs for producers in all 4 countries: $5.12, $9.52, $5.17, and $4.98 per pig, respectively; corresponding differences in market gilts: $1.64, $2.11, $-0.27, and $1.21 per pig, respectively [Table 2]. Feed costs had the highest impact in OWSA, but when changed simultaneously for IM and non-IM pigs in TWSA, the impact on the incremental profitability was relevantly reduced. Compared to non-IM pigs, IM males' profitability improves with higher feed costs, while in IM market gilts lower feed costs increase the incremental profitability [Table 3]. In gilts, the difference in FCR between IM and non-IM market gilts was shown to be a key driver of profitability [Figures 1 and 2].
Discussion and Conclusion

When using the existing health economic model, changes of ± 20% of the performance and economic parameters relevant for pig producers only have little impact on Improvac®'s incremental net profitability. Feed costs and FCR have the highest impact on profitability across countries. Improvac® immunized males are always more profitable for pig producers compared to non-IM males and reduce environmental impact by improving feed efficiency [Figure 1]. In market gilts the immunization benefits are linked to nutritional strategies to balance the additional feed intake and growth with feed efficiency [Figure 2]. These parameters of most influence were comparable over all four countries. In summary, Improvac® improves the sustainability by decreasing or improving the FCR, the key factor of environmental burden in pork production.

References:
1. Batorek, N., et al, 2012. Animal 6, 1330–1338
2. Kress, K., et al, 2019. Sustainability of Pork production with immunocastration in Europe. Sustain. 11, 8–10. https://doi.org/10.3390/SU11123335
3. Poulsen Nautrup, B., et al, 2018. Res. Vet. Sci. 119, 182–195
4. Poulsen Nautrup, B., et al, 2020. Res. Vet. Sci. 131, 159–172

Table 1

Table 1: Overview of the default data considered in the model as valid from the perspective of the producers. Production and carcass parameters were defined for pigs not immunized with Improvac® (non-IM), i.e., physically castrated male pigs or untreated gilts. Efficacy data represent the differences between IM and non-IM pigs as reported in meta-analyses (Poulsen Nautrup B., et al., 2020, 2018). In case of no statistically significant difference in meta-analysis ($P \geq 0.05$), the same value was applied to IM and non-IM pigs (± 0.00). All parameters were subjected to changes in one-way sensitivity analyses (OWSA).

	Default male pigs	Default female pigs
Production and carcass parameters		
Initial weight (kg)	25	25
Final live weight in non-IM pigs (kg)	117	115
Carcass weight in non-IM pigs (kg)	92	92
Feed-conversion ratio in non-IM pigs (kg/kg)	2.76	3.31
Efficacy data (impact of Improvac® on production and carcass parameters)		
Difference in feed conversion ratio between IM and non-IM pigs (kg/kg)	- 0.22	± 0.00
Difference in live weight between IM and non-IM pigs (kg)	+ 2.1	+ 3.8
Difference in carcass weight between IM and non-IM pigs (kg)	± 0.00	+ 3.1

Table 2

Table 2: Results of base case analyses calculated for the 4 countries in local currencies and converted to USD. Outcome was the incremental net return per pig, i.e., the difference in net return between pigs immunized with Improvac (IM) and non-IM pigs, i.e., physically castrated male pigs or untreated gilts.

		Incremental net return (per pig) between IM and non-IM pigs: Base case results	
		Perspective of the producers	
		Male Pigs	Female Pigs
Country	Final live weight in non-IM pigs	Local Currency / USD*	Local Currency / USD*
Canada	135 kg	CAD 6.49 / USD 5.12	CAD 2.08 / USD 1.64
Thailand	135 kg	THB 324.65 / USD 9.52	THB 72.00 / USD 2.11
Brazil	125 kg	BRL 24.34 / USD 5.17	BRL – 1.28 / USD – 0.27
France	121.1 kg	EUR 4.63 / USD 4.98	EUR 1.12 / USD 1.21

*Source of conversion rates: European Commission's official monthly conversion rates for June 2022 (access: June 23, 2022)

Table 3

Table 3: Results of two-way sensitivity analyses (TWSA) conducted for the outcome of incremental net return per pig, i.e., the difference in net return between pigs immunized with Improvac (IM) and non-IM pigs, as calculated for 4 countries from the perspective of the producers. Feed costs were changed over a range of ± 20% simultaneously for IM and non-IM pigs.

	Incremental net return (per pig) between IM and non-IM pigs: Results from TWSA				
	Male Pigs			Female Pigs	
	- 20% Feed costs in IM and non-IM pigs	+ 20% Feed costs in IM and non-IM pigs		- 20% Feed costs in IM and non-IM pigs	+ 20% Feed costs in IM and non-
Canada	CAD 5.75	CAD 7.23		CAD 2.74	CAD 1.42
Thailand	THB 268.40	THB 380.90		THB 115.20	THB 28.80
Brazil	BRL 20.99	BRL 27.69		BRL 1.56	BRL - 4.11
France	EUR 3.82	EUR 5.44		EUR 1.70	EUR 0.55

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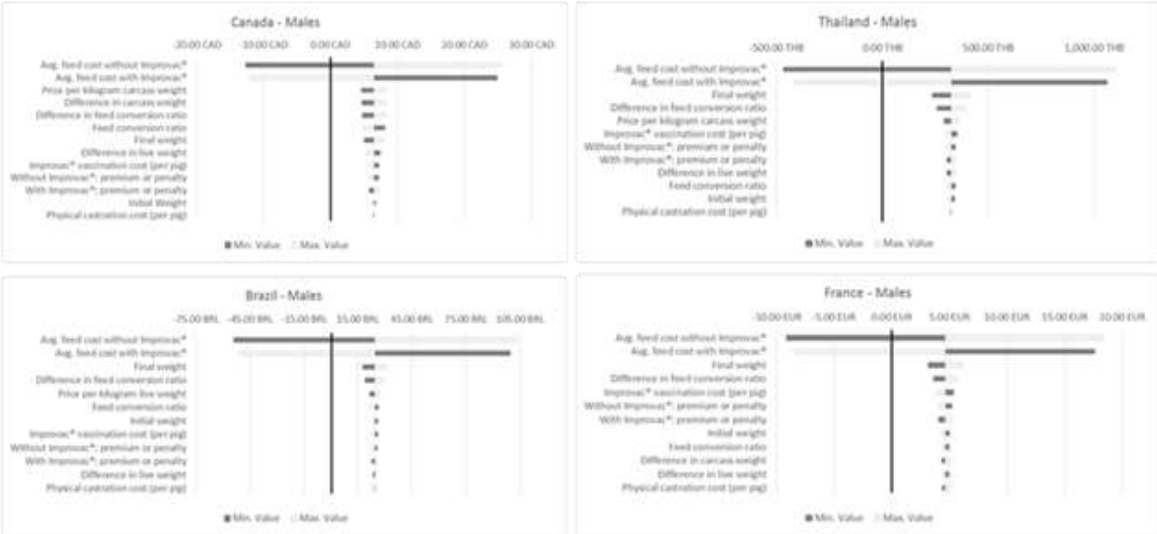
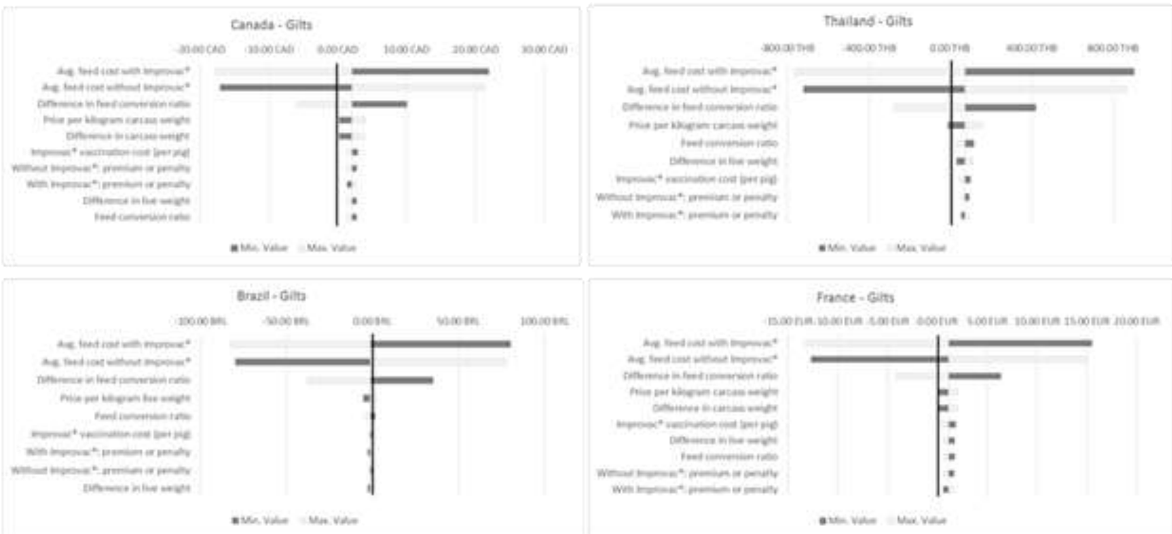
Graphs or Images 1

Fig. 1. Tornado diagrams for one-way sensitivity analyses run for the outcome of incremental net return between **male pigs** immunized with Improvac® (IM) and physically castrated pigs as calculated for 4 countries from the perspective of the producers. Each parameter was changed ±20% of base case values and results depicted as horizontal bars. The black vertical lines represent no difference in net return between IM pigs and non-IM pigs, values to the right (positive figures) indicate higher net return with IM pigs.



Graphs or Images 2

Fig. 2. Tornado diagrams for one-way sensitivity analyses run for the outcome of incremental net return between **female pigs** immunized with Improvac® (IM) and untreated gilts as calculated for 4 countries from the perspective of the producers. Each parameter was changed ±20% of base case values and results depicted as horizontal bars. The black vertical lines represent no difference in net return between IM pigs and non-IM pigs, values to the right (positive figures) indicate higher net return with IM pigs.



P-N-037

EVALUATION OF REPRODUCTIVE PERFORMANCE OF THE USE OF BUSERELIN (PORCEPTAL®) IN A SINGLE FIXED TIME ARTIFICIAL INSEMINATION VERSUS CONVENTIONAL PROTOCOL IN TAIWAN

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Introduction
Fixed time artificial insemination (FTAI) is based on a treatment with GnRH analogue buserelin to induce ovulation in gilts and sows and followed by a single artificial insemination (AI) at defined time intervals. Multiple studies show no significant difference in the reproductive parameters were found between animals following FTAI or a conventional multiple insemination program in European countries. The aim of this study was to demonstrate productive performance of a FTAI protocol using GnRH analogue (Porceptal®, MSD Animal Health) is similar to conventional management with multiple inseminations in commercial pig farm in central Taiwan under subtropical climates.

Materials and Methods
The trial was conducted in a 350 sow farrow-to-finish farm with 3 week batch production in Yunlin (central Taiwan). A total of 986 sows, 697 in control group (C) with conventional AI during Feb to Nov 2020 and 289 in Porceptal group (P) with FTAI during Feb to May 2021 were evaluated.
In C group, AI was performed at estrus onset and repeated every 24 hour (h) while sows were in standing heat. G group was treated with Porceptal® at 86 h after weaning followed by FTAI at 32 h after Porceptal® treatment.
Different reproductive parameters, such as farrowing rate, total born, born alive, stillborn and small/weak piglets were compared via Student t Test.

Results
The reproductive performance showed no significant difference (Table 1). Farrowing rate was not impacted by treatment (C: 77.5% vs P: 72.3%; p >0.05). Prolificacy was also not different (Total born C: 12.1 ± 0.91 vs P: 12.0 ± 0.51 piglets; p >0.05; born alive C: 10.9 ± 0.89 vs P: 10.8 ± 0.45 piglets; p >0.05).
Conclusion

FTAI protocol using buserelin resulted in an identical reproductive performance under subtropical climates compared with conventional management with multiple inseminations. Porceptal® can be considered as a management tool to lower workload, save semen, and even reduce non-productive days.

Keywords

Porceptal, FTAI, Buserelin, Reproductive performance

References

1. A. Martinez et al. IPVS 2016

2. C. Laza et al. IPVS 2016
3. J. Navas et al. IPVS 2016
4. L. Sanjoaquin et al. IPVS 2016
5. M. Krone et al. IPVS 2016

Table 1

Table 1. Reproductive performance of C and P Groups

Group	Control	Porceptal®
No. of Sows	697	289
Farrowed sows	540	209
Farrowing rate	77.5%	72.3%
Total born	6522	2508
Born alive	5864	2249
Small/weak piglets	222	66
Stillborn	436	193
Avg. Total born	12.1	12.0
Avg. Born alive	10.9	10.8
Avg. Small/weak piglets	0.4	0.3
Avg. Stillborn	0.8	0.9

P-N-038

EFFICACY OF YEAST POSTBIOTIC ON INTESTINAL HEALTH AND GROWTH OF NEWLY WEANED PIGS

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Introduction

The jejunum is the major site within the gastrointestinal tract for digestion and absorption of nutrients and for immune protection from the external stressors including allergens, antigens, toxic, pathogens, and numerous anti-nutritional compounds possibly from feeds (1). All these cause intestinal immune reactions, inflammation, oxidative damages, apoptosis in the jejunum which in turn damages growth and health of pigs (2). Selected dietary interventions are shown to be effective to help the intestinal health of newly weaned pigs (3, 4). This study aimed to determine the efficacy of yeast based postbiotics (celluTEIN, Puretein Bioscience LLC, Minneapolis, MN, USA) on the intestinal health and immune status of newly weaned pigs.

Materials and Methods

Thirty-two newly weaned pigs at 21 days of age (16 barrows and 16 gilts) with an initial body weight (BW) of 6.05±0.24 kg were assigned into two dietary treatments based on a randomized complete block design, with initial BW and sex serving as blocks. The dietary treatments consisted of a control group receiving a basal diet and a group supplemented with yeast postbiotics (175 g/ton feed) in the basal diet. The yeast postbiotics was included in the diet by replacing corn and formulated to meet the nutrient requirements. The pigs were fed for 35 days in three phases: P1 (day 1-10), P2 (day 10-19), and P3 (day 19-35). At the end of each phase, the pigs and feed disappearance were individually weighed to determine growth performance parameters. Fecal score was recorded bi-day from day 3 of the experiment. After 35 days of feeding, the pigs were euthanized, and intestinal mucosa was collected to assess immune status and oxidative stress. Jejunal tissue was also collected to measure the expression of genes associated with intestinal barrier markers, cell proliferation and apoptosis. Data were analyzed using SAS 9.4.

Results

The yeast postbiotics did not influence the growth performance of nursery pigs. However, yeast postbiotics reduced ($P<0.05$) the fecal score from d 3-7. Yeast postbiotics did not affect the production of inflammatory cytokines and oxidative damage products whereas it tended to increase the gene expression of IFN-γ ($P=0.071$) and mTOR ($P=0.080$), decrease the gene expression of BAX1 ($P<0.05$), tended to decrease the gene expression of SGK1 ($P=0.066$), increased ($P<0.05$) cell proliferation in the crypts, and tended to increase the villus height ($P=0.078$) and crypt depth ($P=0.052$) in the jejunum.

Conclusions and Discussion

The supplementation of yeast postbiotics (celluTEIN)at 175 g/ton of feed in nursery diets reduced diarrhea within the first week after weaning and provided protection to the villi in the jejunum by enhancing the immunocompetence of nursery pigs, promoting cell proliferation, and reducing the expression of genes associated with apoptosis without affecting inflammatory and oxidative stress status in the jejunum and the growth performance of the nursery pigs.

Acknowledgement

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References

1. Kim SW, Duarte ME. 2021. Understanding intestinal health in nursery pigs and the relevant nutritional strategies. Anim Biosci 34:338-344.
2. Deng Z, Duarte ME, Jang KB, Kim SW. 2022. Soy protein concentrate replacing animal protein supplements and its impacts on intestinal health, mucosa-associated microbiota, and growth performance of nursery pigs. J Anim Sci 100:skac255.
3. Duarte ME, Stahl CH,Kim SW. 2023. Intestinal damages by F18+Escherichia coli and its amelioration with an antibacterial bacitracin fed to nursery pigs. Antioxidants12:1040.
4. XuX, Duarte ME, Kim SW. 2022. Postbiotics effects of Lactobacillus fermentate on intestinal health, mucosa-associated microbiota, and growth efficiency of nursery pigs challenged with F18+ Escherichia coli. J Anim Sci 100:skac2

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