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## DAYS OF VETERINARY MEDICINE 2015



*Proceedings of the 6<sup>th</sup> International Scientific Meeting  
Days of veterinary medicine 2015  
Struga, Macedonia  
September 24-26, 2015*



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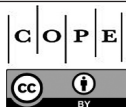
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## **Proceedings of the 6<sup>th</sup> International Scientific Meeting Days of veterinary medicine 2015 Struga, Macedonia September 24-26, 2015**

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**O1****The dual role of farm animals in animal production and translational biomedical research****Antonio Gonzalez-Bulnes***Comparative Physiology Lab, SGIT-INIA. Avda.,  
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Most of the data obtained in biomedical research have been acquired by observational studies, which are usually biased by concurrent factors related to economical, nutritional, behavioral and socio-cultural factors specific to human populations. Thus, research methods must isolate the physiological causes from these factors, which can only be accomplished by interventional studies under well-determined conditions. Interventional research in human beings is obviously banned by ethical reasons. These considerations make the use of animal models essential. Most of biomedical researches using animal models have been performed in rodents. Mice, rats and guinea pigs need little space, are relatively inexpensive to maintain, easy to manage and have a short life cycle. Furthermore, mice and rats have a sequenced genome and are easily modified by genetic engineering. However, there are also a number of severe limitations like marked differences with humans in cell and tissue biology, metabolic and endocrine routes, and developmental patterns and physiology of organs and systems. A second crucial issue is the small body size of rodents, which flaws the translational application of imaging techniques and impedes the serial sampling of large amounts of blood and tissues. Thus, the complete translation of finding in rodents to human medicine is seriously compromised. The use of farm animals (mainly swine, sheep and rabbits) offers numerous profitable characteristics. Necessities for housing, management and clinical care are well-known due to the traditional use of these species in animal production and application of imaging techniques and serial sampling of large amounts of blood and tissues are well-established. Moreover, pathways regulating

metabolic and endocrine routes in large animals are more similar to humans than in rodents. Finally, in the last years, the genomic analysis is well-advanced and it is possible to obtain targeted gene mutations for specific models. From a veterinary point-of-view, research in farm animals is twice interesting due to its dual purpose. In addition to being a recognized biomedical model, farm animals are a key economic resource for either developed or developing or transition regions. Hence, the results obtained in research using farm animals are translational to humans but also of direct application for improving animal production.

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**O2****The relationships between animal welfare, economics, ethics and the law – an uneasy alliance for vets – where do we stand?****David B. Morton***University of Birmingham, United Kingdom*

Animal welfare depends on humans to deliver responsible care to promote good welfare and to avoid poor welfare. Economic pressures often push animal keepers, such as farmers (and also other animal owners) to compromise animal welfare. Occasionally good welfare can promote economic gain, but poor welfare is always bad for the animals and may be economically advantageous or disadvantageous. Farmers need to earn a living, and the public needs to be fed and pay a fair price for their food. Shopkeepers also need to earn money and make a profit in our current societal norms. The law is there to uphold the moral values of society, including protecting animal welfare, but does it? How far should society go to provide animals with a good quality of life? What is the role of vets in this debate - where do we stand and are we equipped to do it?

**O3****The use of *in vivo* imaging to study pregnancy in non-rodent animals**

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**Introduction:** Pregnancy is often considered as a “black box” and access to the fetus or placenta is often limited to the euthanasia of the pregnant dam. Alternatively, *in vivo* imaging enables the longitudinal monitoring of pregnancy.

**Methods and Results:** Before fertilization, gametes can be visualized in the female uterus using confocal laser endomicroscopy. For example, movements of dog spermatozoa at the surface of the endometrium are observed *in vivo* to study their interaction with the endometrium. Ultrasound technologies are considered as the gold standard for *in vivo* imaging of pregnancy. Ultrasound bio-microscopy is used to visualize tissue up to 1cm depth with a 40mm resolution. Applications in the rabbit include *in vivo* visualisation of the cumulus oocyte complex before ovulation. Embryo development is observed before implantation when the embryo is still free in the uterus (early D7), during implantation (late D7) when the embryonic vesicle lays apposed to the reacting uterus, and after implantation (early D8). Ultrasound bio-microscopy guided sampling or injection can also be performed into the blastocoele. After implantation, the fetoplacental unit is monitored using classical ultrasound technologies and 2D-Doppler quantification of blood flows. Reduced perfusion (sheep and rabbit models of intra-uterine growth retardation) can be quantified within placenta by Power Doppler angiography. Ultrafast Doppler, a paradigm shifting ultrasound technology, greatly increases Doppler sensitivity. Through the evaluation of low versus high temporal variation of pulsatility, it was shown to discriminate between maternal and fetal blood flow within the placenta in a rabbit model. Minimally invasive technologies, such as 3D-angiography (X-Ray based technology), can also be used. An indwelling catheter is placed in the uterus via the carotid artery and blood flow is visualized using contrast agent. Angiography can also be used to modify blood flow to the placenta through arterial vessel embolisation. Examples in rabbit and sheep will be shown. Other technologies, such as magnetic resonance, provide fetoplacental images at the histological scale. We are currently exploring how this technique could help develop an “*in vivo*” atlas of fetal rabbit development. Finally, at the time of delivery, shear wave elastography, which is based on ultrafast wave technology, can be used to evaluate cervical ripening in sheep.

**Conclusion:** The use of refined imaging technologies enable researchers to study *in vivo* fetoplacental development and can help reduce the number of animals being used in research. The same techniques will provide useful tools for the veterinary management of domestic animals in practice.

**O4****Culture independent molecular approaches and Next Generation Sequencing in the service of veterinary research**

Georgios Oikonomou

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**Introduction:** Culture-independent studies of the collective set of genomes of mixed microbial communities, often described as metagenomics or microbiomics studies, have been receiving increased attention by the scientific community around the world. Sequencing and analysis of hypervariable regions within the 16S rRNA gene can provide relatively rapid and cost-effective methods for assessing bacterial diversity and abundance and may be useful for pathogen discovery and identification. Such approaches can be proved extremely useful for veterinary research.

**Studies on dairy cattle mastitis:** Mastitis is an important disease in the dairy industry. Different microbial species have been identified as causative agents in mastitis, and are traditionally diagnosed by bacterial culture. Oikonomou et al. (2012) used pyrosequencing of bacterial 16S rRNA genes to investigate bacterial DNA diversity in milk samples of mastitic and healthy dairy cows and compared the results with those obtained by classical bacterial culture. In samples that were aerobic culture negative, pyrosequencing identified DNA of bacteria that are known to cause mastitis, DNA of bacteria that are known pathogens but have so far not been associated with mastitis, and DNA of bacteria that are currently not known to be pathogens. A possible role of anaerobic pathogens in bovine mastitis was also suggested. In a recent study (Boulding et al., 2015) we explored the potential use of the MiSeq Illumina sequencing platform for mastitis diagnosis. Our approach allowed us to identify the potentially causative bacteria in 53/65 (82%) of our cases. As expected, well-known mastitis-causing pathogens such as *Streptococcus uberis* were identified in the majority of the cases. However, we were also able to identify potential new mastitis pathogens such as the anaerobic bacterium *Sneathia sanguinegens* and pathogens which are difficult to isolate such as *Listeria innocua* and *Rhodococcus* spp. Given that DNA sequencing technology has advanced at an incredible pace in recent years, leading to astonishing decreases in sequencing cost, such culture independent approaches based on next generation sequencing may eventually provide with powerful diagnostic tools for mastitis and subclinical mastitis.

**The gut microbiome:** The combined genome of the microbiota of the mammalian gastrointestinal tract (GIT) exceeds the length of the host's genome by a factor of approximately 150,000. The gut microbiota is known to have a role in shaping key aspects of postnatal life, such as development of the immune system, and influencing the host's physiology, including energy balance. Oikonomou

et al. (2013) described the intestinal microbiota of neonatal calves and its association with health and growth. It has also been recently shown that the structure of the rumen fluid microbiome shifts between the pre-partum and first week post-partum periods and that the profile of pre- and post-partum microbiomes could be used to accurately predict production traits (Lima and others 2015).

Boulding et al. 2015. *BCVA Annual Conference*. Southport.

Lima et al. 2015. *Applied and Environmental microbiology*. 81:1327-1337.

Oikonomou et al. 2012. *PLOS One* 7(10): e47671. doi:10.1371/journal.pone.0047671.

Oikonomou et al. 2013. *PLOS One* 8(4): e63157. doi:10.1371/journal.pone.0063157.

## O5

### Morphology of the tongue in fish species of commercial interest

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**Introduction:** The oral cavity of vertebrates plays a very important role in many functions strictly related to the food processing. The relationships between the capacity of adaptability to the environment and the anatomical modifications developed in the oral cavity and especially the numerous differences in the dorsal part of the tongue among teleosts, related to their nutrition in various ecological systems were studied, considering also the important role of the tongue during the intra-oral transport and the swallowing of food, but scarce data are present in literature about its characteristics. Two species, sea bass *Dicentrarchus labrax* and seabream *Sparus aurata*, are mainly involved in the aquaculture industry in the mediterranean area, moreover another species, the white seabream *Diplodus sargus sargus* was chosen as the best candidate for the diversification of marine fish species of commercial interest.

**Material and methods:** The present investigation was carried out, using the standard procedures for light and scanning electron microscopy.

**Results:** In the sea bass *Dicentrarchus labrax* three different areas were observed on the dorsal lingual surface: a triangular apex, a body and a root adjoining the branchial structures. Numerous canine-like teeth, organized in pads, were present along the dorsal surface. The teeth were curved and their tips were oriented towards the esophagus as well as the tongue long axis. The presence of numerous taste buds, scattered on the tongue, was showed by means of light microscopy. In the seabream *Sparus aurata* the tongue can be divided in three main parts, an apex, a body and a root. The apical part of the tongue is inserted in a pouch. A medial ridge is characteristic on the lingual body surface. Numerous taste buds were scattered on the whole tongue surface especially on the lateral parts and in the medial part of the body and the root, and also along the medial ridge

surface. Different papillae were showed throughout the lingual dorsal surface. In the white seabream *Diplodus sargus sargus* an apex, a body and a root, were clearly observed too, with a pouch partially covering the apex. In the pouch, cylindroid and foliate-like papillae can be distinguished. Numerous fungiform, cylindroid and cone like papillae are present along the whole lingua surface, especially on the medial and lateral parts, from the apex to the root. On the dorsal surface of some papillae taste buds were showed.

## O6

### Genome-wide SNP analysis of genetic diversity in Western Balkans and Central European sheep breeds highlights the presence of a strong phylogeographic gradient

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**Introduction:** Due to their geographic position at a major crossroads between Europe and the Near East, Balkan countries represent an interesting target to study past routes of sheep dispersal from the center of domestication into the Europe. Genetic structure and relationships among breeds from this area have been previously investigated using microsatellites. Here we present the first report



on the genetic relationships among Western Balkan and Central European sheep breeds in a worldwide context, investigated using genome-wide SNP genotypes.

**Material and methods:** A total of 119 animals from 20 populations from Western Balkan (Slovenia: Bovec sheep, Jezersko-Solcava sheep, Bela Krajina Pramenka, Istrian Pramenka; Croatia: Cres Island sheep, Krk Island sheep, Lika Pramenka, Pag Island sheep, Rab Island sheep, Dalmatian Pramenka; Bosnia and Herzegovina: Privorska, Dubška; Serbia: Lipska Pramenka; Montenegro: Pivska Pramenka, Zuja, Sora; Republic of Macedonia: Ovchepolean Pramenka, Karakachanska Pramenka) and Central Europe (Czech Republic: Sumavska, Valachian) were genotyped using the Illumina OvineSNP50 BeadChip. Data from 2491 animals from 123 populations sampled worldwide were also available from the ISGC dataset.

**Results:** On a world-wide scale, MDS and ADMIXTURE analyses highlighted a strong relationship of Western Balkan sheep with Mediterranean sheep (Spanish and Italian non Merino breeds). Interestingly, the two breeds from Czech Republic (Sumavska and Valachian) displayed a stronger relationship with Central (Swiss and German) and Northern European (Netherlands) breeds than with Mediterranean breeds. A faint “Merino” influence (ranging 2-9% of the total genomic component) was observed in Ovchepolean Pramenka. Analyses carried out at the regional scale confirmed that genetic variability was mainly distributed along a phylogeographic gradient. However, Valachian sheep appeared to be differentiated from all other breeds, including the Czech Sumavska, thus likely supporting the hypothesis that they were introduced into Czech territories between the 15<sup>th</sup> and 16<sup>th</sup> century by immigrating Valachs. Karakachanska Pramenka also clustered separately, this result likely being the consequence of the high inbreeding observed ( $N = 6$ ; average  $F = 0.18 \pm 0.05$ ). TREEMIX analysis highlighted that several migration events among Western Balkan and Central European breeds are plausible. Most of the inferred migrations involved breeds from the same country or from neighboring countries.

**Conclusion:** These molecular evidences support the hypothesis that ancient domestic sheep populations spread into Europe along two main geographical axes, a SE/NW land route and a SE/SW Mediterranean route. Due to their geographical position, Western Balkan sheep seem to have played a role in stock dispersal mainly following the Mediterranean route, while Central European sheep contributed more to the land route. Genetic differentiation among populations was very low, likely reflecting recurrent gene-flow in historical times.

## O7

### Comparison of staining methods for PMN cell counts in endometrial cytology samples to diagnose subclinical endometritis in dairy cows

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**Introduction:** A high-quality staining method is mandatory to yield an objective and accurate evaluation of the PMN/epithelial cell ratio in endometrial cytology samples. Modified Wright-Giemsa (Diff-Quick®) is currently the most widely used staining method to evaluate endometrial cytology slides in dairy cows. In order to evaluate the accuracy of Diff-Quick® to stain PMNs, it was compared to Naphtol-AS-D-chloroacetate-esterase (CIAE), an enzyme-histochemical staining in which PMNs appear bright red after staining considered to be the gold standard for identification and counting of these cells.

**Material and methods:** Holstein Friesian cows ( $n=114$ ) were used to take cytology samples using a CytoBrush (CB) adapted to a conventional AI catheter. After sampling, two microscope slides were prepared by rolling half of the CB circumference on one slide (first duplicate) and the other half on another slide (second duplicate) obtaining a suitable and equally distributed quantity of cellular material on both slides. Before staining, smears were air-dried. First duplicates were stained with Diff-Quick® and their counterparts with the CIAE method. After staining, slides were evaluated by light microscopy. For each slide, a total of 300 cells was counted by one observer and the PMN/epithelial cell ratio was assessed at X 400 magnification.

**Results:** Average PMN% for the Diff-quick® samples was  $14.17 \pm 20.42\%$  while for the CIAE samples it was  $15.47 \pm 24.91\%$ . Concordance correlation coefficient (CCC) analysis was used to assess the agreement between both staining methods. Agreement between PMNs percentage in both staining techniques was good ( $\rho=0.84$ ;  $0.78-0.89$ ) with a standard error of only 2%. Nevertheless, when the number of PMNs increased, the divergence between both staining methods grew. Large differences between CIAE and Diff-Quick® are only considerable when very high amounts of PMNs have to be evaluated, largely exceeding the threshold for SCE.

**Conclusion:** To conclude, Diff-Quick® was confirmed as an easy, fast and high-quality staining technique, which can be routinely used to stain bovine endometrial cytology samples.



**O8****Examination of antioxidant effect of ellagic acid on rat testis exposed to tobacco smoke**

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**Introduction:** Toxic substances in tobacco smoke which are carried to every part of the body by blood are likely to disturb the balance between oxidant - antioxidant systems on tissues. In this case, increased free radicals cause tissue damage. Ellagic acid is a polyphenol compound that found in fruits such as pomegranate, strawberry and grape. In this study, it is aimed to examine the protective effects of ellagic acid, which is a powerful anti-oxidant, against oxidative damage on testis tissue caused by the chemicals in tobacco smoke.

**Material and methods:** Twenty-four male adult (8 weeks old) Sprague-Dawley rats were divided randomly into 4 equal groups: group I (Control), group II (Tobacco smoke), group III (Tobacco smoke + corn oil) and group IV (Tobacco smoke + ellagic acid). The rats in group II, III and IV were exposed to tobacco smoke 1 hour twice a day for 12 weeks. In addition to tobacco smoke exposure, 12 mg/kg ellagic acid (dissolved in corn oil), was applied to the rats in group IV by oral gavage. Equal amount of corn oil used in solving ellagic acid was applied to the rats by oral gavage in group III. At the end of the experimental period rats were decapitated. Testis tissues, blood and sperm samples were taken. The testis slides were stained by H&E, PAS and Masson's Trichrome methods. Also eNOS stain and TUNEL methods were applied. Biochemical and sperm analyzes were performed.

**Results:** In histological examination of group II testis slides compared with the group I; degeneration in seminiferous tubule germinative epithelium, cell debris in the seminiferous tubule lumen, separation in basement membrane, atrophic tubules, vascular congestion and edema in interstitial area were observed. The results observed in group II was found to significantly decrease in group IV. The increased apoptotic cells and eNOS immune reactivity were observed in group II and III. Also ellagic acid administration was decreased eNOS immune reactivity and apoptotic cells. MDA levels increased but CAT and GSH-Px levels decreased in group II and III compared to group I. In contrast, MDA levels decreased, CAT and GSH-Px levels increased in group IV. A significant increase in the amount of abnormal sperm was detected in group II and III compared with group I. The reduction in the amount of abnormal sperm was found in group IV.

**Conclusion:** These results indicate that exposure to tobacco smoke led to markedly alterations on testes structure and treatment with ellagic acid might prevent the testes from these toxic effects.

**O9****The effects of pralidoxime, atropine, N-asetyl cysteine on liver and kidney of malathion exposed rats**

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**Introduction:** The organophosphorus (OP) compound malathion is extensively used as insecticide and acaricide in agricultural, veterinary, medical and public health practices. OP compounds are primarily recognized for their ability to induce toxicity in mammals through inhibition of acetylcholinesterase. OP produce oxidative stress in different tissues through the formation of reactive oxygen species. We aimed to investigate the effects of Pralidoxime, Atropine, N-Asetyl Cysteine triple combination on Malathion exposed rat liver and kidney.

**Material and method:** 30 Wistar Albino rats randomly divided into 5 groups; Control (group-I), Pralidoxime+Atropine (Group-II), Malathion (Group-III), Malathion+Pralidoxime+Atropine (Group-IV), Malathion+Pralidoxime+Atropine+N-Asetyl Cysteine (Group-V). Group-I provided with standard rat chow and tap water ad libitum during the experiment period. Group-II received 40 mg/kg IP (intraperitoneal) pralidoxime and after cholinergic effects occurred Atropine was applied at the dose of 2 mg/kg in between 2 and 6 hours. Group-III received 1375 mg/kg malathion, dissolved in corn oil by oral gavage. Group-IV received same malathion application with Group-III afterwards 40mg/kg pralidoxime and 2 mg/kg IP Atropine were applied. Group-V received 100 mg/kg IP N-Asetyl cysteine in addition to Group-IV application. After the experiment period of 24 hours rats were decapitated, liver and kidney tissues were taken. Tissues' MDA and Catalase levels were measured spectrophotometrically.

**Results:** Liver and kidney MDA levels increased in group-III compared with group-I. On the other hand in group-IV and V MDA levels relatively decreased compared to group-III. Group-III catalase activities were lower than group-I. Group-IV and group-V catalase activities were higher than group-III. Moreover group-V catalase activities were higher than group-IV.

**Conclusion:** These results indicate that malathion exposure caused oxidative stress in rats kidney and liver. Pralidoxime, atropine and N-Asetyl Cysteine triple combination might have protective effects on both kidney and liver tissues.

**O10****Implementing behavioral assessment programs in veterinary practice for standardized evaluation of canine and feline temperament and diagnostics of behavior disorders**

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**Introduction:** Modern global veterinary practice has incorporated Applied Animal Behavior science into the health care of pets, where veterinarians who specialize in animal behavior are working side by side with fellow clinicians, thus providing overall physical and mental care of animals. Today, in their practice, veterinarians use research - based behavior assessments in order to diagnose behavior disorders or to determine critical points in relationship between an owner and a pet. These assessments are useful tool when collaborating with breeders, owners, shelters or NGO's, where veterinarian can determine the level of socialization or adoptability of a dog or a cat by using different tests.

**Material and methods:** Behavioral assessments are conducted throughout a series of standardized tests where we can assess a temperament of a pet, comfort level to restraint or touch, reaction to novel stimuli, bite inhibition, food and resource guarding behavior, willing to interact with other animal or human, arousal level, cause and type of aggression, fears, phobias, anxiety disorders, stereotypic or obsessive compulsive behavior.

**Results:** The overall test results in case studies showed that in 47 tested dogs most common behavioral disorders were- aggression towards people, fear of various things, destructiveness in the house, aggression towards animals, biting, excessive vocalization, problems between new pet and other pets, leash aggression, general disobedience. In 13 tested cats the problems were mainly related to urine marking, aggression towards the owner, excessive night vocalization, house soiling outside the litter box. The results showed us that pets prone to behavioral disorders are the ones who didn't have proper socialization or habituation period at breeders, and the ones who were adopted to a new home from a shelter or NGO without any prior adjustment or adoptability assessment.

**Conclusion:** Animal behavior problems often have detrimental effects on the relationships between pets and their owners and, consequently, function as important determinants in relinquishment decisions. Therefore, an intervention strategy for educating owners and breeders, may contribute to the reduction of development of behavior disorders, as well as finding a professional help for even the smallest change of behavior in pets. Applied Animal Behavior science also has a significant impact on the welfare of animals in shelter environment, or in foster care, providing the ability to treat aggression and reduce stress by ensuring necessary mental stimulation and teaching the animal an alternative, more adaptable forms of behavior with the use of behavior modification protocols in order to increase adoption rate and decrease euthanasia.

**O11****Attitudes of key stakeholders towards cattle welfare in commercial dairy farms in Macedonia**Miroslav Radeski<sup>1\*</sup>, Egzon Zhitia<sup>3</sup>,  
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**Introduction:** Society's expectation regarding animal welfare standards is of major importance. Therefore, the main objective of this study was to outline the attitudes of relevant stakeholders regarding welfare of dairy cattle, at what level the society is ready to accept certain welfare standards and to determine the main welfare priorities for intervention in commercial farms in Macedonia.

**Material and methods:** For the purpose of this study, a special questionnaire was developed. It consisted of 40 questions divided in four main sections: Personal data; Welfare of dairy cattle; Perspectives; Evaluation and feedback. The items comprised of multiple or ordinal questions and response scales. The developed questionnaire was spread electronically or as a hard copy to seven key stakeholders. The collected data were processed using descriptive (median, range) and comparative statistics of ordinal and categorical data.

**Results:** The total number of respondents was 98 from 35 different locations in Macedonia. Most respondents were 20 to 50 years old (87.5%) with a slightly higher number of male respondents (62.1%). The most prevalent respondents were Veterinary practitioners, Educators or scientists, Consumers and Governmental bodies and Official Veterinarians, with occurrence of 27.1%, 24.0%, 19.8% and 15.6%, respectively. The majority of the respondents (89.6%) describe animal welfare as good feeding, housing, health and appropriate behaviour of animals and 94.8% consider that animal welfare needs special attention and further development. Additionally, high percentages were found for the preference for loose housing system over tie-stall system (94.8%), and the importance of providing access to loafing areas (99.0%) or the implementation of milk SCC testing (94.6%). The median varied between different stakeholders regarding acceptable levels in dairy farms for very lean animals (10-15%), animal cleanliness (0-30% dirty animals), lameness (0-10%), dystocia, digestive disorders, mortality (0-20%). Higher level of agreement between the stakeholders was found for acceptable level of mastitis incidence (10%); the importance of good feed, water provision, good comfort and animal health were rated with the highest scores on the scales.

**Conclusion:** The key stakeholders understand the essence of animal welfare. The importance of the commonly



known main factors affecting the welfare state were rated as highly important, while the acceptance level of certain conditions in dairy farms fluctuates between different stakeholders. The findings of this study could be used as a baseline for defining future objectives and developing improvement strategies for animal welfare in dairy farms in Macedonia.

## O12

### **Update on activities of European Union Reference Laboratory for *Listeria monocytogenes*: a road-map toward European harmonization of typing of food and clinical strains of *Listeria monocytogenes***

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Since 2006, EURL *Lm*, hosted by ANSES and with financial support of European Commission (DG SANTE), coordinates and manages a network of 37 National Reference Laboratories (NRLs) in 29 EU Member States. Most of these NRLs are in charge of typing of strains isolated from food, environment and animals.

In the past few years, the NRLs have reinforced and consolidated their typing activities, by participating to annual EURL training sessions, workshops and Proficiency Testing trials (PT trials)<sup>1,2</sup>. In 2014, a total of 21 NRLs performed PFGE, according to PFGE and PFGE profile interpretation standard operating procedures (SOPs)<sup>3,4</sup> developed by EURL. These protocols were compared with those used for typing of clinical strains in the frame of collaborative projects between the major actors involved into the European surveillance of *Lm*: ECDC, EFSA, SSI and EURL<sup>4,5</sup>. Consequently, the need to exchange typing data has emerged. EURL has thus set up a European molecular typing database, EURL *Lm* DB, to centralize and share within the NRL network PFGE profiles of food strains, as well as associated epidemiological data<sup>6</sup>. EURL SOP for database curation has been recently compared with the SOP used by SSI and ECDC for profiles of human strains. EURL *Lm* DB has strongly contributed to the recent development of a joint ECDC-EFSA database of molecular typing data gathering human and non-human circulating *Lm* strains. EURL *Lm* is part of steering committee of this new database. In the coming months, EURL will be in charge of curation and cluster investigation for the non-human *Lm* strains.

EURL *Lm* has been collaborating with other EURLs, such as EURL VTEC and *S. almonella*, as to harmonize the PFGE/DB curation SOPs for the new ECDC-EFSA DB<sup>5</sup>. Finally, EURL *Lm* has focused its research activities on

the genetic structure of populations of *Lm* food strains using PFGE, MLST and WGS, in particular through a close partnership with DTU. EURL has also reinforced its expertise in WGS and protocol harmonization within three different multi-annual European projects founded by EFSA and European Commission (Horizon 2020).

EFSA : European Food Safety Authority

ECDC: European Center for Disease prevention and Control

DTU: Technical University of Denmark, Copenhagen

SSI: Serum Statens Institut, Copenhagen, Denmark

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## O13

### **Veterinary drug residues in food matrices: analysis, EU regulation and trends**

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Veterinary drugs have been widely used for animal food production to prevent or treat diseases or as growth promoters (forbidden in Europe). However, (mis)use of these veterinary drugs may result in the occurrence of veterinary drug residues in food matrices of animal origin, like for example meat, milk, eggs and honey. Seen that their presence may impose health risks for consumers, regulations for these residues have been established. In Europe, Maximum Residue Limits (MRLs) for veterinary medicinal products in food of animal origin are stipulated in the European Council Regulation (EEC) No. 2377/90, a regulation that was repealed and replaced by Regulation (EC) No. 470/2009. A list of pharmacologically active substances and their classification regarding MRLs in foodstuffs of animal origin are consolidated in Commission Regulation (EU) No. 37/2010.

In order to monitor and guarantee the food safety for consumers, there is a need for sensitive and validated analytical methods to detect/quantify veterinary drug residues in food and feed matrices. A trend towards detecting and/quantifying as much as possible compounds within one analytical run can be observed (i.e. multi-residue methods). Over the years, this has led to a myriad of methods, ranging from screening methods to confirmatory methods. While traditional fast screening methods can be used to distinguish between suspected (possible non-compliant) and negative (i.e. compliant) samples, confirmatory methodology allows indisputable identification and/or quantification. For the latter, low-resolution liquid chromatography – tandem mass

spectrometry (LC-MS/MS) is a frequently used technique in view of targeted analysis of residues of veterinary drugs. Although not yet routinely used, high-resolution MS (HRMS) coupled to LC offers the possibility to perform “untargeted” analysis of veterinary residues as well due to its high resolution and mass accuracy. Additionally, retrospective analysis without “a priori” knowledge and identifying unknowns are feasible with HRMS. However, criteria for method validation and identification criteria for HRMS are currently under discussion.

In order to ensure that the quality of analytical results generated by means of such chemical detection methods is guaranteed, the methods developed should be properly validated. Guidelines for the performance characteristics that should be addressed during validation of qualitative/quantitative screening and confirmatory methods are stipulated in Commission Decision (CD) 2002/657/EC. These concern for example determination of the decision limit, detection limit, trueness/recovery, precision, selectivity/specificity and ruggedness/stability. Commission Decision 2002/657/EC is supplemented with a guideline document for the validation of screening methods for residues of veterinary medicines (Community Reference Laboratories Residues (CRLs) (20/1/2010)).

It is obvious that the field of veterinary drug residue analysis has evolved tremendously and that it will continue to evolve both methodologically and regulatory.

#### O14

##### **Multi-annual national control plan (MANCP) in R. of Macedonia**

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Multiannual national control plan (MANCP) is a strategic document setting out the functions related to the systems for official controls and control measures on food trade, veterinary health and plant health are set out in the Multiannual national control plan (MANCP) covering the official controls under the competence of the Food and Veterinary Agency, State Agriculture Inspectorate and Phyto-sanitary Directorate.

MANCP has been produced in accordance with the relevant national legislation with is in line with the applicable EU acquis and the planning cycle is set out on 5 years.

The main purpose of the MANCP is to ensure comprehensive and integrated system for official controls contributing to protection of the public health and animal health and welfare, plant health and protection of consumer interests. It covers the mechanisms to be implemented for improvement of the efficiency of implementation of the control systems at all stages of food production, animal health and welfare and plant health.

Organization of official controls has EU based approach and they are implemented through application of strategic

document, annual surveillance and control plans and programmes for control and eradication of animal diseases which are of interest for Republic of Macedonia; monitoring of residues of VMP in food of animal origin; monitoring of food safety; programme for monitoring of import.

MANCP contributes to effective implementation of the relevant national legislation and it ensures verification that the competent authorities perform monitoring and ensure effective maintenance of the official controls systems “from farm to table”.

In interest of the relevant business sector and for purposes of enabling free circulation of goods, FVA within the framework of its activities also performs registration and/or approval of food business operators thus guaranteeing compliance with the relevant EU and national requirements.

#### O15

##### **Genotyping and phenotyping of isolates from human salmonellosis**

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Pulsed-gel field electrophoresis (PFGE) typing and antimicrobial susceptibility testing was performed over 38 clinical human isolates of *Salmonella enterica* subsp. *Enteritidis* obtained in the period 2011-2013. All strains were isolated in several public health laboratories in Republic of Macedonia, which were later submitted to the Faculty of veterinary medicine-Skopje for further testing. During the analysis of the results, the epidemiological data for the year and place of isolation of the human isolates was taken into consideration. As can be seen from the results, 5 indistinguishable PFGE patterns were found, with asimilarity among the patterns of 7%. Despite the global genetic similarity of the isolates of *S. enteritidis*, generic and temporal relatedness among cases of salmonellosis could be established, which could be used to trace the sources in the food chain. Phenotyping analysis was conducted with testing of minimal inhibitory concentration against 18 antimicrobial substances using automated system with commercially available gram negative susceptibility card. Resistance against *Nitrofurantion*  $\leq 32$  µg/ml was detected in 89% of tested strains and intermediate resistance against



*Ertapenem*  $\leq 0.25$   $\mu\text{g/ml}$  in all tested strains. This *in vitro* data could be taken into account during treatment of clinical salmonellosis.

The results from genotyping and phenotyping of the isolates of *S. enteritidis* showed that they are independent and not connected.

## O16

### Profile of the antimicrobial resistance of *Salmonella* spp. isolates from raw meat in R. Macedonia

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**Introduction:** The antimicrobial resistance of the pathogenic bacteria, such as *Salmonella* is in increase the last few decades, first of all because of the inappropriate use of the antimicrobial substances in human and in veterinary medicine, presents significant issue for public health all over the world. Our research was conducted in order to determine the prevalence and antimicrobial resistance of the individual strains of *Salmonella* spp. isolated from different types of raw meat (pork, turkey, poultry and mechanically deboned meat).

**Material and methods:** In a period of one year, 822 samples of different types of meat were analyzed, from which 72 strains of *Salmonella* were isolated and serotyped using Kaufman White scheme. After that they were tested for antimicrobial resistance towards 8 antimicrobials with Kirby Bauer test and with automated Vitek 2 Compact system.

**Results:** The analysis showed that 50 isolates (69.4%) had resistance towards at least one anti microbial, 10 strains (13.9%) were resistant towards two antimicrobials, and there stof the 12 isolates (16.7%) were multi-drug resistant (resistance toward more then 2 antimicrobials).

**Conclusion:** The results illustrate that the occurrence of the antimicrobial resistant *Salmonella* spp. found in food is wide spread all over the world and presents a potential risk for the consumers health. This is a reason for introduction of active monitoring on the development of the antimicrobial resistance considering this pathogen.

## O17

### *Campylobacter* spp. prevalence in broilers at slaughter after chilling

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**Introduction:** Broilers and poultry meat are the most common source of infection with *Campylobacter* spp. in humans. The latest data from all over the world reveal that *Campylobacter* spp. is the leading cause of foodborne diarrhea. They are most prevalent in chickens with caeca, colon and cloaca of the birds as the main areas of colonization. The aim of this study was to determine the prevalence of *Campylobacter* spp. in poultry carcasses at slaughter post chilling using carcass swab samples.

**Material and methods:** In this study 84 samples of swabs from broilers were tested for *Campylobacter* spp. prevalence. Upon processing of the broilers and after the chilling process was completed 84 carcasses were sampled and the swabs were placed in a sterile plastic bag. The bags were sealed, transported in a cool box packed with ice to the laboratory and cultured within four hours. The samples were enriched in Preston selective broth and incubated microaerophilically on 42°C for 24 hours. The enrichment was streaked on modified charcoal cefoperazone deoxycholate agar (mCCDA) and incubated under microaerobic conditions at 37°C for 48 h. Isolates were identified using standard parameters including Gram staining, oxidase and catalase testing, temperature tolerance, morphology, indoxyl acetate test, hippurate hydrolysis, growth and production of H<sub>2</sub>S on triple sugar iron agar.

**Results:** *Campylobacter* spp. was detected in 46.4% of the samples. A total of 39 isolates were recovered, with *C. jejuni* being the predominant species with prevalence of 29.8%. *C. coli* showed prevalence of 11.9%, *C. lari* 2.4% and *C. upsaliensis* 2.4%.

**Conclusion:** The findings of this study indicate high prevalence of *Campylobacter* spp. and this is a sign that the implemented slaughter process had a limited effect on the final products. This may represent a potential risk to consumers, if proper cooking practices are not employed. The prevalence of *Campylobacter* spp. determined during this study appears to be similar to those reported elsewhere in the world.

**O18****Optimization and validation of LC-MS/MS method for multiresidual analysis of  $\beta$ -agonists in urine**

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**Introduction:**  $\beta$ -agonists are synthetically produced compounds which are frequently used in animals for treatment of pulmonary diseases. They have been illegally used to improve production performance of food-producing animals in 5- to 10-fold of the therapeutic doses. On the other hand  $\beta$ -agonists are transmitted in meat and meat products and present a risk for public health. For these reasons the European Union banned the use of these substances as growth promoters in farm animals. The aim of this study was optimization and validation of LC-MS/MS method for determination of four  $\beta$ -agonists: clenbuterol, salbutamol, terbutaline and ractopamine.

**Material and methods:** The extraction of  $\beta$ -agonists residue from urine samples was performed according to the confirmatory method from the reference laboratory for  $\beta$ -agonists from Berlin, Germany. Validation of the method was performed according to the Commission Decision 2002/657/EC. Linearity of the method, Decision limit ( $CC\alpha$ ), Detection capability ( $CC\beta$ ), accuracy, precision and reproducibility of the method were evaluated.

**Results:** The calculated values for coefficient of correlation ( $r^2$ ) were from 0.987 to 0.997. The obtained values for  $CC\alpha$  and  $CC\beta$  for clenbuterol were 0.120  $\mu\text{g/L}$  and 0.139  $\mu\text{g/L}$ , respectively. For salbutamol  $CC\alpha$  was 0.534  $\mu\text{g/L}$  and  $CC\beta$  was 0.565  $\mu\text{g/L}$ . The calculated results for  $CC\alpha$  for terbutaline and ractopamine were 1.625  $\mu\text{g/L}$  and 0.591  $\mu\text{g/L}$ , and for  $CC\beta$  were 1.768  $\mu\text{g/L}$  and 0.680  $\mu\text{g/L}$ , respectively. The accuracy of the method was evaluated by determining the recovery of spiked urine samples on three concentration levels (at 0.5, 1 and 1.5 times the Minimum Required Performance Level (MRPL)). The method recovery ranged from 89.83% to 97.40%. The precision of the method ranged from 3.49 to 11.49 ( $RSD_r$ , %) and the reproducibility of the method was from 6.40 to 17.64 ( $RSD_R$ , %).

**Conclusion:** According to the data obtained from the validation procedure the method showed good linearity, good precision, recovery and reproducibility and the method is applicable for determination of  $\beta$ -agonists in urine.

**O19****Effects of caffeic acid phenethyl ester on liver in high-fructose corn syrup drinking rats**

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**Introduction:** High-fructose corn syrup (HFCS), is used as a common sweetener in many beverages and foods over the past few decades largely, induces metabolic syndrome. Caffeic acid phenethyl ester (CAPE), an active component of propolis from honeybee hives, is known to have anti-inflammatory and anti-oxidant properties. The purpose of the study was to determine the effect of CAPE on liver in HFCS drinking rats.

**Material and methods:** Eighteen male 8 week aged Sprague-Dawley rats were randomly divided into three groups ( $n=6$ ): Control, HFCS and HFCS+CAPE. Metabolic syndrome was induced by HFCS 30% in tap water for six weeks. CAPE applications at the dose of 50  $\mu\text{mol/kg/day}$  for two weeks, were initiated after four weeks of fructose consumption. After the experimental period of six weeks, rats were decapitated and livers were taken for the evaluation of biochemically and histopathologically. Liver tissues malondialdehyde (MDA), glutathione (GSH) levels and catalase (CAT) activities were quantified by spectrophotometry. Also liver tissue specimens were embedded in paraffin blocks. Sections obtained from paraffin blocks were used for immune detection of eNOS and HSP70.

**Results:** In comparison with control group, MDA level was significantly higher, but GSH level and CAT activity were significantly lower in HFCS group. CAPE administration led to a significant decrease in MDA level and an increase in both CAT activity and GSH level when compared to HFCS group. Also, in comparison with control group, HSP70 immunoreactivity increased in HFCS group, but decreased in the HFCS+CAPE group than HFCS group. Significant decreases were observed in eNOS activity in HFCS group than control group. On the other hand, no significant differences were found in the eNOS immunoreactivity in HFCS+CAPE group than HFCS group.

**Conclusion:** These results indicate that the overconsumption of HFCS may promote hepatic damage and lead to oxidative stress which can cause inflammation in the liver. On the other hand CAPE supplementation had beneficial effects and might play an anti-inflammatory and anti-oxidant role on liver in HFCS drinking rats.



**O20****The effect of corn oil addition on physical, chemical and sensory acceptability of culled layer hens sausages**

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A study about the effect of corn oil addition, on physical, chemical and sensory acceptability of culled layer hens sausages, was carried out with objectives to assess the right of corn oil percentage on physical, chemical and sensory quality of culled layer hens sausages. Different factors during processing have an impact on quality characteristics of final product. Using of culled layer hens in further processing, are popular in some countries. Chicken sausages considered as very popular and highly consumed in many countries. Fresh culled layer hens sausages were manufactured using some concentration of corn oil (5%, 10%, 15%, 20% and 25%). This work used an experimental, with completely randomized design 5 x 4 (CRD), with 5(five) different percentage of corn oil, and 4 (four) replications. The effect of fat content on the physical, chemical and sensory quality was studied. Samples were analyzed immediately (day zero) in room temperature. Results indicated that the moisture and fat content has significance results, but the sausage pH are not significant between the treatments. The conclusion is, that the corn oil addition has improved the quality of fresh culled layer hens sausages.

**O21****Canine leishmaniosis – an overview**

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**Introduction:** Canine leishmaniosis (CanL) is a major zoonotic disease endemic in more than 70 countries in the world. It is enzootic in regions of southern Europe, Northern Africa, the Middle East, Central Asia, China, South and Central America and has sporadically emerged also in the USA. CanL is also an important concern in non-endemic countries where imported disease constitutes a veterinary and public health problem. Dogs are the main animal reservoir for human visceral leishmaniosis and the disease is usually fatal if not treated in people. Phlebotomine sand flies are the vectors of *Leishmania infantum*, the causative agent of CanL in the Old World and for its New World synonym *L. chagasi*.

**Clinical findings:** The typical history reported by owners of dogs with CanL includes the appearance of skin lesions, ocular abnormalities, or epistaxis. These

are commonly accompanied by weight loss, exercise intolerance and lethargy. Dogs from all breeds can be infected with leishmaniosis. The most consistent serum biochemistry findings in dogs with clinical CanL are serum hyperproteinemia with hyperglobulinemia and hypoalbuminemia. Proteinuria and some degree of renal pathology is frequently present in dogs with CanL and subsequent renal failure due to immune-complex glomerulonephritis eventually develops and is believed to be the main cause of death in dogs with CanL.

**Diagnosis:** Leishmania amastigotes can be demonstrated by cytology from the skin, lymph nodes, spleen or bone marrow. Various serological methods for the detection of anti-Leishmania antibodies have been developed. These include indirect immunofluorescence assays (IFA), enzyme-linked immunosorbent assay (ELISA), and direct agglutination assays (DAT). Detection of parasite-specific DNA in tissues by PCR allows sensitive and specific diagnosis. Several different assays with various target sequences using genomic or kinetoplast DNA (kDNA) have been developed for CanL.

**Therapy:** The main drugs used for treatment of CanL include the pentavalent antimony meglumine antimoniate (Glucantime®). Treatment with these drugs is frequently combined with meglumine antimoniate administered for 4 weeks and allopurinol used for long term therapy. Miltefosine (Milteforan®) is an additional oral anti-leishmanial drug that can be used for the first month of treatment in combination with allopurinol instead of meglumine antimoniate.

**Prevention:** Commercial vaccines against CanL has been approved in Brazil and Europe, however they do not prevent infection but rather decrease the occurrence of clinical disease. The use of topical insecticides against CanL in collars or spot-on formulation containing pyrethroids has been shown to be effective in reducing disease transmission.

**O22****Dairy cattle lameness. Overview and recent advances**

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**Introduction:** Lameness is undoubtedly one of the most important issues the modern dairy industry faces today. It is a debilitating, painful condition with significant animal welfare implications as it has been associated with hyperalgesia (Whay et al. 1998) and described as one of the most representative indicators of compromised welfare. Apart from being an important animal welfare issue, lameness can be extremely costly too. The severe impact of lameness on milk yield, fertility, survivability,

nutrition and carcass quality is well established and has been recently reviewed (Huxley 2013). Average total cost estimates per lameness case in the UK range between £180 and £273. At a current incidence of 80 new cases per 100 cows per year, lameness may be costing the UK dairy industry more than £200 million annually.

Lameness prevalence around the world. Unfortunately, despite the known and well accepted negative impact of lameness on welfare and productivity, lameness prevalence in dairy herds worldwide remains at unacceptably high levels. In an epidemiological study on lameness prevalence that was conducted approximately 20 years ago in the UK the mean annual herd prevalence over the whole study period was 20.6 % with a range from 2.0 to 53.9 % (Clarkson et al. 1998). More recent studies report consistently increased prevalence estimates (Barker et al. 2010, von Keyserling et al., 2012). Data obtained during herd health visits in dairy farms in the Wirral, Cheshire and North Wales (Oikonomou 2015, unpublished) are shown in an average herd lameness prevalence of 39% (ranging from 17 to 62%).

Recent advantages on the aetiopathogenesis and treatment of main lameness causing lesions. The main causes of lameness in dairy cattle are associated with foot lesions of infectious (mainly digital dermatitis, and interdigital phlegmon) and non-infectious (mainly sole hemorrhages, sole ulcers and white line disease) aetiology. Housing, cow comfort, nutrition, early identification and treatment of lameness causing lesions, routine foot trimming, and stockmanship can all be associated with lameness prevalence and any lameness control program should focus greatly on improvements on all the above mentioned areas. Recently published studies regarding lameness causing lesions aetiopathogenesis and treatment may potentially lead to better understanding and eventually confrontation of the condition (Bicalho and Oikonomou 2013, Thomas et al. 2015).

Opportunities for genetic/ genomic selection. Evidence exists suggesting that genetics has also a role to play in dairy cattle lameness control and prevention. Low to moderate heritability estimates, ranging from 0.01 to 0.22, have been reported for lameness, locomotion scores and specific foot lesions. Oikonomou et al. (2014) recently reported that digital cushion thickness is moderately heritable and genetically strongly correlated with sole ulcers and white line lesions.

Bicalho and Oikonomou. 2013. *Livestock Science*. 156: 96-105.

Huxley. 2013. *Livestock Science*. 156: 64-70.

Oikonomou et al. 2014. *J. Dairy Sci.* 97: 532-536.

Thomas et al. 2015. *J. Dairy Sci.* 98:1-10

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Whay et al. 1998. *The Veterinary Journal*. 156, 23-29.

## O23

### Ultrasound measurement of digital cushion thickness in dairy cows with sole ulcer

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**Introduction:** The digital cushion plays an important role of dampening the compression of the corium tissue beneath the third phalanx. Several authors reported increased levels of hoof damage lower BCS, lower overall digital cushion thickness (DCT) in older cows. The prevalence of sole ulcers and white line diseases is significantly associated with thickness of the digital cushion.

**Material and methods:** In order to investigate the association between sole ulcers and the thickness of digital cushion a field cross-sectional study was carried out. A total of 35 lactating Holstein dairy cows (19 with diagnosed sole ulcer and 16 with healthy hoofs) were enrolled in the study. The thickness of the digital cushion was measured by ultrasonography at the typical ulcer site, after the routine claw trimming. Measurements were done using the portable ultrasound scanner (Mindray DP-50 Vet) on the lateral claws of the hind feet at two sites: at the tip of the distal phalanx (site 1) and below the tuberculum flexorium (site 2). The 7.5 MHz linear transducer was placed 1-2 cm axial of the imaginary medial line of the sole at the typical site for sole ulcer. The digital cushion was identified as a thin echogenic line between the inner margin of the corium of the sole and the solear surface of the distal phalanx. Before the beginning of the study the ultrasonography DCT measurement was validated using 32 digits from cadavers. The ultrasound measurements were compared with the caliper measurements of the digital cushion after dissection of the hooves.

**Results:** The mean DCT values at site 1 and 2 measured on healthy hoofs were significantly higher than then hoofs with sole ulcer (4,07±0,42 mm and 4,31±0,49 mm and 3,93±0,45 mm and 3,33±0,47 mm, respectively). Significant difference (p<0,05) was observed at site 2 between dairy cows with healthy hoofs and those with a sole ulcer.

**Conclusion:** The results of our study support the concept that the prevalence of sole ulcers was significantly associated with DCT and cows with low DCT have a higher risk of claw horn lesions. DCT measurements using ultrasound, after routine claw trimming, can be useful in predicting sole ulcer formation during early lactation.



**O24****Prevalence of *Rhodococcus equi* in meat animals in Poland**

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**Introduction:** *Rhodococcus equi* is a well-known equine pathogen responsible for foal rhodococcosis. However, *R. equi* infection in other species than horse has raised considerable interest due to similarity to tuberculosis and frequent isolation of *R. equi* from tissues of apparently healthy domestic and wild animals intended for human consumption. Moreover, an increasing number of *R. equi* infections in humans are observed and *R. equi* is considered as an emerging zoonotic pathogen. Probably the most important route of infection is foodborne transmission. The study aimed to investigate the molecular epidemiology of *R. equi* in swine, cattle and horses carcasses intended for human consumption in Poland.

**Material and methods:** Submaxillary lymph nodes were obtained from pigs (n=398) and cattle (n=234). Lymphocentrum retropharyngeum and middle tracheo-bronchial lymph nodes from horses (n=198) were collected. *R. equi* isolation, phenotypic and genotypic identification of isolates was conducted as described previously (1).

**Results:** Totally 1028 lymph nodes samples were investigated. *R. equi* was isolated from lesion-free submaxillary lymph nodes of 26.6% (105/395) of pigs and 1.3% (3/234) of cattle. From horses *R. equi* was isolated only from 0.5% (1/198) of middle tracheo-bronchiales lymph nodes. The purulent lesion were observed only in 0.7% (3/398) pigs submaxillary lymph nodes samples and in two of them *R. equi* was detected. All bovine (100%) and 98.1% of pigs isolates were *vapB*-positive. Single horse isolate was *vapA*-positive and carried plasmid 85-kb typ I.

**Conclusion:** The prevalence of *R. equi* in healthy pigs intended for human consumption is very high. To the authors' best knowledge, this is the first epidemiological report of *R. equi* detection in lymph nodes of apparently healthy cattle and the first description of *R. equi* epidemiology in slaughtered horses. Risk of *R. equi* transmission by consumption of beef or horsemeat seems to be marginal, but it is possible especially in case of consumption of undercooked or raw meat.

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**O25****Prevention of early embryonic death by rapid milk progesterone test in a dairy herd**

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Over the past two decades, numerous studies have been conducted into the use of progesterone (P4) levels as a fertility management tool. In order for the embryo to implant into the uterine wall, the cow must have progesterone levels greater than 5ng/ml. After fertilization, the rate of a successful pregnancy is determined by the survival of the embryo. Early embryonic death occurs prior to the period of corpus luteum (CL) maintenance in cow at days 13-18 of the reproductive cycle. The aim of present study was to determine P4 with "P4 Rapid" test at day 7-8 post AI. For this purpose, the current study has conducted on 155 repeat breeding Holstein cows (< 3 AI) with prolonged estrus cycle (< 26 days). At day 7 and 8 post AI, the milk progesterone levels were measured (P4 Rapid test). The cows with low P4 (n= 104 or 67%) were divided in two groups each n=52. In Group I the PRID DELTA devices were inserted for two weeks and control Group II without progesterone devices. At day 50 post AI all animals (n=104) were submitted to ultrasound pregnancy detection. Pregnancy was registered in 27 animals (51,9%) for Group I and in 16 cows (30,7%) for Group II. Measurement of P4 level in milk 7-8 days post-insemination (AI) is practical method of a herd management plan and placing a progesterone device for 2 weeks may be enough to sustain that pregnancy until the placenta takes over progesterone production.

**O26****Current clinical experience with the use of adipose derived mesenchymal stem cells in horses**

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**Introduction:** In the present study, treatment of selected cases of osteoarthritis (OA) and tendonitis in horses with adipose derived mesenchymal stem cells (MSC) is presented. In all treated patients other methods (hyaluronic acid, arthroscopic debridement of the joint, PRP treatment) previously failed or didn't give expected results.

**Material and methods:** About 1 cm<sup>3</sup> of adipose tissue was collected from the radix caudae region of horses in the local infiltrative anaesthesia. Adipose tissue was enzymatically digested and cells were grown in special

MSC media for 7 to 10 days. About 5 millions of cells were suspended in PBS and injected intra/articularly or intra-tendinously. Horses were evaluated two weeks, two months and every six following weeks with clinical examination, x-ray imaging and ultrasound (for soft tissues) by experienced veterinarian.

**Results:** During the past 3 years, 15 horses were treated, 12 with diagnosis OA of different joints, one with superficial flexor tendon injury and one with suspensory ligament injury. One horse was treated for both, OA and suspensory ligament injury. Out of 13 horses treated for OA, 11 horses improved clinically (reduced lameness) and 3 of these horses returned to previous sport activity 4 – 6 months after treatment. Six horses successfully returned to previous activity as leisure horses 2 – 6 months after treatment and 2 horses with very severe clinical lameness (grade 3-4) improved their conduct and showed lameness reduced to grade 3. In 2 out of 13 horses (15,4 %) there was no improvement after 24 months with lameness remaining at the same level as before treatment. All three horses treated for injured tendons improved significantly after stem cell applications to the injury with marked improvement in their lameness and general mobility 4 - 6 months after the treatment.

**Conclusion:** Our results demonstrate that the use of adipose derived mesenchymal stem cells in OA and tendon injuries in horses is promising and should be further investigated. Almost all of our patients improved clinically, while two horses with OA that did not show any improvement after the treatment had also mechanical problems which could not be resolved with such treatments and were most likely, at least partially responsible for no response to treatment.

## O27

### Abscess diseases in goats

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**Introduction:** Common name abscess diseases is used to describe two etiologically distinct diseases; Morel disease caused by *Staphylococcus aureus* subsp. *anaerobius* and caseous lymphadenitis (CLA) caused by *Corynebacterium pseudotuberculosis*. Both diseases have similar epizootiology and are characterized by abscess formations in or near major superficial lymph nodes. The major difference is that, unlike in Morel's disease, in CLA abscesses can also develop internally in organs, although that visceral form is relatively rare in goats. In addition, awareness of the Morel disease is very limited, at least in Croatia, making the possibility of misdiagnosis with the more common and very well documented CLA highly probable. Objective of this study was to evaluate

clinical and epizootiological findings of Morel's disease and caseous lymphadenitis in two naturally infected goat herds in Croatia.

**Material and methods:** All animals in both herds were clinically examined by inspection and palpation for the presence of abscesses. Epizootiological data and clinical features, including the localization, number and size of the abscesses, were recorded and compared. Samples were taken from each detected abscess for bacteriological examination.

**Results and conclusion:** The study showed that in the early epizootic stage both diseases equally affect both young and adult goats. The main differences in the clinical picture were related to the smaller average number and size of abscesses per animal in caseous lymphadenitis. However, these differences are still considered to be insufficient to distinguish individual cases and in a case of an outbreak, confirmatory diagnosis of the disease should always be based on microbiological examination.

## O28

### Seroprevalence and geographical distribution of small ruminant lentiviruses in sheep and goats in Macedonia

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**Introduction:** Maedi Visna in sheep (MV) and Caprine Arthritis encephalitis in goats (CAE), are diseases caused by a group of closely related viruses which share a large number of mutual characteristics, both biological and structural, and because of that, nowadays they are referred to as small ruminant lentiviruses (SRLV). They cause latent, persistent infections, with slow progression, but always with lethal outcome for the host. Main characteristics of MV are alterations of the respiratory tract (Maedi), wasting, chronic udder inflammation, arthritis and nervous signs (Visna), while CAE is mainly characterized with joint alterations - arthritis, and nervous form in young kids (2-4 months of age), though it can cause mastitis and pneumonia. These diseases are of great economical importance, especially for the countries with developed and organized farming system of sheep and goat breeding. The aim of this epidemiological study was determination of seroprevalence of small ruminant lentiviruses, as well as their geographical distribution in Macedonia.

**Material and methods:** During the years 2010 and 2011, in total 2022 serum samples, from sheep and goats older than 1 year of age, were collected for serological testing. Samples derived from 195 villages spread across



the territory of the eight official statistical/administrative regions of Macedonia. For detection of specific antibodies to MVV and CAEV, an indirect ELISA method was used, according the manufacturer's protocol. The true prevalence (TP) was calculated with correction of the results for the apparent prevalence (AP), for the sensitivity (Se) and the specificity (Sp) of the test, declared by the manufacturer.

**Results:** Test results revealed high percentage of positive animals (32.25%) with wide geographical distribution across the country and great regional variation in the seroprevalence. Significantly higher seroprevalence was observed in sheep in comparison to goats. Detailed results about the total seroprevalence, seroprevalence on species level as well as regional variations and distribution are further discussed.

**Conclusion:** High seroprevalence and wide distribution of SRLV across the country indicate that SRLV infections have enzootic character, while the low seroprevalence in goats was expected and reflects the extensive condition of goat breeding in R. Macedonia.

## O29

### Application of fluorescence based molecular assays for improved detection and typing of *Brucella* strains in clinical samples

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**Introduction:** Bacteria from the genus *Brucella* are causative agents of brucellosis – a zoonotic disease which affect many wild and domestic animal species and humans. Taking into account the significant socio-economic and public health impact of brucellosis, its control is of great importance for endemic areas. The chosen control strategy could be successful only if adapted to the current epidemiological situation. This implies that a choice of appropriate diagnostic procedures for detection and typing of *Brucella* strains are of essential importance. Significant advancement of molecular techniques and their advantages compared to classical methods gives strong arguments in promotion of these techniques as a powerful tool for comprehensive diagnostics of brucellosis.

**Materials and methods:** Real time PCR test targeting IS711 has been implemented for *Brucella* spp. detection. Assay performances were assessed by testing tenfold dilutions of bacterial DNA, corresponding to a concentration range between 1.7 ng/μL and 0.17 fg/μL. Molecular typing system based on amplification and

fluorescent based detection of loci from the MLVA-16 scheme was also implemented. Comparative analysis of analytical sensitivities of both systems was performed and evaluated using clinical samples obtained from ruminants positive on serological tests for brucellosis.

**Results:** The observed lower limit of detection for implemented IS711 real time PCR was 4.2 fg of bacterial DNA. The assay amplified broad range of DNA concentrations (4.2 ng to 4.2 fg) with excellent linearity ( $R^2 = 0.997$ ) and 85.5% reaction efficacy. Implemented MLVA-16 typing system showed equal analytical sensitivity with the IS711 real time PCR indicating the theoretical possibility for successful typing of all *Brucella* strains detected by IS711 real time PCR. This was confirmed with successful detection and typing of *Brucella* in clinical samples with variable load of bacterial DNA.

**Conclusion:** Implemented fluorescence based system for molecular detection and typing of *Brucella* spp. is highly sensitive and therefore very suitable for use with clinical samples. Moreover it is safe and relatively easy to perform and provides quick and objective results. Therefore, its' application as routine diagnostic tool could significantly improve the diagnostics of brucellosis.

## O30

### Phylogenetic analysis as a tool to better understand an epizootic of Newcastle disease in Macedonia

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**Introduction:** Despite regular vaccination of commercial poultry and mandatory vaccination of backyard poultry, many outbreaks of Newcastle disease (ND) have occurred in Macedonia. In the recent past Macedonia has reported outbreaks of ND every year from 1999 to 2006. In the absence of avian influenza it is the most important economic disease of poultry in Macedonia. The objective of this paper was to study epidemiological situation of ND during the country wide epizootic in 2005-2006 in Macedonia using sequencing and phylogenetic analysis.

**Material and methods:** Newcastle disease viruses (NDV) isolated from five different outbreaks of ND in 2005 to 2006 were analyzed in this study. In total, four strains were detected from backyard poultry, one strain was detected from farm poultry and one strain was detected from wild birds. Nucleotide sequencing of the part of the

fusion gene (nt 47 - 420) of NDV encompassing cleavage site (CS) was performed on all strains. Alignment of the nucleotide sequences was performed using BioEdit software and construction of phylogenetic tree was done using MEGA software.

**Results:** Amino acid motif of the CS of all strains was typical for virulent NDV strains. All viruses isolated from different outbreaks belonged to a single genotype VIIId. Phylogenetic data in correlation with field situation on country and regional level are further discussed.

**Conclusion:** Sequencing and phylogenetic analysis represent valuable epidemiological tools to better understand NDV outbreaks. They help in recognizing endemic presence of a disease in a country or region, explore possibilities of sources of introduction of an outbreak virus and establish proofs of inter-species transmission. These contribute towards improved risk analysis and adequate implementation of control measures.

### O31

#### Presence of CTX-M type extended-spectrum beta-lactamase producing *Escherichia coli* in healthy laying hens

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**Introduction:** Transmission of antibiotic resistant *E. coli* from chicken to human may occur by different ways. Thus, carriage status of chickens for important antibiotic resistant bacteria should be known. In this context, extended-spectrum beta-lactamases (ESBL) producing *E. coli* is important to monitor in chicken because ESBLs confer resistance to all penicillins, 1<sup>st</sup>-4<sup>th</sup> generation cephalosporins and monobactams and ESBL genes can be transferred from commensal *E. coli* strains to pathogen and zoonotic bacteria. For this purpose, in the present study, it was aimed to determine the presence of extended-spectrum beta-lactamase (ESBL) producing *E. coli* in intestinal microflora of healthy laying hens in central district of Burdur city in Turkey and to detect and characterize the genes encoding ESBLs.

**Material and methods:** In the study, 3 laying hen farms were visited and 200 fecal samples were collected randomly. After application of an enrichment procedure to the fecal samples in pepton buffered buffer, they were spread on Brilliance *E. coli*/Coliform selective agar media supplemented with cefotaxime (2 µg/ml) or ceftazidime (2 µg/ml). The strains grown on the medium were identified as *E. coli* based on biochemical tests and genotypic confirmation by PCR targeting 16S rRNA gene of *E. coli*. ESBL production of the strains was determined by

phenotypic confirmation test as recommended by CLSI. Then, the *E. coli* strains were investigated for major ESBL gene classes (TEM, SHV and CTX-M) by PCR and DNA of ESBL genes were sequenced for determination of types of enzymes. Finally, the susceptibility of the strains to various classes of antibiotics were determined by agar disc diffusion test.

**Results:** ESBL producing *E. coli* was isolated from 12 (6.0%) laying hen fecal samples. CTX-M type beta lactamase gene was found in all strains (12/12, 100%) and TEM type was found in two strains (2/12, 12.6%) but SHV type ESBL was not detected in any strains. DNA sequence analysis of TEM and CTX-M genes identified them as TEM-1, CTX-M -3 and CTX-M-15.

**Conclusion:** In conclusion, this study revealed that ESBL producing *E. coli* exists in laying hens in Burdur city of Turkey and the risk exists for public health. Infection due to ESBL producing *E. coli* may result in treatment failure in both human and animal.

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### O32

#### Bacteria associated with postpartum dysgalactia syndrome in farmed sows in the Republic of Macedonia

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**Introduction:** Postpartum dysgalactia syndrome (PDS) in sows is a pathological condition characterized with fever and reduced milk production in the first 12 to 48 hours postpartum, where bacteria play a major role in its etiology. The aim of our study was to determine the species and prevalence of bacteria in PDS affected farmed sows in the Republic of Macedonia.

**Material and methods:** One-hundred-and-sixty sows from 5 pig farms were clinically examined for PDS 24 hours after farrowing. Milk samples and vaginal swabs for bacteriological testing were taken from PDS affected (n=30) and PDS unaffected (n=86) sows. To determine the statistical differences between the groups, we performed the Chi square-test and the results were considered statistically significant at p<0.05.



**Results:** *Escherichia coli* was the most frequent isolate found in 73.3% (22/30) of affected and in 31.4% (27/86) of unaffected sows. *Staphylococcus* spp. in unaffected sows was isolated in 24.4% (21/86) versus 13.3% (4/30) in affected sows. Additionally, *Streptococcus* spp. was present in 20% (6/30) of affected and 10.5% (9/86) in unaffected sows. In affected sows, *Escherichia coli* was detected in 66.6% (20/30) of vaginal and in 23.3% (7/30) milk samples, where in unaffected sows it was found in 29.1% (25/86) in vaginal and in 10.5% (9/86) in milk samples. *Staphylococcus* spp. in unaffected sows was detected in 17.4% (15/86) vaginal and in 16.4% (14/86) milk samples, whereas 6.7% (2/30) of the affected sows were also found positive. *Streptococcus* spp. was isolated in 13.3% (4/30) of the vaginal and in 6.7% (2/30) of the milk samples in affected sows, and in 3.5% (3/86) of the vaginal and 7% (6/86) of the milk samples from unaffected sows.

**Conclusion:** We found that the most prevalent bacterium strongly connected with the clinical appearance of PDS in affected sows is *Escherichia coli*. The high percentage of *Escherichia coli* found in vaginal swabs of affected sows is possibly associated with fecal contamination of the uterus shortly after farrowing and the clinical manifestation of PDS. The low prevalence of *Staphylococcus* spp. in affected sows suggests its minor role in the etiology of PDS.

### O33

#### Features of canine leptospirosis in Croatia

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**Introduction:** Canine leptospirosis is a well known zoonotic infection with worldwide distribution. The serovars Canicola and Icterohaemorrhagiae have traditionally been responsible for most cases of canine leptospiroses. The use of widely available bivalent vaccines containing those two serovars has greatly reduced canine leptospirosis. However, re-emergence of the disease has been detected in Europe and North America, partly due to changes in the infecting serovars. The aim of this study was to determine the prevalence of the presumed infective serovars and to analyse main epidemiological and clinical features among clinically ill dogs in Croatia.

**Material and methods:** A retrospective analysis of archive data obtained from NR Laboratory for leptospires and Clinic for infectious diseases was conducted. During a period of four years (January 2010 to October 2014), 311 canine sera were submitted to the Laboratory for

leptospires, Faculty of Veterinary Medicine, University of Zagreb. Using a microscopic agglutination test (MAT) with cut-off point of 1:800, isolation procedures and/or PCR - 67 (21,5%) positive animals were detected.

**Results:** The most prevalent presumed infective serovars, in decreasing order, were Pomona (33,3%), Icterohaemorrhagiae (26,7%), Grippotyphosa (21,7%) and Bratislava (11,7%) and infection was mainly detected in large breed male dogs from suburban and urban areas. Detailed clinical data of 27 dogs that were presented at the Clinic for Infectious diseases was additionally analysed. Most common presenting clinical signs included lethargy (89%), anorexia (85%), vomiting (70%), muscle weakness (63%), icterus (59%) and abdominal pain (59%). Major haematological and biochemical abnormalities were azotemia (93%), thrombocytopenia (70%), bilirubinemia (70%), anaemia (59%) and leukocytosis (56%). Although acute renal and hepatic failures were the most frequent clinical manifestations in dogs with leptospirosis a very high percentage of dogs with pulmonary involvement (41%) was also detected. Eight dogs were euthanized because of financial reasons connected with the high costs of intensive care while survival rate in treated animals was 63%. Negative outcome was mainly associated with leptospirosis pulmonary haemorrhagic syndrome (LPHS) and/or severe acute kidney injury (AKI).

**Conclusion:** In this study, an increasing overall incidence of canine leptospirosis was detected, as well as increasing incidence of severe cases with high mortality due to severe AKI and pulmonary involvement. In addition, results showed that most infections were caused by serovars not covered by the currently used vaccines raising questions concerning its efficacy in preventing leptospirosis in dogs.

### O34

#### Transfusion in dog

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Blood transfusion is an important and useful tool in veterinary critical care and emergency medicine. It is a medical treatment defined as intravenous therapy with fresh whole blood or blood products (fresh plasma, fresh frozen plasma, platelet rich plasma, packed red blood cells, plasma cryoprecipitate and cryosupernatant). Sometimes, blood transfusion is the only essential method in treatment if anemia occurs caused by haemorrhage, haemolysis or reduced erythropoiesis and also coagulopathies, sepsis, disseminated intravascular coagulopathy and deficiencies of specific coagulation factors. Blood transfusion is often

mandatory supportive therapy in demanding surgical procedures as well as in comprehensive trauma. Dogs' erythrocyte antigens (DEA) determine blood groups and 8 of them are internationally accepted. The most important is to determine whether the donor and the recipient are positive or negative to the blood group DEA 1.1. DEA 1.1 test is available in form of in-house typing kits. Donor and recipient should be type matched otherwise, recipient is at risk for transfusion reactions which can cause severe illness or even death, so its welfare could be jeopardized. An ideal blood donor should meet further criteria: it should be healthy, large breed dogs weighing at least 25 kg, between ages 1 and 7 years, well-tempered, clinically healthy, free of contagious disease and not pregnant. A standard blood donation in the dog is 450ml ('one canine unit') and this can safely be obtained from a 25kg dog. Transfusion reactions can be immune-mediated (immediate and delayed) or non-immune-mediated. Immune-mediated transfusion reactions can occur when typing and cross-matching of donor and recipient blood is not carried out. Immediate immune-mediated reactions are usually hyperthermia and vomiting, and delayed (between 2 and 21 days post transfusion) is indicated by a falling PCV (packed cell volume). Non-immune-mediated reactions are caused by improper handling of blood or poor infusion technique and subsequently can cause septicemia in recipient. Strict transfusion policy, the use of proper blood type, and careful monitoring blood transfusion could minimize the risk of an adverse reaction and maximize the benefits of transfusion and improve health care quality.

### O35

#### Premedication and preparation before anesthesia in dogs and cats

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**Introduction:** Appropriate premedication and preparation before anesthesia decreases the risk of unwanted reactions and lethal reactions with cats and dog during anesthesia. To form proper and complete anesthesia plan general health condition of the patient needs to be known, beside the necessary knowledge of the effects and possible combinations of the drugs for premedication and anesthesia. Individualized anesthetic plan is of high importance for sick and geriatric patients. Age, breed, body weight, temperament and health condition of the patient have big influence in management of the perianesthetic period. One of the main purposes of this article is to emphasize the need of history, complete clinical examination and if necessary additional clinical examinations before every anesthesia in cats and dogs. The objective of this study was to evaluate the need of adequate premedication and analgesia, adaptation of the

anesthetic plan as well as the consumption of Propofol, as induction agent according to the patient conditions in 18 dogs and 4 cats.

**Material and methods:** Four groups of animals were observed. Premedication was achieved with combination of phenothiazines, benzodiazepines,  $\alpha_2$ -agonist and opioids whereas induction was achieved with different doses of Propofol. Premedication in the first group (n=6 dogs) was with Xylazine (0.5-1.1mg/kg), Diazepam (0.1-0.2mg/kg) and Tramadol (1.0-1.5mg/kg); second group (n=8 dogs) with Acepromazine (0.02-0.1mg/kg), Morphine (0.3mg/kg) and Diazepam (0.1-0.2mg/kg); the third group (n=4 dogs) where premedicated with Morphine (0.3mg/kg) and Diazepam (0.1-0.2mg/kg) and in the fourth group (n=4 cats) premedication was achieved with Xylazine (0.5mg/kg) and Ketamine (2mg/kg).

**Results:** The induction with Propofol significantly differed between the groups. Doses needed for induction with Propofol in the first group ranged from 2.0-3.8mg/kg, in the second group 3.2-8.3mg/kg in the third group doses of Propofol ranged between 3.8-7.2mg/kg while in the fourth group the induction was achieved with 2.8-5.5mg/kg of Propofol.

**Discussion:** Proper preanesthetic medication decreases the stress of the patient, ensures analgesia and decreases the anesthesia induction and maintenance dosage. Since every surgical procedure is painful, analgesia is primary segment of the balanced anesthesia. Premedication with opioids significantly decreases development of central hypersensitization. Proper pain control is part of the balanced anesthesia concept with pre-emptive analgesia and multimodal analgesia with opioids,  $\alpha_2$ -agonist, NSAID's and local anesthetics.

### O36

#### Multimodal anesthesia with zoletil/ medetomidine/butorphanol/ propofol / ketamine for dental procedures in brown bears (*Ursus arctos*)

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**Introduction:** Immobilizing and the surgery procedures of the brown bears are a challenge for every surgeon. The anesthesia should ensure safety work and stable physiology of the animal to minimize the risk of complications. The main purpose of this study was to monitor the physiological effects of multimodal anesthesia in brown bears during the dental procedures.

**Material and methods:** Physiologic variables (body temperature, heart rate, respiratory rate and saturation) in twelve brown bears, anesthetized with multimodal drug combination were evaluated for ninety minutes during



the dental procedures. The animals were kept in the Park for dancing bears Belitza, Bulgaria. The premedication of the animals was made with zoletil 1 mg/kg i.m. and medetomidin 0,003 mg/kg i.m.; Induction of anesthesia was made by ketamine 2 mg/kg i.v. and propofol 2 mg/kg i.v. like as bolus plus butorphanol 0,05 mg/kg im. The anesthesia was maintained with constant rate infusion (CRI) with ketamin 0,8 mg/kg/h and propofol 0,4 mg/kg/min.

**Results:** Rectal body temperature decreased significantly during the anesthesia, whereas pulse rate, respiratory rate and saturation were stable and there were no significant changes in these parameters during the whole period of anesthesia.

**Conclusion:** The examined bears were stable with consistent physiological variables during the anesthesia period. This anesthetic drug combination is suitable for oral surgery with middle duration.

## P1

### Preliminary study from the implementation of timed artificial insemination (TAI) protocols in dairy herds in Macedonia

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**Introduction:** Hormonal treatments in the estrus-synchronization protocols are implemented to increase the probability of estrous detection and insemination, and to subsequently increase the number of pregnant cows in dairy herds. In the majority of the dairy farms in Macedonia, the most common protocol used for estrus synchronization is still based on the application of a single prostaglandin ( $\text{PGF}_{2\alpha}$ ) injection followed by insemination 72 hours or at detected estrus. Since high producing dairy cows have reduced expression of estrous behavior and hence the lower estrus detection rate, an important tool and strategy for increasing the % of cows for AI is the implementation of timed-AI (TAI) synchronization protocols without the needs of estrus detection. In this study, we have described the results from two TAI protocols that were initially introduced in two dairy farms in Macedonia.

**Material and methods:** Total of 70 cows were included in the study and divided into two groups. In the Presynch-Ovsynch group (Group 1,  $n=35$ ) the cows were pre-synchronized with 2 injections of  $\text{PGF}_{2\alpha}$ , 14 days apart and 12 days later submitted to an Ovsynch 56 protocol (GnRH at  $70 \pm 3$  DIM,  $\text{PGF}_{2\alpha}$  7 d later, GnRH 56 h after  $\text{PGF}_{2\alpha}$ , and TAI 16 h later at  $80 \pm 3$  DIM) for first TAI. The remaining ( $n=35$ , Group 2) cows received only an Ovsynch 56 protocol at the random stage of the cycle after being previously diagnosed non pregnant (these cows had at least one insemination). Pregnancy diagnosis has

been performed at 35 days after TAI. Twelve cows from both (Ovsynch) groups that came into estrus, during the hormone administration were inseminated the same day.

**Results:** All 58 cows from both groups were inseminated 16 h after the second GnRH injection. However, 7 cows from the Group 1 did not have a corpus luteum at day 35 post insemination, and were classified as acyclic cows. These cows were further submitted to GGPG protocol (GnRH, 7 d before first GnRH in the Ovsynch). For the remaining cows, the conception rate were 60.86 % (14/23), 32.14% (9/28) and 58.33% (7/12) in Group 1, Group 2 and in cows inseminated at detected estrus, respectively.

**Conclusion:** Implementation of the TAI protocols might improve the reproductive management by increasing the percentage of cows receiving AI with subsequent increase of the reproductive efficiency and fertility in the herds. Further investigations are needed involving greater number of cows in order to confirm the preliminary results.

## P2

### The influence of cryoprotectant concentration on motility of cryopreserved boar spermatozoa

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**Introduction:** Despite the widespread of use of cryopreserved semen in cattle, its utilization in swine is limited, mostly as a result of the poor cryo-survival of boar spermatozoa. In order to overcome the mentioned problem, most of the work so far was focused on designing a suitable cryo-protocol and devising of suitable extenders for boar semen cryopreservation, with adequate concentration of cryoprotectant. The effectiveness of glycerol, as a cryoprotectant, depends on a number of properties: the compound is highly soluble in water and remains so at low temperatures in order to produce a profound depression of the freezing temperature; it is able to penetrate into the cells; and it has a low toxicity so it can be used in the relatively high. The objective of this study was to determine the appropriate concentration of glycerol in freezing extender, in order to ensure successful cryopreservation of boar spermatozoa.

**Material and methods:** The ejaculates were processed according the procedure by Westendorf et al. (1975), modified by Thurston et al. (2001). The pre-extended ejaculates, without centrifugation were chilled to 5°C within 1.5 hours and were extended to final concentration with isothermal (5°C) with freezing extender (CryoGuard™-CF Minitub, Tienfenbach, Germany), which contained 3% of glycerol ( $n=396$ ) for

the first group, or 8% of glycerol (n=40) for the second group; packed in transparent PVC 0,5 mL straws, and frozen using computer controlled freezer (IceCube 15 M, Minitüb, Tiefenbach, Germany). Total motility and the subpopulation composition of spermatozoa according to its velocity were assessed by CASA (IVOS Ver.14, Hamilton-Thorne Research, MN, USA).

**Results:** The percent of motile spermatozoa after the thawing in the Group1 was  $28,81 \pm 0,56\%$ ,  $SD=11,32$ ,  $CV=128,17$ ; compared to Group2 ( $10,95 \pm 0,60\%$ ,  $SD=3,80$ ,  $CV=14,45\%$ ). The differences between the groups were highly significant ( $p < 0,00001$ ), as well as the analysis of variance. The differences found in the subpopulation of spermatozoa were as follows between group 1 and 2: rapid  $25,02 \pm 0,63/7,10 \pm 0,57\%$ ; medium  $4,20 \pm 0,18\%/3,60 \pm 0,40\%$ ; slow  $9,62 \pm 0,37\%/16,17 \pm 1,47\%$  and static  $61,37 \pm 0,70/73,37 \pm 1,49\%$  with high significant differences in all subpopulations ( $p < 0,00001$ ).

**Conclusion:** Regarding the obtained results, the following conclusions were drawn: the higher content of cryoprotectant is decreasing the motility of cryopreserved spermatozoa ( $p < 0,00001$ ). Also, the differences in the subpopulation constitution of the ejaculates are significantly different ( $p < 0,00001$ ).

### P3

#### Suitability of centrifugation based technique for spermatozoa isolation

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**Introduction:** Sperm plasma membrane integrity is the first-line hallmark of cellular biological integrity. Spermatozoa motility and fertilizing capability are directly correlated with this trait, and therefore it is of highest importance to be kept unaltered in laboratory conditions. Even though it is widely accepted that centrifugation and washing of spermatozoa have detrimental effects on cellular structure, still there are many reports of its use in laboratory practice. In order to determine the degree of these negative effects, an aim was set to evaluate the most suitable post-washing dilution rate for spermatozoa after centrifugation.

**Material and methods:** Ejaculates (n=42) have been collected from eight subjects (rams, Ovchepolean

Pramenka, age of 2-4 years, and average body mass of 50 kg  $\pm 10$ ) by artificial vagina. Total and progressive motility parameters were obtained on computer analyzer (CASA), while membrane functional and structural integrity tests included hypoosmotic swelling test (HOST), and supravital staining (Mortimer). Following the obtainment of primary results for the fresh ejaculates (control group), they were diluted with Ringer (Ca<sup>2+</sup> free) solution and centrifuged in two phases (250 G for 3 minutes, then 700 G for 12 minutes). After extraction of the supernatant, the washed cells were diluted in ratio 1:1 and then separated in three aliquots that were joined in the according groups (A, B or C), depending on the additional dilution rate (one, three or six times, respectively). Each group was evaluated for the same parameters and then analyzed for significant differences. Kruskal-Wallis comparison of ranks model was used to exclude subject dominance effect in the control group, after which samples were analyzed by groups.

**Results:** Comparison of ranks between subjects in the control samples found small differences between no more than two rams for most of the used parameters which was insufficient to be regarded as significant bias in the control group. Nonparametric analysis between treatment groups revealed significant difference ( $P < 0.05$ ) for total, progressive motility and velocity parameters. In regards of viability and sperm plasma membrane integrity, significant difference was found comparing the control and two of the treatment groups (B and C,  $P < 0.0001$ ).

**Conclusion:** Regarding the significant differences between the control and treatment groups, it can be concluded that centrifugation and washing of spermatozoa decreases their viability and membrane integrity. This is especially augmented with higher dilution rates after centrifugation. Therefore, if no alternative method is possible for spermatozoa isolation, centrifugation should be followed with lowest dilution rate in order to decrease the detrimental effects on their integrity.



**P4****Association analyses of DNA polymorphisms in bovine PRL and DGAT1 genes with milk production traits in Simmental cows**

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**Introduction:** The selection of dairy cattle is orientated mostly towards the improvement of milk traits – milk yield, fat and protein yield and content. Genotyping tests give breeders the opportunity to use previously unavailable information to increase the frequency of beneficial alleles in their herds. Acyl-CoA: diacylglycerol acyl transferase 1 (DGAT1) enzyme has a role in the synthesis of triglyceride with the catalyzation of the reaction of diacylglycerol and fatty acid. Previous studies reported *DGAT1* gene placed on BTA14 as a potential candidate gene, with a nonconservative substitution of lysine by alanine (Lys232Ala) producing a major effect on milk composition and yield. Prolactin (PRL) plays an important regulatory function in mammary gland development, milk secretion, and expression of milk protein genes. *PRL* synonymous SNP G8398A, mapped on chromosome 23, has become a popular marker used for genetic characterization of cattle populations regarding milk composition and yield. To examine their possible applications in the genetic improvement of dairy cattle productivity, we herein investigated the effects of the *DGAT1* Lys232Ala and *PRL* G8398A SNPs on milk, fat and protein yield, as well as fat and protein percentage in the milk

**Material and methods:** The 51 Simmental cows from one dairy farm were genotyped. Information about milk traits for each cow were provided by the Croatian Agricultural Agency which is responsible for milk recording system according to ICAR rules. DNA was isolated from milk and genotyping of *DGAT1* and *PRL* genes was performed using PCR-RFLP. Frequencies of alleles and genotypes were calculated and association analysis was performed using general linear model calculations.

**Results:** In *DGAT1* gene allele Ala had higher frequency (0,54), and the most common genotype was LysAla (0,67). There was no association found between *DGAT1* gene polymorphism and milk performance traits ( $P>0,05$ ). For the *PRL* gene, the allele G had higher frequency (0,95).

The genotype AA wasn't found and the genotype AG was found in low frequency (0,11). No significant differences were detected between the two found genotypes ( $P>0,05$ ). **Conclusion:** Although in most of the previous studies SNP Lys232Ala showed significant impact on fat yield and percentage, in the studied population no association was found. The prevalence of the allele G in the *PRL* gene could explain the lack of significant differences between two found genotypes for the examined traits ( $P>0,05$ ).

**P5****Effects of tannin supplementation on duodenum morphology and cell proliferation in fattening boars**

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**Introduction:** Boar taint is an off-flavour appearing in meat of some entire male pigs, which is primarily caused by high levels of androstenone and skatole. Androstenone is a steroid produced by Leydig cells of the testes, whereas skatole originates from cell debris of the gut mucosa and is the product of intestinal bacterial metabolism of amino acid L-tryptophan. There are different ways to influence skatole levels either by altering the microbial nitrogen metabolism or by changing the intestinal content, the rate of passage and absorption. Numerous studies investigated the effects of different dietary ingredients on morphological characteristic in small intestine: dietary fibers, chickpea seeds, glutamine, glucose, faba bean, cereal, distillers dried grains, and cowpea. The objective of the study was to investigate the effect of supplementing boars' diet with tannins (sweet chestnut wood extract) on duodenal morphology and cell proliferation in fattening boars.

**Material and methods:** A total of 23 boars (Landrace x Large white) were assigned to four treatment groups, a control (T0 fed mixture with 13.2 MJ/kg, 15.6% crude proteins) and three experimental groups for which T0 feed was supplemented with 1% (T1), 2% (T2) and 3% (T3) of sweet chestnut extract of tannins. Boars were kept individually with *ad libitum* access to feed and water and slaughtered at 193 days of age and 122±10 kg live weight. Morphological and immunohistological alteration of duodenum was analysed.

**Results:** No significant effect of tannin supplementation was observed on duodenum cell proliferation. Boars in T3 group had significantly increased most of the investigated duodenum morphology characteristics in comparison to control T0 group. However, the size of villi width

and crypt depth was not significantly different between groups. Results indicated that 3% tannins supplementation in boars diet had significant influence on improved duodenum small intestinal morphology.

**Conclusion:** This study demonstrated that morphologic changes in duodenum were affected with different supplements of tannins in boars' diet. Further studies are necessary to determine the function of tannins as a feed supplement on possible boar taint reduction.

## P6

### Different concentrations of hydrolysable tannins fed to entire males affect salivary glands

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**Introduction:** Boar taint is an off-flavour in the meat of boars and is primarily caused by high levels of androstene and skatole. Piglets are therefore being surgically castrated without any anaesthesia and analgesia, the practice that is strongly criticised. Due to initiative to abandon this practice in EU by the year 2018, the alternatives and factors that could reduce boar taint are being investigated. One of them is the effect nutrition, i.e. specific ingredients. Thus we studied the effect of hydrolysable tannins in the diet of fattening boars with the focus on its effect on salivary glands morphology.

**Material and methods:** The study was conducted on 24 boars, crosses of Landrace x Large White allocated within litter to four treatment groups: control (T0 fed mixture with 13.2 MJ/kg, 15.6% crude proteins) and experimental diets for which T0 diet was supplemented with 1%, 2% and 3% tannin of hydrolysable tannin-rich extract Farmatan®. Experiment lasted 10 weeks and pigs were slaughtered at 122 ± 10 kg. Left parotid and mandibular glands were dissected, weighed and stored in the 10% buffered formalin. Tissue samples were stained with haematoxylin/eosin (HE) and Goldner. Morphometric analysis was performed on microtome sections of both glands, regarding acinar area and secretory ductal cells on 20 microscopic fields per sample.

**Results:** Tannin supplementation affected ( $P < 0.0001$ ) weight of parotid gland and tended to affect ( $P < 0.10$ ) the weight of mandibular gland. Boars fed diet with 3% tannin supplementation had 2.5-fold heavier parotid glands and 20% lower weight of mandibular gland compared to control group.

**Conclusion:** The histological assessment designated parotidomegaly on the basis of larger acinar area and bigger acinar cells due to diet with 3% tannin supplementation.

## P7

### The skull of the “Ruda” sheep in Albania: Morphological and morphometrical data Sokol Duro<sup>1\*</sup>, Ilirjan Bakiasi<sup>2</sup>

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**Introduction:** The sheep are important animals for Albania's economy because they provide valuable products such as meat, milk and wool. “Ruda” sheep is a native breed that is found mostly in the north-eastern Albania and occupies about 6-7% of the total numbers. Their head presented some special features: their eyes are big and protruding, head is without wool and a quite convex profile.

So far in the literature there are no investigations concerned with morphometric parameters of the skull and eyes of this mutton. The aim of this study was to identify crucial morphologic and morphometric parameters of its skull and to compare with other ruminants.

**Material and methods:** In the period January - March 2015 were measured and evaluated totally 36 osteometric parameters of 17 skull of “Ruda” sheep (8 females and 9 males) aged over 2 years, after the slaughter. The skulls are prepared in Anatomy Lab. by using the boiling maceration techniques for skeleton preparation described by Simoens et al. (1994). Measurements were made by metric rules and, electronic calibre. All results were evaluated and presented as average and standard deviations.

**Results:** The skull total length, skull width, skull total height, cranial length, cranial width, facial length and facial width of the “Ruda” sheep were 258.22 mm (±9.01), 109.35 mm (±5.53), 108.96 mm (±3.28), 135.76 mm (±4.65), 67.78 mm (±1.91), 80.60 mm (±6.01) and 32.53 mm (±1.1), respectively. Distance of infraorbital foramen from processus alveolaris maxillae and tuber maxillae were 23.14 mm (±2.75), 26.77 mm (±1.07), respectively. Foramen magnum high and foramen magnum width were 19.39 mm (±1.39) and 21.88 mm (±1.67) respectively. Also supraorbital foramina distance, nasal length and the highest point of nasal bone were 50.64 mm (±3.67), 100.48 mm (±6.13) and 94.33 mm (±4.89), respectively. The angle between two axes of eyes was 122.56 ° (±2.35).

**Conclusion:** This study provided us valuable data on morphologic and morphometric parameters about skull of “Ruda” sheep. Our investigation shows no significant statistical difference for the measured parameters. These data will serve us a base for comparison study with other ruminates species and important data on the clinical anatomy.



**P8****Clinical anatomy of the lower jawbone of the “Ruda” sheep in Albania**Sokol Duro<sup>1\*</sup>, Ilirjan Bakiasi<sup>2</sup><sup>1</sup>*Department of Morphofunctional Matters, Faculty of Veterinary Medicine, Agricultural University, Tirana, Albania*<sup>2</sup>*Department of Clinical subjects, Faculty of Veterinary Medicine, Agricultural University, Tirana, Albania*

**Introduction:** “Ruda” sheep is a native breed that is found mostly in the north-eastern Albania. In Albania has any data about morphometric parameters of the skull of this mutton.

The purpose of this study was to identify and evaluate some mandible parameters with clinical importance of “Ruda” sheep.

**Material and methods:** In the period January - March 2015 were measured and evaluated mandibles of 17 head of “Ruda” sheep (8 females and 9 males) aged over 2 years, after the slaughter. The mandibles are prepared in Anatomy Laboratory by using the boiling maceration techniques for skeleton preparation described by Simoens et al. (1994). Measurements were made by metric rules and, electronic Calibre. All results were evaluated and presented as average and standard deviations.

**Results:** The total length of the mandible in sheep was 197.25 mm ( $\pm 11.76$ ), and in rams 201 mm ( $\pm 9.58$ ). Maximum mandibular height in sheep was 116.63 mm ( $\pm 5.68$ ), and in rams 122.44 mm ( $\pm 5.03$ ). Diastema in sheep was 50.03 mm ( $\pm 3.47$ ), and in rams 51.06 mm ( $\pm 2.71$ ). The distances of mental foramen and mandibular foramen from caudal border of mandible are 166.75 mm ( $\pm 10.48$ ), 18.66 mm ( $\pm 3.42$ ) in sheep and, 163.68 mm ( $\pm 8.72$ ), 17.78 mm ( $\pm 2.08$ ) in rams respectively.

**Conclusion:** This study provided us valuable data on morphometric and clinical parameters of mandible of “Ruda” sheep, which will serve us a base for comparison study with other ruminates species and important data on the clinical anatomy, especially in regional anaesthesia.

**P9****Muscle variations – from embryonic development to clinical importance**Ivan Alić<sup>1\*</sup>, Tajana Trbojević Vukičević<sup>1</sup>, Martina Đuras<sup>1</sup>, Dražen Vnuk<sup>2</sup>, Snježana Kužir<sup>1</sup>, Vesna Gjurčević Kantura<sup>1</sup><sup>1</sup>*Department of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*<sup>2</sup>*Clinic of Surgery, Orthopaedics and Ophthalmology, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

**Introduction:** Muscle variations are defined as the appearance of additional muscle bundle or belly, unusual muscle origin or termination, as well as complete absence of a muscle. Muscle variations are very interesting regarding their embryonic development, but their importance increases during some surgical procedures. In our previous studies we described morphology and appearance of muscle variations in a dog, but for complete understanding of muscle variations it is necessary to investigate their embryonic development on the level of single cell.

**Material and methods:** All animals were conserved with 4% formalin fixative solution and dissected within a gross anatomy course at the Faculty of Veterinary Medicine University of Zagreb. The animals were handled in accordance with the Croatian Animal Protection Act (2006), and the study protocol was approved by the Ethics Committee of the Faculty of Veterinary Medicine.

**Results:** In our investigations muscle variations were observed in 22.8% cases. Although, muscle variations were observed in specific body regions, mostly in pectoral girdle muscles (brachiocephalicus, omotransversarius, rhomboideus capitis and serratus ventralis cervicis), neck muscles (sternocephalicus, sternohyoideus, stylohyoideus and scalenus dorsalis) and hindlimb muscles (sartorius), all observed variations were connected with the limbs. Described muscle variations were present as thin additional muscle bundle. Additional muscle bundles were innervated like a muscles from which they originate and the same innervation suggests the same embryonic origin.

**Conclusion:** Most of described muscle variations were connected with the limbs. According to this, we consider that during limb development, which commences during the fourth week in dogs, some myotomal cells fuse with the neighbour muscles during limbs outgrowth. Moreover, in limb development very important events were series rotations which participated in specification of the limb axes. According to this facts we conclude that limb rotation contribute in development of muscle variations. Although, muscle variation have embryonic importance, their most importance was topographic position during some surgical procedures.

**P10****Perinatal growth of skeletal muscle cells of pheasants hatched from eggs of different eggshell colour**

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**Introduction:** Eggshell colour of pheasant eggs can vary significantly, so the eggs could be dark brown, light brown, olive, blue, white, etc. In studies so far it was shown that blue pheasant eggs had low quality characteristics, and also that these eggs have some structural abnormalities. The aim of this study was to examine the characteristics of growth of skeletal muscle cells of pheasants hatched from eggs of different eggshell colour during their perinatal development.

**Material and methods:** A total of 400 eggs were divided in four groups according to the colour of their eggshell (dark brown, light brown, brown/green, blue/green) and incubated under the standard conditions. For histological examinations, on 17<sup>th</sup> day of embryonic development and on 1<sup>st</sup> day after hatching, the samples of leg (*M. biceps femoris*) and breast (*M. pectoralis superficialis*) skeletal muscles were taken. Samples were taken from 10 pheasants from each group. Histological preparations of taken samples were used for determination of diameter of skeletal muscle cells.

**Results:** Results show that on average, diameter of leg muscles of pheasants hatched from blue/green eggs was smaller by 18.3% ( $p < 0.05$ ) compared to pheasants hatched from brown/green eggs on 1<sup>st</sup> day after hatching. Also, on the same day, diameter of breast muscle cells of pheasants hatched from blue/green eggs was smaller by 15.2% ( $p < 0.01$ ) compared to pheasants hatched from light brown eggs. These results can be explained by lower quality of blue/green eggs which eggshell is thinner and more porous. This leads to higher evaporation from these eggs and greater water loss during incubation. Consequently, chickens hatched from these eggs have lower viability, which can be reflected to weaker development of certain tissues and organs.

**Conclusion:** It was concluded that eggshell colour of pheasant eggs is related to development of skeletal muscle tissue. During the perinatal period of development, in pheasants hatched from blue/green eggs the smallest growth of skeletal muscle cells was recorded, which could affect on further development of skeletal muscle tissue during the postnatal period of development.

**P11****Developmental programming of the gonadotropic cells by glucocorticoids**

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**Introduction:** Glucocorticoids play a very important role in the final maturation of fetal organ systems, acting at the tissue morphology and function, in preparation for extrauterine life. Transplacental glucocorticoid diffusion is normally limited by placental enzyme 11 $\beta$ -hydroxysteroid dehydrogenase type 2. However, the synthetic glucocorticoid dexamethasone (Dx), is often used in obstetric practice when risks of preterm delivery persist, passes through the placenta and promotes differentiation and maturation of fetal tissues. Overexposure to glucocorticoids retard fetal growth and may alter the trajectory of developmental process in glucocorticoid sensitive tissues. The aim of this study was to determine whether exposure to Dx during fetal period programmed development and function of gonadotropic cells in peripubertal (38-day old) female offspring.

**Material and methods:** The gravid females were randomized into a control and an experimental group, each consisting of ten animals. On day 16<sup>th</sup> of pregnancy, experimental dams received subcutaneously 1.0 mg Dx (Dexamethasone phosphate)/kg b.w., followed by 0.5 mg Dx/kg b.w./day on day 17<sup>th</sup> and 18<sup>th</sup> of gestation. The control gravid females received the same volume of saline. On postnatal day 38<sup>th</sup> female pups were randomly chosen from each of ten control litters and from ten litters of Dx treated mothers and sacrificed under ether narcosis. Pituitary glands were prepared for further immunohistochemical, immunofluorescence and stereological examinations. Serum concentration of FSH and LH were also determined.

**Results:** Volume of the pituitary gland was estimated using Cavalieri's principle and have shown decrease by 43% ( $p < 0.05$ ) in peripubertal female offspring prenatally exposed to DX in comparison to control values. Using a fractionator-physical disector method, we have shown that exposure to dexamethasone in the fetal period caused a reduction in total number of FSH and LH cells *per* pituitary gland by 37% and 35% ( $p < 0.05$ ), respectively, in 38-day old female offspring, followed by a decrease in serum concentrations of FSH by 45% ( $p < 0.05$ ) and LH by 49% ( $p < 0.05$ ). The relative intensity of FSH and LH immunofluorescence signal remained unchanged.



**Conclusion:** We can conclude that overexposure to dexamethasone during fetal development induces developmental programming of female reproductive system at the pituitary level, which may be associated with impaired reproductive function.

## P12

### Measurement of physical parameters of air quality and the level of harmful gases in laying hens housing

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**Introduction:** Ensuring optimal environmental condition of the poultry houses during their exploitation is very important, because it can influence the performance of the hens. Maintaining a proper ventilation can be costly for the farmers but without it, losses due to poor bird health and performance due to poor air quality can be much more detrimental to net returns. The goal of this study is to measure the air quality in the laying hens houses and to determine the differences in the air quality in various areas inside the houses.

**Material and methods:** Ten houses with laying hen conventional battery cage were measured for the air quality. The measurements were performed inside the houses at 9 points at multiple cage heights were measured. The measurements of O<sub>2</sub>, CO, H<sub>2</sub>S and NH<sub>3</sub> were done with portable detector MultiRAE and the temperature, relative humidity, CO<sub>2</sub>, airflow and lighting was measured with TESTO measurement instrument with multiple sensor probes.

**Results:** The mean values for the temperature, relative humidity, light intensity and airflow were in the range of 15,31°C and 25,6°C, 48,03% and 81,12%, 1,83 and 28,25 lux, between 0,05 and 0,51 m/s respectively. The deviation in temperature in a house was depended of heights of the battery and the air flow. Different cages were getting different amounts of light the ones that were closes to the light source were lighter were as the lower cages got the lowest light. The mean values for NH<sub>3</sub>, O<sub>2</sub> and CO<sub>2</sub> were between 0,39 and 8,17 ppm, 19,365 and 21,22%, and 696,2 and 1466,56 ppm, respectively. On one of the house at a spots the air flow was at minimum as low as 0,01 m/s, and at this spots were measured the highest the concentration of NH<sub>3</sub> (26 ppm). In one of the farms the alarmingly low levels (18,5%) of O<sub>2</sub> was measured, it was the same farm that had the lowest ventilation rate. In none of the houses CO nor H<sub>2</sub>S were detected.

**Conclusion:** It was concluded that the measurement of the air quality is in a house can vary depending of the places this measures are taken. The measurement of the air quality in different positions in the houses is of great

importance not only to the animal welfare and safety of the staff because it can show us the areas in the house where there is reduced or no air ventilation and the level of the harmful gases can be elevated.

## P13

### Wireless sensors for heard health monitoring and production management in dairy farming

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**Introduction:** Monitoring of the herd health status is of a paramount importance for successful and profitable farming. Real-time information can help both the farmers and veterinarians into applying measures which can prevent occurrence of health threats. The main objective of a good monitoring system is to be stable, reliable in data transmission, energy efficient and financially applicable. For this purpose, the aim of this investigation was to test different routing protocols on computer generated (CG) wireless sensor network (WSN).

**Material and methods:** An open source computer software (NS-2 simulator) is used to simulate WSN composed of wireless sensor (WS) nodes in a predetermined surface area, that had randomized dynamics, depicting animal subjects (CG-cattle). A monitoring application was custom programmed for this trial which was used for data collection. 360 tests were performed in order to evaluate routing protocols used by the WSNs (AODV, DSDV, DSR, each tested for n=15, 35 and 75 nodes), using the following variables and scenarios: Va.1 (n), Va.2 (%), Va.3 (ms), Va.4 (%) and A, B, C, D, respectively).

**Results:** The three WSN's showed the following ranges of variable means, respective to the group order: AODV: 0-215 (n), 89.24-99.96(%), 8.48-1143.99(ms) and 7.82-60.78(%); DSDV: 0-93 (n), 2.44-2.96 (%), 31.24-1995.77 (ms) and 64.61-78.8 (%); and DSR: 0-37 (n), 12.43-99.37 (%), 9.97-1137.74 (ms) and 0.02-0.65 (%). Highest peaks in respect of the variables for the different WSN's and scenarios are detected in the following groups: AODV: C, B, A and A; DSDV: C, B, A and A; DSR: B, C, A and A.

**Conclusion:** Following the results for the three types of WSN's, we can conclude that DSR is the most reliable and most efficient for transmission and retrieving data on free-stall dairy farms. DSDV having the lowest peaks for the respective variables, would be least recommended for our aim. AODV showed some moderate quality in data exchange, but still contains some of the flaws of the second WSN and should be further evaluated.

**P14****Determination of sampling number and localisation about hygienic control of cheese vat, press clot and canvas**

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**Introduction:** In cheese production, the surface of various tools and equipments such as cheese vat, press cloth and canvas are used as control points for hygienic controls. Also, cheese vat (66 000 cm<sup>2</sup>), press cloth and canvas (125 000 cm<sup>2</sup>) are very large surfaced tools and equipments. In some standarts (BS 4285, ISO 8086), it is indicated that sample is taken from the 900 cm<sup>2</sup> surface area. Nevertheless, there is no information about sampling number and localisation. Therefore, in this study we aimed to determine sampling number and localisation of cheese vat, press cloth, and canvas.

**Material and methods:** Washed vat, press cloth and canvas surface areas were divided into 900 cm<sup>2</sup> sampling areas 74, and 139, respectively. Then samples were taken from each sampling area separately by cotton swabs. This sampling was done in the same way after vapor application on the vat, press cloth, and canvas surface. All of the samples were analysed immediately for aerobic mesophilic count, micrococci-staphylococci, *Enterobacteriaceae*, coliform bacteria, yeasts and moulds. The microbiological results evaluated one way ANOVA and Duncan Test.

**Results:** We detected that there were statistically significant differences about microbiological results for each of the cheese vat, press cloth, and canvas among 12 sampling areas. These areas of vat localized on the side of where the staff mostly passed corridor. The different areas for press cloth and canvas were located to touched points where press cloth and canvas were inserted into vat by staff. Also, the microbiological levels of vat, press cloth, and canvas were significantly decreased after vapor application.

**Conclusion:** According to our results, it should be taken 12 samples from vat, press cloth, and canvas. These sampling localizations for the hygienic control should be preferred from the possible touched points near corridor which of passed by staff frequently. Moreover, it is important that vapour application to vat, press cloth, and canvas before use in manufacturing process.

**P15****Studies on mycotoxin contamination level in pig feed**

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**Introduction:** Mycotoxins, a series of secondary metabolites generated from molds, widely contaminate many agriculture commodities such as grain and feed, either in the field or during storage under favorable conditions. The most prevalent and predominant mycotoxins are aflatoxins, ochratoxin A and zearalenone. These toxins represent a serious threat to both human and animal health exerting carcinogenic, nephrotoxic, immunotoxic, teratogenic, genotoxic and mutagenic effects. Swine are generally the most sensitive among the animals. IARC made classification and most of mycotoxins are carcinogen to humans. Content of mycotoxins in feedstuffs is regulated by legislation worldwide with MRL in the range of 20-3000 µg/kg.

**Material and methods:** Total of 22 complementary feeding stuffs for fattening pigs, 23 feed samples for sow, 12 samples for in-pig sow, 15 samples for piglets, 15 corns and 10 wheat samples were analyzed in our laboratory for presence of mycotoxins. The HPLC-FLD with immunoaffinity column clean-up was the method used for determination of all three mycotoxins. The extraction and purification of samples was done according to several modified AOAC and ISO methods. The results were evaluated according to Macedonian legislation (Official Gazette 47/2012; 149/2012; 53/2013) which are in accordance with European regulations.

**Results:** Total of 97 pigfeed samples were analyzed for mycotoxin contamination level. Among them 48 samples were with aflatoxins, OTA and ZEA concentration below LOD (49.5%). One (1) corn sample was positive on aflatoxins in accordance with legislation (MRL is 20 µg/kg) and 1 feed sample for piglets was also positive (MRL is set on 10 µg/kg). Forty nine (49) feed samples showed presence of aflatoxins in the concentration range of 2.72-24.69 µg/kg; OTA in the range of 2.95-17.58 µg/kg and ZEA in the concentration range of 58.9-172.31 µg/kg. Most of the samples, especially complementary feeding stuffs for fattening pigs, show co-occurrence for all three mycotoxins (5 of 9 samples) and feed samples for sow (3 of 6 samples).

**Conclusion:** Forty nine (49) pigfeed samples (50.5%) were found to be contaminated with mycotoxins (aflatoxins, OTA and ZEA) with levels ranging from 2.72-172.31 µg/kg for different commodities and different mycotoxin. Although only 2 samples surpassed the legislation limits suggested by the official agencies, it should not

be neglected the overall presence of mycotoxins in feed samples. The following attention and strategies should be directed to reduce the exposure of humans and animals to mycotoxins in the continuous food chain for providing food and feed safety.

## P16

### Determination of radioactivity exposure in terms of radium equivalent and radiation risk index in the surrounding of the city of Skopje

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**Introduction:** The naturally present radionuclides have biological, radiotoxic and radio-pathogenic effects on the human organism. For this reason it is necessary to determine the content of radionuclides in the environment, and furthermore to calculate the dose that humans receive. Even though the majority of the population is settled in the city of Skopje and its surrounding, so far such studies have not been conducted; therefore this type of investigation is of particular interest.

**Material and methods:** The objective of this study was to determine the exposure to radiation due to distribution of  $^{226}\text{Ra}$ ,  $^{232}\text{Th}$  and  $^{40}\text{K}$  in the soil in the surrounding of the city of Skopje. Data were used from already measured activity concentrations of  $^{226}\text{Ra}$ ,  $^{232}\text{Th}$  and  $^{40}\text{K}$  in 14 soil samples using HPGe gamma spectrometer and the technique for registration of the fission monitoring. The exposure to radiation was defined in terms of radium equivalent -  $\text{Ra}_{\text{eq}}$  (Bq/kg) and radiation risk index -  $\text{H}_{\text{eks}}$ , calculated for each sampling location with the formula proposed by Beretka et al. (equation 1 and 2):

$$\text{Ra}_{\text{eq}} \text{ (Bq/kg)} = A_{\text{Ra}} + 1.43A_{\text{Th}} + 0.07A_{\text{K}} \quad (1)$$

$$\text{H}_{\text{eks}} = A_{\text{Ra}}/370 + A_{\text{Th}}/259 + A_{\text{K}}/4810 \quad (2)$$

**Results:** The data obtained show that the mean value of radium equivalent revealed in this research is 142.81 Bq/kg and is far below the value of 370 Bq/kg, which corresponds to a dose for the population of 1 mSv. However this value is somewhat higher than the world's average, being 129.7 Bq/kg. The mean value of the radiation risk index is 0.40, which shows that there is no high radiation risk for the population in the city of Skopje. By comparison of the results from this study and values measured in other countries, it was concluded that there is no significant difference in the radioactivity exposure in terms of  $\text{Ra}_{\text{eq}}$  and  $\text{H}_{\text{eks}}$ .

**Conclusion:** The knowledge for the concentration of natural radioactivity is essential for the assessment of the present and estimation of the future radioactive pollution in the environment. On the basis of data measured and calculated, one may conclude that there is no high radiation risk for the population in the city of Skopje. However, continuous and systematic examination is necessary in order to assess any changes in the level of natural and artificial radioactivity. The results obtained within this study are useful as basis for radiological mapping of the area studied, as well as for enrichment of the world's data bank.

## P17

### Overview over chemical composition of some selected feeds for sheep and lambs

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**Introduction:** The proper nutrition plays a major role in the overall productivity, health, and well-being of the sheep flock. The daily diet of the youth must be adapted to their need for development and the nutrient requirements of the sheep vary with differences in age, body weight, and stage of production. During the grazing season, sheep are able to meet their nutrient requirements from pasture and additional nutrient supplementation is required during the winter period. Poor nutrition can lead to reduced fertility, poor lamb survival, low growth rates and can contribute to ewe and lamb mortality.

**Material and methods:** As an object of analysis in this research are 20 randomly selected fodder mixtures from 8 different manufacturers in Republic of Macedonia in which are included 14 fodder mixtures for lambs and 6 fodder mixtures for sheep, by examining the most significant parameters in accordance with the Regulation for Quality of animal feed such as: protein concentration (ISO standard 5983-2:2005), moisture content (ISO standard 6496:1999), mineral matter (ISO standard 5984:2002), fiber content (ISO standard 6865:2000) and fat content (ISO standard 6492:1999).

**Results:** The results from this study show a clear picture about the quality of the animal feed which is used during different stages of ewe's production and lamb growth. After lambing, the energy and protein requirements of the ewe increase by 30 and 55 %, respectively. Reduced intake may results in excessive body weight loss, low milk production, mismothering, and poor lamb gains. In examined samples, total protein content is between 13.3-18.7% which is in accordance with the Regulation for Quality of animal feed. Fibers are an energy source that is important for the rumen function and it's concentration in the tested samples is between 4.9-12.2% depending



of the type of the animal feed, which is in accordance with the Regulation for Quality of animal feed. Adding minerals in the feed is usually required at the time of high production and pregnancy, its content is between 4.4-7.2%, the moisture concentration is between 9.5-13.2% and the fat content is between 2.1-3.4%, which shows that each fodder mixture fulfills the requirement for quality with the Regulation for Quality of feed (Official gazette of Republic of Macedonia, No.54/2014).

## P18

### Effect of dietary eicosapentaenoic and docosahexaenoic acid supplementation during last month of gestation on colostrum fatty acids composition in Charolais cows

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**Introduction:** Eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids are essential nutrients. Their significance is especially important in the perinatal period of the calf for the development of central nervous and immune system. The aim of the study was to explore whether it is possible to alter cow colostrum fatty acid composition with a low level of fat supplement, high in EPA and DHA.

**Material and methods:** In feeding trial were included a total of 20 Charolais cows during the last month of their gravidity. Cows were divided into 2 groups: a control group (Control) and an experimental group (DHA + EPA), each group consisting of 10 animals. All the animals were fed a basal diet, consisting of haylage and corn concentrate, and had constant access to drinking water. For the period of one month before expected calving, cows in experimental group (DHA + EPA) were supplemented with fat supplement, consisting of 9.1g/cow/day of EPA and 7.8 g/cow/day of DHA. Milk samples were collected on the 1st day (6 hours after calving, colostrum). Analysis of fatty acid composition was performed by gas chromatography.

**Results:** Fatty acid composition of colostrum in experimental group (DHA + EPA) was significantly altered: we found higher concentrations for fatty acids: EPA, DHA, docosapentaenoic, oleic and stearic acid. Summed profile of saturated fatty acids (SFA),

monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) was not altered but summed profile of n-3 long chain PUFA was significantly higher in experimental group. Also, addition of fat supplement significantly decreased concentration of myristic acid in colostrum.

**Conclusion:** These results showed that fat supplement, high in DHA and EPA, modified the fatty acid profile of colostrum milk fat and increased the proportion of fatty acids beneficial for health of calf in perinatal period.

## P19

### Optimization and validation of UHPLC tandem mass spectrometry method for determination of multi-class pesticide residues in bovine liver applying QuEChERS sample preparation

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**Introduction:** The determination of pesticide residues in matrices of animal origin is a matter of high necessity in view of the proven toxicity and stability thereof. Traditional sample preparation techniques usually are time-consuming, labor-intensive, complicated and expensive. Lately, it is a common opinion that many chemically different compounds have to be determined with one method, rather than single class of compounds. The QuEChERS (Quick, Easy, Cheap, Easy, Effective, Rugged, and Safe) approach for sample preparation offers unique possibility for multi-residual and multi-class determination of pesticides applying single method of analysis. For the purpose of monitoring pesticides within the National control program for food of animal origin, we optimized and validated UHPLC-tandem mass spectrometry method for determination of carbamates, pyrethroids and organophosphorus pesticides in bovine liver.

**Material and methods:** The UHPLC-mass spectrometry method in ESI+ mode was optimized for the following pesticides: carbaryl, carbofuran, fenvalerate, permethrin, cypermethrin, deltamethrin, malathion, parathion, dichlorvos and diazinon. The initial extraction with acetonitrile was followed by matrix-dispersive sample purification with 1.2 g of mixture from magnesium sulfate, PSA and C18 in ratio 6:1:1. Validation parameters were determined in accordance to the SANCO 12571/2013 document requirements. The established linearity was in

the range from 5 to 250 µg/kg. The LODs and LOQs were estimated at concentration levels with S/N ratio of at least 3:1 and 10:1, respectively. Accuracy was tested spiking blank bovine liver at two concentration levels, 10 and 50 µg/kg (n=5).

**Results:** The linearity determination revealed regression coefficients values higher than 0.99 for all pesticides tested. The estimated LODs and LOQs were lower than 10 µg/kg, with exception of diazinone, for which, LOQ value of 12.4 µg/kg was obtained. However all values estimated are lower than the established maximum residue levels for liver. Recoveries obtained were in the range 71.9 -115.9 % and 70.2 – 98.8 %, for spiked levels of 10 µg/kg and 50 µg/kg, respectively. The determined precision for all pesticides within the method scope was lower than 20%. Validation parameters estimated are in line with the respective legislative requirements, proving that the method is suitable for performing official pesticides control.

**Conclusion:** It was presented that QuChERS sample preparation, with some modifications, may be successfully applied for pesticides determination in liver samples with UHPLC-Tandem mass spectrometry. In accordance with the legislation requirements, the method proposed may be used for pesticide analysis within the Monitoring program for residues in animal products.

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## P20

### Aflatoxins occurrence in peanuts and products containing peanuts

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**Introduction:** Aflatoxins are highly toxic, cancer causing metabolites of certain strains of the fungi *Aspergillus flavus* and *A. parasiticus*, that cause immune-system suppression, growth retardation, liver disease and death in both humans and domestic animals throughout the world. They are a group of closely related compounds with small differences in chemical composition. Aflatoxins of concern are designated B1, B2, G1, G2 and are usually found together in various foods and feeds in various proportions, of which aflatoxin B1 is considered the most prevalent and the most potent form. In accordance to EU Commission Regulation (165/2010) groundnuts (peanuts) intended for direct human consumption or use as an ingredient in foodstuffs, should comprise maximum concentration of 2.0 µg/kg of AFB1 i.e. 4.0 µg/kg of sum of B1, B2, G1 and G2.

**Material and methods:** HPLC method with fluorescence detection was used for investigation of quantitative

determination of aflatoxins in peanuts and products containing peanuts, after their clean-up on immunoaffinity columns. This method is in accordance with ISO 16050 and AOAC Official Method 991.31. In duration of 21 consecutive months (2013, October – 2015, June), 38 samples of peanuts and products containing peanuts were analyzed. Among them: 19 samples raw peanuts, 7 samples roasted peanuts, 6 samples salty sticks with peanut butter, 4 samples peanut flips, 1 sample peanut paste and 1 sample peanut skins. Limit of detection (LOD) is 0.005 µg/kg.

**Results:** Aflatoxins concentration was below LOD in 26 (68.42%) samples: in 21 out of 26 peanuts samples and in 5 out of 12 products containing peanuts. Aflatoxins were detected in 12 (31.58%) samples, in a concentration range between 3.21 µg/kg and 61.42 µg/kg. Aflatoxins concentration detected was: 3.21 µg/kg, 10.6 µg/kg, 12.8 µg/kg and 14.2 µg/kg in 4 samples raw peanuts; 2.12 µg/kg in a roasted peanuts sample; 61.42 µg/kg in a peanut paste; 4.98 µg/kg and 5.7 µg/kg in 2 samples salty sticks with peanut butter; 16.83 µg/kg, 21.11 µg/kg, 22.68 µg/kg and 37.2 µg/kg in 4 samples peanut flips. Ten of the samples exceeded maximum limit of aflatoxins concentration i.e. 26.31% of total investigated samples.

**Conclusion:** Relative high percent of aflatoxin occurrence in investigated samples points out that further examinations on this topic are necessary. Thus it is highly recommended to increase the monitoring of aflatoxins in commodities. Beside this, proper agricultural and agronomic practices have to be imposed to reduce susceptibility and exposure of commodities to fungal invasion during pre-harvest, storage and processing periods.

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## P21

### Comparison of extraction methods in HPLC-FD analysis of ochratoxin-A in swine kidney

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**Introduction:** Ochratoxin A (OTA) is mycotoxin which is produced by fungi *Aspergillus* and *Penicillium* species. OTA is found in food commodities and several animal products. The toxin is known as etiologic agent of Balkan Endemic Nephropathy disease. OTA was classified in Group 2B as a possible carcinogen in humans by The International Agency for Research on Cancer. The aim of this study was to make comparison between three extraction methods (immune-affinity column extraction (IAC), liquid-liquid extraction (LLE) and solid phase

extraction (SPE), prior to HPLC-FD analysis of swine kidney.

**Material and methods:** The study was performed using OTA free swine kidney samples. IAC clean-up procedure was done according to study of Jorgensen K and Petersen A (2002) and LLE and SPE analysis were done according to the work of Monaci et al. (2004). HPLC-FD method was validated in the respect of EU Decision (2002/657/EC) in our laboratory. The validation parameters were: specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy and precision (repeatability and reproducibility).

**Results:** The linearity of the method at concentration range of 0.1 to 10 ng/ml was satisfactory with high value for coefficient of correlation ( $r^2 > 0.9999$ ). The chromatogram obtained when IAC clean-up procedure was employed, was very clean, without matrix effect and retention time was between 5-6 minutes. Accuracy was evaluated by recovery levels which were 77.6 and 103%. Repeatability ( $RSD_p$ ) levels were 8.8 and 9.4%; reproducibility ( $RSD_R$ ) levels were 3.24 and 8.8% for the concentrations at 1 and 5 ppb. LLE method with small amount of sample (2.5g) and extraction solvent (5ml) was resulted with low recovery (average 26%). LLE with high amount of sample (20g) and extraction solvent (100ml) gave the high recovery level as average 69.5-112%. LLE was resulted in a poor quality of clean chromatogram due to matrix effect. There was no acceptable recovery levels (0.9%) of SPE method.

**Conclusion:** The validation of the HPLC-FD method with previous IAC extraction procedure was achieved complying with the EU Decision (2002/657/EC). LLE technique was not adequate to provide pure chromatograms in compare to IAC clean-up procedure, but it was also efficient, with satisfactory validation parameters, rapid, less expensive and easy to perform as well. It should be extraction method of choice in analysis of OTA in kidney if the laboratory is dealing with big number of samples.

collected by honey bees from various plant sources and its composition and properties depends on geographic region and extraction procedure. This extraction process should remove the inert material and preserve the polyphenolic (flavonoid and other phenolic compounds) fraction, which is considered to contribute more to the observed healing effects than the other propolis constituents. The influence of different extraction methods and solvents on total phenolic compounds, antioxidative capacity and chemical composition of CNP extracts were in focus of this study.

**Material and methods:** Three different methods of extraction were used: maceration, reflux, and microwave-assisted extraction (MAE), as well as three different aqueous solutions of ethanol. Total polyphenol content and antioxidant activities were evaluated for all CNP extracts using respectively Folin-Ciocalteu and 2,2-diPhenyl-1-Picrylhydrazyl (DPPH). The reduction of this radical by an antioxidant compound results in a decrease in absorbance and is proportional to the number of electrons absorbed, indicating the antiradical capacity of the substances in study. Afterwards, extracts were analysed by GC-MS to quantify chemical compounds responsible for differences in antioxidative capacity.

**Results:** Propolis extracts showed differences in antioxidative capacity determined by DPPH method with the best results for 2 hours, room temperature and ethanol/water ratio 70:30. Extracts also differed in quantity and composition of different compounds responsible for antioxidative properties and biological activity determined by GC-MS.

**Conclusion:** Extraction procedure as well as ratios of solvents has effects on composition of phenolic acids and esters as well as flavonoids and medicinal properties of propolis. All extracts of CNP showed respectable antioxidant activities in comparison to standards (trolox, vitamin C). These data should be considered when using propolis in veterinary medicine.

## P22

### Antioxidative properties and GC-MS analyses of Croatian native propolis for implementation in veterinary medicine

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**Introduction:** The aim of this study was to evaluate antioxidative properties and chemical composition of Croatian native propolis (CNP) as a part of the project: „Intramammary propolis formulation for prevention and treatment of mastitis in dairy ruminants”. Propolis is

## P23

### Microbiological quality of soft, semi-hard and hard cheeses during the shelf-life

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**Introduction:** Cheeses as ready-to-eat food should be considered as potential source of foodborne pathogens, primarily *Listeria monocytogenes*. The aim of present study was to determine microbiological quality of soft, semi-hard and hard cheeses during the shelf-life, with particular reference to *L. monocytogenes*.

**Material and methods:** Five types of cheeses were sampled at different time-points during the cold storage



and analyzed for presence of *Salmonella* and *Listeria monocytogenes*, as well as lactic acid bacteria, *Escherichia coli*, coagulase positive staphylococci, yeasts, molds, sulfitereducing clostridia and *L. monocytogenes* counts. Water activity, pH and NaCl content were monitored in order to evaluate the possibility of *L. monocytogenes* growth. Challenge test for *L. monocytogenes* was performed in soft cheese, to determine the growth potential of pathogen during the shelf-life of product.

**Results:** All analyzed cheeses were compliant with microbiological criteria during the shelf-life. In soft cheeses, lactic acid bacteria increased in course of shelf-life period (1.2 - 2.6 log increase), while in semi-hard and hard cheeses decreased (1.6 and 5.2 log decrease, respectively). Soft cheeses support the growth of *L. monocytogenes* according to determined pH values (5.8-6.5), water activity (0.99-0.94), and NaCl content (0.3-1.2 %). Challenge test showed that *L. monocytogenes* growth potential in selected soft cheese was 0.43 log<sub>10</sub> cfu/g during 8 days at 4 °C. Water activity in semi-hard and hard cheeses was limiting factor for *Listeria* growth during the shelf-life.

**Conclusion:** Soft, semi-hard and hard cheeses were microbiologically stable during their defined shelf-life. Good manufacturing and hygienic practices must be strictly followed in production of soft cheeses as *Listeria*-supporting food and be focused on preventing (re) contamination.

## P24

### Antilisterial activity of spices in vitro

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**Introduction:** Bacteria *Listeria monocytogenes*, with its ubiquity, and increased ability to grow and survive in adverse conditions, presents a significant challenge in food production. On the other hand, spices are known to have antimicrobial properties, in addition to their ability to improve sensory characteristics and digestibility of food. The aim of present study was to determine antibacterial activity of spices against *L. monocytogenes*.

**Material and methods:** Fifteen spices (chilly, clove, cinnamon, coriander seed, cumin, curry, garlic, ginger, oregano, paprika, black and white pepper, rosemary, thyme and turmeric) were collected from retail from the same producer, and analyzed for presence of *Listeria monocytogenes*. For screening of antibacterial activity *L. monocytogenes* ATCC 7644 strain was used. The overnight culture of *L. monocytogenes* in BHI broth was subsequently diluted, to the level of 10 cfu/ml and all dilutions (1-7 log cfu/ml) were used for further screening of antibacterial activity. 5% suspension of spices and herbs was made in distilled water. 100 µL aliquot of

suspension of each spice was put in wells of two micro-titer plates and incubated at 5 or 37 °C for 30 min. Then 100 µL of serial dilution of *L. monocytogenes* were inoculated to each well with suspensions of spices, and plates were incubated at 5 °C and 37 °C. After incubation 100 µL of aliquot was spread onto Palcam agar and incubated 24 h at 37 °C.

**Results:** *L. monocytogenes* were not detected in any spice samples. From 15 spices only cinnamon and clove showed strong antibacterial activity against *L. monocytogenes* at both temperatures, while at 37 °C strong antimicrobial activity was determined also for garlic, oregano, rosemary and thyme. Weaker antimicrobial activity (1-2 log reducing in number compared to control) was noticed for curry and rosemary at 5 °C and chilly, ginger and thyme (2-3 log decrease) at 37 °C. Spices which showed strong antibacterial activity, were not only preventing growth of *L. monocytogenes*, but killed all bacteria even in concentration of 7 log cfu/ml (there was no viable bacteria on the agar).

**Conclusion:** Our results indicate potential that spices have as antimicrobial agents, and further research should be focused on determination on minimal inhibitory dose, and optimization of combination of spices.

## P25

### Comparison of marketing authorisation procedures of veterinary drugs in EU and USA and regulatory perspective in Macedonia

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The primary purpose of the rules governing veterinary drugs is to protect the health and improve production of the animals and the most important, to safeguard public health. The Marketing Authorization Procedures (MAP) of Veterinary Drugs (VD) in the EU and USA are the most demanding in the world. The aim of our work was to make a basic comparison of the MAP of VD for EU and the USA, and for Macedonia, as well. The main objective was to provide a general understanding of the similarities and differences of both market authorization systems. In addition, the level of harmonization of the MAP in Macedonia with the MAP in EU was discussed. The comparison was made through the deep insight of the current legislation in both the EU and USA for several aspects as, MAP, governmental involvement and technical requirements. Competent authority responsible for the MA of VD in USA is FDA, so there is only one procedure

for MA. In the EU national competent authorities of each member state are responsible for the MA of VD. The European Medicines Agency (EMA) operates as a decentralised scientific agency, as opposed to a regulatory authority of the EU. There are four procedures in which a VD can obtain marketing authorisation in the EU: centralized, national, mutual recognition and decentralized procedure. The choice of which procedure to follow depends on the number of countries in which the VD is going to be marketed and the type of VD concerned. Each procedure, as well as different types of application, were reviewed and discussed. In the EU many problems have arisen in connection with the free circulation of medicinal products due to different national procedures for marketing authorisation. Even if the European directive is completely implemented, the harmonisation process appears difficult in consideration of the different social, political and economical characteristics of the different countries.

Since Macedonia is a candidate country for membership into the European Union, it must follow the EU legislation. The national procedure is type of authorization for medicines which is only valid in Macedonia. In Macedonia, applicants have to submit an application to the Food and Veterinary Agency. Law on Veterinarian drugs is harmonized with the standards defined within the EU legislation, through Directive 2001/82/EK of the European Parliament and with the Directive of the Council from 6 November 2001 regarding the Codex for veterinarian drugs of the Community (32001L0082).

## P26

### Validation of GC-MS method for determination of organochlorine pesticides in honey

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**Introduction:** Pesticides play a beneficial role in agriculture because they are intended for preventing, destroying or controlling any pest in plants. Honey may be contaminated with pesticides on two ways: during collection of pollen and nectar by bees (cross-contamination) and through treatment of bee hives when the pesticides can migrate into honey (direct contamination). The residues of organochlorine pesticides in honey are a potential risk for human health. Due to this fact, their usage in agriculture is banned. The aim of this study was validation of GC-MS method for determination of organochlorine pesticides in honey.

**Material and methods:** For extraction of organochlorine pesticide residues from honey samples, multiresidue

QuEChERS method was used. In this study the following pesticides were included: aldrin, dieldrin,  $\alpha$ -hexachlorocyclohexan, hexachlorocyclohexan, lindane, heptachlor, metoxychlor, DDT,  $\alpha$  and  $\beta$  endosulfan, chlordane, endrin. Validation of the method was according to the Sanko 12571/2013. During the validation procedure linearity, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy of the method were investigated. Analyses were performed on GC-MS (7890, Agilent, USA). For determination of organochlorine pesticides 74 samples of honey from R. Macedonia were analyzed.

**Results:** The linearity of the method showed good correlation for all standards and  $r^2$  was from 0.9677 to 0.9999. LOD was between 0.40  $\mu\text{g/kg}$  and 7.04  $\mu\text{g/kg}$ , and LOQ was from 1.22  $\mu\text{g/kg}$  to 21.30  $\mu\text{g/kg}$ . Determined values for LOQ for all pesticides were less than Maximum Residue Level (MRL). The accuracy of the method was evaluated by determining the recovery of spiked honey samples on two concentration level at 10  $\mu\text{g/kg}$  and 50  $\mu\text{g/kg}$ . The recovery was from 78.03 to 114%, and from 74.01 to 107.14%, respectively. In addition, method showed good precision and coefficient of variation was from 6.02 to 12.06%. In the honey samples these pesticides aren't detected.

**Conclusion:** In our study the GC-MS method for determination of organochlorine pesticides in honey samples was validated. The performance of the method can meet the requirements of the domestic and international legislation and it's applicable in official control laboratories for determination of these pesticides in honey samples.

## P27

### *Fusarium* mycotoxins and toxigenic moulds occurrence in maize from Albania

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Natural contamination of feedstuff with mycotoxins, toxic secondary metabolites produced by fungi is a permanent challenge in animal nutrition. Their presence would compromise both the animal health and the quality of derived animal food products. The aim of the presentation was the study on *Fusarium* mycotoxins and the toxigenic moulds occurrence in maize from Albania. *Fusarium* mycotoxins are formed at the field prior to harvesting. Their presence in feed is regulated by Commission Recommendation 2006/576/EC on the presence of mycotoxins in products intended for animal feeding. Albania has adopted the EU legislation on

food and feed contaminants. Investigation of *Fusarium* mycotoxins in maize revealed high levels of FB<sub>1</sub> and FB<sub>2</sub>, but much lower levels of DON and ZON.

For the determination of mycotoxins, an LC-MS/MS method was applied. FBs contamination incidence in maize harvested in 2014 reached 92%. The highest determined level for the sum of FB<sub>1</sub> and FB<sub>2</sub> was 50800 kg/kg, which means the EU recommendation level of 60000 kg/kg was not exceeded. DON was the second most frequent mycotoxin with an incidence rate of 42%, with maximum determined level of 799 kg/kg. Compared to the EU guidance level of 8000 kg/kg for animal feeds, the concentration in all the analysed maize samples was below this value. ZON contamination was found with low incidence, 8%. The maximum level detected was 263 kg/kg, and compared to the EU guidance levels of 2000 kg/kg, all the analysed maize samples were below the MRL.

Within microbiological examination, *Fusarium* sp., *Penicillium* sp., *Aspergillus* sp., *Alternaria* sp., *Cladosporium* sp., and *Mucorales* sp. were found. The *Fusarium* sp. colony forming units (CFU) found in maize was from 2.5 to approx. 100 x 10<sup>4</sup> UFC/g, whereas other moulds were present: *Aspergillus* sp. in range 2 x 10<sup>3</sup>–200 x 10<sup>4</sup> UFC/g; *Penicillium* sp. 2.5–500 x 10<sup>3</sup> UFC/g. In studied maize samples was encountered also the presence of yeasts.

## P28

### Levels of lead and cadmium in cereal products commercially available in Macedonia

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**Introduction:** Lead and cadmium are major components of the biosphere that penetrate into living organisms through all types of exposure pathways. The main sources of toxic heavy metals are active mines, fossil fuels, electronic wastes, fertilizers and pesticides. Prolonged exposure to these contaminants is manifested by concerned dangerous effects on several organ systems, including the nervous, circulatory and immune system. In order to prevent these adverse health impacts it is important to assess the concentrations of heavy metals in cereal products, as the most used food for consumption over the territory of Macedonia.

**Material and methods:** Individually 46 wheat flour, 34 pastry and 15 corn flour samples were dry mineralized in quartz crucibles in a muffle furnace at 550°C. Nitric acid (0.1 M w/v HNO<sub>3</sub>) and double-distilled water (grade 2 according ISO 3696) were used for dilution of the completely mineralized cereal products. Quantitative determination of heavy metals was performed by Zeeman

electro thermal atomic absorption spectrometry (Model AAS 600). The absorbency of the measured solutions was converted to mass concentrations through calibration curve and the results were statistically analyzed using computer program Excel 2007 for Windows.

**Results and discussion:** Among the analyzed samples, corn flour showed slightly higher mean lead concentration, but almost three times lower maximal value in comparison with wheat flour. Contrary, the pastry was found to contain the lowest mean concentration of cadmium. It was described as an issue of the differences in manufacturing techniques among all three varieties of cereals.

Compared with available literature from different parts of the world, the obtained levels of lead and cadmium in the particular cereals showed similar results. According to the Commission Regulation (EC) No 1881/2006 the maximal permissible values for both toxic metals were not exceeded in researched samples.

**Conclusion:** The examined samples in present study were characterized by lower concentrations of selected heavy metals compared to valid legal regulations. Taking into account that the levels of Pb and Cd in commercially available cereal products are within the acceptable limits, they should be considered not health-threatening of the population in Macedonia.

## P29

### Pulsed – field gel electrophoresis used for epidemiological study of extended spectrum β lactamases - producing *Escherichia coli* colonizing respiratory tract in children

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**Introduction:** The extended spectrum β-lactamases (ESBL) are enzymes capable of hydrolyzing broad spectrum of antibiotics. Despite extensive studies of ESBL-producing organisms in adult patients, there is a lack of information about the epidemiology and spread of ESBL - organisms in pediatric population. The aim of this study was to elaborate gastrointestinal tract as endogenous reservoir for the respiratory tract colonization with ESBL- *E.coli*.

**Material and methods:** Study population – hospitalized children with respiratory tract infections, in period from January 2015 to June 2015. Modified disc diffusion test was performed for detecting ESBLs. Strains of ESBL – *E.coli* from sputum samples were compared with strains of ESBL – *E.coli* from feces, obtained from the



same patients. Resistotyping was performed using the antimicrobial susceptibility patterns. Pulsed – field gel electrophoresis was used as a "gold standard" for molecular typing of the strains (n=60). 25 isolates were from sputum samples, 22 from feces samples, taken from clinical cases, while 13 samples were taken from healthy patients, as control.

**Results:** Clinical isolates (n=47) and 13 isolates from control patients, were PFGE typed using XbaI digestion. Resulting band profiles of clinical isolates demonstrated presence of 7 groups with 100% similarity, while in control group a genetic diversity was present, not related with clinical cases. 60.86% (14/23) of the compared pairs from clinical cases (each pair contains ESBL- *E. coli* strains detected from sputum and feces in the same patient) showed 100% similarity of the PFGE patterns.

**Conclusion:** According to the PFGE comparison of all isolates, it can be concluded that the GUT is the main reservoir of ESBL-*E. coli*. Small age is a risk factor for translocation of bacteria, enabling the colonization of the respiratory tract.

### P30

#### Targeting counterfeit and substandard food and food supplement behind the illicit trade in Republic of Macedonia

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Recently, the economical crisis in Europe has transformed the criminal segment in to new modalities of illegal activities related to counterfeit of food and food supplements (F&FS). The illegal activities are driven predominantly by economical profit, infrequently by other factors such as lack of attentiveness, negligence, bad hygienic and production practices etc. In Macedonia, as in other countries in Europe, non-infectious diseases are causing approximately 85% of deaths in human population and bad and unsafe food together with smoking and alcohol consumption are considered as major hazards. The key goal of our investigation was to identify the scope of illegal activities with F&FS and to raise the public awareness about the dangers of counterfeit and substandard F&FS in Macedonia.

The investigation was carried out during the period 2012 to 2014, based on the consumers complaints submitted to the Consumers Organisation of Macedonia about unfit and unsafe food at the markets. There were 35 complaints in 2012 (3 for expired date, 3 for false declaration, 2 for pure quality and 27 rest), 188 in 2013 (56 for expired date, 32 for false declaration, 70 for pure quality and 30

rest) and 76 in 2014 (11 for expired date, 22 for false declaration, 26 for pure quality and 17 rest).

According to the data analysis on rising number of consumers complaints, it can be concluded that consumer awareness about food safety have increasing trend. The collected investigation data included the comparison of data on offences registered by the police and customs in collaboration with private sector.

The most common causes of the consumers complains in Macedonia related to F&FS are: irregularities in implementation of the control systems; incorrect labelling/declaring of the products; expired date; presence of contaminants and unidentified ingredients in the food; frauds and mistakes related to food; changes in organoleptic characteristics of the products and suspicions on poisoning mostly in the restaurants.

The results of our investigation are indicating the global character of this type of fraud and necessity to find modalities to fight this type of crime on national and international level. It is critical to keep the focus on this area of illegal activities, because gathered information is suggesting that most common illegal activities in F&FS are related to expiration date, false declaration and poor quality of F&FS. At the same time it is necessary to raise the awareness among consumers in Macedonia on risks coming from counterfeit and prohibited food.

### P31

#### Trend in cow mastitis pattern in Croatia

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**Introduction:** Cow mastitis is a permanent problem in dairy production due to several reasons such as plenty of possible pathogens, possibility of transmission among cows, contaminated cow environment, highly producing cows prone to infection etc.

**Material and methods:** Total of 385 cows from 15 farms were included in the research. Farms were visited at evening milking time during the period from February to April 2015. Samples for bacteriological examination were taken from previously prepared udder quarters of all lactating cows at the time of visit and transported to the laboratory on ice. California mastitis test and bacteriological examination were carried out according to the National mastitis council recommendation. Obtained data concerning the occurrence of udder infection, occurrence of pathogens, distribution of infection among quarters was compared with results from similar researches formerly carried out.

**Results:** CMT positive reaction was noticed in 211 (13.7%) individual udder quarters. 145 out of 385 (37.3%) cows had at least one CMT positive or permanently

dried off quarter. From 175 udder quarters (11.56%) mastitis pathogens were isolated. Among 363 cows with all functional udder quarters, 106 or 29.2% had at least one infected udder quarter. The most commonly isolated udder pathogen was *Staphylococcus aureus* (69 or 4.56%), followed by the group of streptococci (37 or 2.44%), *Trueperella pyogenes* (26 or 1.7%) and *Corynebacterium* spp. (26 or 1.6%). There was no statistical difference in the level of infection between front and rear udder quarters. CMT results and results of microbiological examination were moderately correlated (Kappa coefficient 0.4662). Positive trend in udder health is obvious if results are compared with the results of former similar researches carried out 1997 and 2004. CMT positive reactions in those researches were noticed in 34% and 28% of examined quarters. Mastitis pathogens were isolated from 21.5% and 22% of quarters, respectively.

**Conclusion:** Udder health improvement in cows on Croatian dairy farms is influenced mainly by adoption of EU legislation regarding milk quality whose implementation in Croatia started in 2001.

### P32

#### Antimicrobial activity of non-alcoholic propolis solution against common bovine mastitis pathogens

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**Introduction:** Bovine mastitis causes significant cost to dairy industry and is the main reason for antimicrobial use on dairy farms. Discarded milk following antimicrobial therapy of mastitis causes additional losses and may lead to presence of residues in milk. The aim of our study is to evaluate the *in vitro* antimicrobial activity of non-alcoholic solution of Croatian propolis against common bovine mastitis pathogens.

**Material and methods:** *In vitro* antimicrobial activity of propolis was evaluated by agar-dilution method according

to CLSI guidelines. Minimum inhibitory concentrations (MICs) were determined for approximately 100 isolates of common mastitis pathogens.

**Results:** Propolis showed good activity against *Trueperella pyogenes*, streptococci, *Staphylococcus aureus* and coagulase-negative staphylococci, with minimum inhibitory concentrations ranging from 8 to 64 mg/L. Significantly lower activity was determined against *E. coli* and *Pseudomonas aeruginosa* with MICs  $\geq 5000$  mg/L.

**Conclusion:** Our results indicate that non-alcoholic propolis solution has good *in vitro* activity against gram-positive bacteria causing bovine mastitis. Further clinical trials are conducted to assess its *in vivo* efficacy for the intramammary treatment of mastitis.

### P33

#### Relationship between milk urea nitrogen and fertility in dairy cows

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**Introduction:** Milk urea nitrogen (MUN) indicates the amount of urea found in milk and these values are closely correlated with the concentration found in the blood. The advantage of using MUN instead of blood urea nitrogen is noninvasive technique used for milk sampling. MUN is used for evaluation of diet composition and feeding disorders. It may be used to investigate metabolic and fertility problems related to excess dietary nitrogen. Although, high dietary protein stimulates milk production, increased protein has been found to be detrimental to reproductive performance of the animal. The aim of this study was to investigate the relationship of MUN with fertility measured as interval from calving to conception (CCI) and number of inseminations to conception (NINS).

**Material and methods:** Ninety clinically healthy Holstein cows at day 30 of lactation were chosen from three dairy farms (30 cows per farm) with different feeding regimens.

**Results:** Average milk production was  $43.07 \pm 1.60$  L at first farm;  $42.87 \pm 1.77$  L at second and  $39.97 \pm 1.82$  L at third farm, with no significant difference between farms. Mean MUN levels were  $4.90 \pm 0.18$  mmol/L at first farm;  $6.27 \pm 0.07$  mmol/L at second and  $7.88 \pm 2.17$  L mmol/L at third farm. MUN level at first farm was significantly lower than at second and third farm ( $p < 0.001$ , respectively) and MUN level at second farm was significantly lower than at third farm ( $p < 0.001$ ). Cows at

third farm had longest CCI ( $164.93 \pm 13.71$  days), while cows at first farm had shortest CCI ( $154.70 \pm 1.62$  days). CCI at second farm was  $160.37 \pm 2.25$  days. CCI at first farm was significantly lower than at second and third farm ( $p < 0.001$ , respectively). NINS was 2.27 at first, 2.40 at second and 2.37 at third farm.

**Conclusion:** Our results indicate that high MUN values are not associated with mean milk yield but are strongly associated with depressed fertility. It may be concluded that diets balanced to achieve greater efficiency of nitrogen utilization and lower milk urea concentrations have with positive impact on reproductive parameters and have no negative effect on milk production.

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### P34

#### Presence of virulence genes, biofilm production and antibiotic susceptibilities in *Trueperella pyogenes* isolated from cattle

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**Introduction:** *Trueperella pyogenes* (formerly *Arcanobacterium pyogenes*) is a commensal bacterium present on upper respiratory and urogenital tract mucosa of domestic and wild animals. It is also an opportunistic pathogen and isolated from the infections of cattle, such as mastitis, metritis, arthritis and pneumonia. The purpose of this study was to investigate virulence genes, biofilm production and antibiotic susceptibilities of *Trueperella pyogenes* isolated from cattle.

**Material and methods:** A total of 44 *T. pyogenes* was isolated from various specimens (milk=27, vaginal fluid=6, synovial fluid=6, abscesses=3, pleural fluid=1, aborted fetus=1) of cattle. The virulence genes (*plo*, *nanH*, *nanP*, *cbpA*, *fimA*, *fimC*, *fimE*, *fimG*) of the isolates were investigated by conventional PCR. Biofilm production were analysed by bioassay. Susceptibilities of the isolates to 14 antibiotics which are used commonly in veterinary medicine were determined by disc diffusion test.

**Results:** *Plo* and *fimA* genes were detected in all isolates. *CbpA*, *nanH*, *nanP* genes were determined in 6.8%, 61.3%, 84.1% of the isolates, respectively. However, *FimC*, *fimE* and *fimG* genes associated with adhesion and colonisation were found in 81.8%, 81.8% and 34.1% of the isolates, respectively. Biofilm production was determined in 39 (88.6%) *T. pyogenes* isolates. All strains were detected susceptible to amoxycillin clavulanic acid.

The percentage of the strains susceptible to cefoperazone was 97.7%, to amoxycillin, ampicillin, florfenicol and penicillin 95.45%, to enrofloxacin and cloxacillin 75%. All isolates were resistant to neomycin, 84.1% to oxytetracycline, 86.4% to gentamycin, 47.7% to erythromycin.

**Conclusion:** In conclusion, it is stated that majority of the isolates has ability to produce biofilm and this can cause difficulties in the treatment of the infections caused by *T. pyogenes*, even though they are found sensitive to antibiotics *in vitro*. Also, the presence of the virulence genes (*plo*, *nanH*, *nanP*, *cbpA*, *fimA*, *fimC*, *fimE*, *fimG*) in *T. pyogenes* isolates indicates their important roles in occurrence of the infections.

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### P35

#### Investigation of *Listeria monocytogenes* outbreak on a dairy cattle farm

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**Introduction:** Gram positive bacteria of the species *Listeria monocytogenes* are ubiquitously present in the environment. They are important human foodborne pathogens, which also affect the ruminants. Listeriosis, in both humans and the livestock animals, is associated with various clinical manifestations, especially the septicemia, abortion, gastroenteritis and infections of the central nervous system (CNS). In Slovenia, an increase of the outbreaks of listeriosis in ruminants has been recorded in the past years. Herein, we describe a case recorded in one of the organic farms.

**Material and methods:** The outbreak occurred in the spring 2014 on a farm with 68 dairy cattle, surrounded by forest. One of the cows died showing clinical signs of the affected CNS. The isolation of bacteria was performed from the brain sample and *L. monocytogenes* was obtained. In order to determine the potential sources of infection, additional samples were collected on the farm, including the individual milk samples and milk from the bulk tank. In addition, faecal, silage, stable tap water, drinking trough water, wallow water, reservoir drinking water and the deer faecal samples were collected. The feeding and farm management practices were also inspected. *L. monocytogenes* was isolated from the milk of nine cows, silage, all water samples, including the water reservoir, and deer faeces. All isolates were serotyped, using both the classical determination of somatic and flagellar antigens and the PCR typing. Since *L. monocytogenes* is a genetically highly diverse bacterial species and is widely spread in the environment, the genotyping with pulse-



field gel electrophoresis (PFGE) was also performed as enabling the inspection of epidemiological connections between the obtained isolates.

**Results and Conclusion:** The PFGE analysis showed eight different subtypes of *L. monocytogenes*. Genotyping did not provide a definitive answer on the source of infection. However, the obtained results showed that the silage can be considered as one of the possible, but probably not the only source of infection for dairy cattle. On the other hand, the farm water could also play a role and was possibly contaminated, together with the silage, from the external source (deer) or from the farm source (cattle). As the farm was self-sustaining regarding the herd progeny, the existence of external source of infection is giving a much more plausible explanation. Further investigations of such outbreaks could give additional insights into the transmission routes of *L. monocytogenes* between the environment and ruminants.

### P36

#### Some descriptive epizootiological features of 223 cattle positive on bovine tuberculosis in epizootiological area of Kumanovo

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**Introduction:** In 2007 in the Republic of Macedonia was adopted and started implementing the multiannual national program for eradication of bovine tuberculosis (bTB) in cattle where the object of mandatory annual testing with single tuberculin test (STT) are all cattle older than 6 weeks. Epizootiological area (EA) of Kumanovo consists of 3 municipalities: Kumanovo, Lipkovo and Staro Nagorichane and cover area of 1212 km<sup>2</sup> which is 4,7 % of total area of the Republic of Macedonia.

**Material and methods:** Retrospective and descriptive analysis of 223 positive cattle on comparative tuberculin test (CTT) (which represents 9,6% of the total 2329 cattle positive on CTT in Republic of Macedonia for the period 01.01.2007-march 2015) in EA Kumanovo has been carried out. Data for the suspected/positive cattle on single tuberculin test (STT) were obtained from private veterinary stations. The data for positive cattle on CTT were obtained from the reports of Faculty of veterinary medicine in Skopje, while data for movements of cattle, date of birth and size of holdings from the Food and Veterinary Agency system for identification and registration of animals.

**Results:** Increasing tendency of registered positive cattle on CTT was observed in EA Kumanovo. From a total of 756 reported cattle positive/suspected on STT, retested

with CTT were 676 cattle (89.4%) where 223 (33%) were declared as positive. Majority of positive cattle on CTT belonging to the age group of 13-24 months (40,17,9%) and only one of the age group 157-168 months (1,0,4%). Median age of positive cattle was 60.9 months, SD±39.3, ranging 4-160 months. There is a strong negative correlation between age and number of registered positive cattle. With increasing age up to 24 months the number of positive cattle is increases and after that positive cases in cattle going down,  $r=-0,82$ ,  $p=0,0003$ . The male/female ratio is 15/208 (6.7/93.3%). In total of 139 positive holdings, the greatest number of positive holdings or 98 (70,5%) are in size 1-10 cattle. Fifty-eight percentages of positive cattle are born on the positive holdings and 42% are imported from other holdings. Sixty-two percentages have no movement and 38% have movement through livestock market before being found positive on CTT.

**Conclusion:** There is no decline tendency of bTB after 8 years of implementation of the new multiannual program from 2007. Biosecurity measures for small holdings and movements of cattle must be strongly applied on the field.

### P37

#### Outbreaks of listeriosis in small ruminants with different clinical manifestations in the Republic of Macedonia

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**Introduction:** Listeriosis is a zoonotic infectious disease occurring in a large variety of animal species, but most clinical cases appear in ruminants. The clinical manifestations in sheep and goats are in the form of encephalitis, abortions and septicemia. The disease is caused by *Listeria monocytogenes* although *Listeria ivanovii* has been associated with abortions and meningoencephalitis in sheep. The aim of our study was to diagnose the causative agent of abortions, neurological disorders and mortality in the affected sheep and goat herds.

**Material and methods:** The material originated from five outbreaks occurring in different regions of the country. Two outbreaks were characterized with abortions and mortality in one sheep and one goat herd and the other three were characterized with neurological disorders and mortality in sheep. The samples included material from aborted fetuses (abomasal fluid, spleen, liver) and adult animals (brain tissue, spleen, liver and kidney). The samples were processed by isolation of the agent on artificial media, biochemical identification on VITEK 2, and

molecular detection of *Listeria monocytogenes* by PCR.

**Results:** The isolation was successful in all five outbreaks resulting in typical colonial morphology for *Listeria*. The biochemical identification successfully identified *Listeria monocytogenes* and *Listeria ivanovii* subsp. *ivanovii* and the PCR successfully detected *L. monocytogenes*. *Listeria ivanovii* subsp. *ivanovii* was identified as the causative agent of abortion in sheep and *Listeria monocytogenes* as causative agent of meningoencephalitis and mortality in sheep and abortions and mortality in goats.

**Conclusion:** This study shows that *L. monocytogenes* and *L. ivanovii* subsp. *ivanovii* are the causative agents of listeriosis in small ruminants in R. Macedonia. The implemented diagnostic protocols successfully identified the two pathogenic species of *Listeria*. Additional investigations should be made in order to identify the strains circulating in R. Macedonia and their possible connection.

### P38

#### Partial sequencing reveals the existence of one BTV-4 strain on whole territory of Macedonia during 2014 outbreak

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**Introduction:** Bluetongue (BT) is non-contagious, insect-borne, disease of ruminants caused by bluetongue virus (BTV). BTV is transmitted between susceptible animals by some species of *Culicoides* midges. The disease has major international significance and economic impact. Since 1998 BTV become frequently present in different parts of European continent, causing few wide epidemics. First occurrence of bluetongue in Macedonia was recorded in 2001, with clinical cases in sheep throughout the country. BT reoccurred in July 2014 when whole country was a part of the big BT epidemics in the Balkans. Out of 26 officially recognized BTV serotypes, only serotype 9 and serotype 4 have been confirmed in Macedonia. The purpose of this research was to clarify and better understand the molecular epidemiology of the disease and the possible presence of different BTV serotypes on the territory of Macedonia.

**Material and methods:** EDTA treated blood from sheep with clinical signs was used for detection of BTV RNA using Pan BTV real time RT-PCR (Toussaint et al. (2007)). Total RNA was isolated with QIAmp Viral RNA Mini Kit (Qiagen) according to manufacturer's instruction. Positive samples were further tested with serotype 4 - specific conventional RT-PCR which targets segment-2 of the viral RNA. Reverse transcription and PCR step were performed with Qiagen One Step RT-PCR kit. A selection

of 24 BTV-4 positive samples from 20 municipalities was sequenced using Big Dye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems). Raw sequence data was edited in BioEdit and sequences alignment was performed in MEGA 6.

**Results:** Nucleotide similarity analysis, querying nucleotide data available in GenBank, confirmed that all nucleotide sequences are part of BTV-4 genome and showed 100% similarity with BTV -4 strain (KP268815) isolated in Hungary in 2014. Twenty two nucleotide sequences were identical and only two sequences had the same nucleotide mutation (A-G) at position 631.

**Conclusion:** Macedonia experienced severe BTV outbreak during 2014 which had great direct and indirect economic impact, especially in sheep production. The epidemics in 2014 present a first, officially recorded, introduction of BTV-4 in Macedonia, which spread across the whole country within one month. Based on nucleotide data from partial sequencing of Segment 2, we have concluded that the same BTV-4 strain is present all over the country. This implied that the outbreaks had same origin and were appearing as a result of the infection spreading wave.

### P39

#### Ocular fibrosarcoma in a sheep

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**Introduction:** Fibrosarcoma is malignant tumor of the connective tissue which originates from fibroblasts. It has variable presentations depending on species, age, site, and etiopathogenesis. Fibrosarcomas usually localized under the skin but it may be originated from visceral organs. Although this tumor occurs in all animals, it is most commonly observed in old cat and dogs.

**Material and methods:** This report describes a case of an ocular fibrosarcoma in a 4-year-old, female, sheep.

**Results:** A big tumor was protruding the outside of the eye but not invaded the surrounding tissue and easily removed surgically. The grayish colored mass was hard and 19x13x8 cm in size. The surface of the tumor was infected and necrotic layer covered the mass. Marked hemorrhage and necrotic areas were present across the whitish cut surface. Histopathologically, the mass was composed of spindle shaped, anaplastic pleomorphic cells. Massons trichrome staining revealed collagenous matrix in the tissue. Immunohistochemically, the mass was positive for vimentin and proliferating cell nuclear antigen and negative for smooth muscle actin, desmin, glial fibrillar acidic protein and S100 protein antibodies.

**Conclusion:** According to histopathological and immunohistochemical findings the tumor diagnosed as fibrosarcoma. To the authors' knowledge, this is the first report of ocular fibrosarcoma in a sheep.

#### P40

### Pathological examination on hearts of lambs with white muscle disease

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**Introduction:** White Muscle Disease (WMD) or Nutritional Muscular Dystrophy is a degenerative muscle disease of all ruminants and it is caused by deficiency of selenium and vitamin E. WMD is mostly seen in newborns or fast growing ruminants. Although skeletal muscles affected by the disease, lesions occur mostly in cardiac muscles.

**Material and methods:** In this study, 52 lambs heart with WMD examined pathologically and histopathologically.

**Results:** Owners stated that animals have difficulty in breathing, a frothy nasal discharge, fever and sometimes sudden death. Mortality rates were between 35-55% in flocks. At necropsy, white areas were seen in myocardium. The lesions were commonly localized on left side of the heart. In addition auricular, musculus papillaris, and septal wall of the heart were affected. In severe cases all of the myocardium became pale and whitish. At the histopathological examination marked degeneration and necrosis present in myocardial cells. Calcification was commonly observed especially in severely affected cases.

**Conclusion:** This study showed that WMD can cause severe pathological findings and mortality in lambs.

#### P41

### Haemogram changes in lactating compared to non-lactating Assaf dairy sheep

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**Introduction:** Assaf dairy sheep originated from Israel and is crossbreed between Awassi and East Friesian breeds. Improving production, through increasing milk

production, causes changes in homeostatic regulatory mechanisms including hematological parameters.

**Material and methods:** Fifty animals of Assaf sheep, kept under intensive breeding and at the same environmental conditions, and fed with the diets appropriate for productive stages were included in the study. Average lamb production was 1.57 and daily milk production in lactating animals was from 2.3 to 2.5 liters. Sheep were divided in two groups: lactating dairy sheep (n=30) and control group (n=20). Blood samples were taken in EDTA vacutainers from *v. jugularis* from lactating animals twice: at early lactation (EL) stage and in the peak lactation state (PL), as well as from control animals twice, at the same time points as from lactating animals. Results obtained from lactating animals and its controls were compared. Complete blood count was performed on Automatic Hematology Analyser, Exigo (Sweden), on veterinary program for sheep.

**Results:** In lactating sheep, red blood cells count (RBC  $10^{12/L}$ ) were  $8.64 \pm 1.57$  in EL and  $8.13 \pm 0.86$  in PL group, hematocrit values (HCT %) were  $22.91 \pm 3.17$  in EL and  $17.61 \pm 1.44$  in PL group and hemoglobin value (HGB g/dl) were  $11.42 \pm 1.67$  in EL and  $9.45 \pm 0.73$  in PL group. All previously mentioned parameters were significantly lower ( $p < 0.001$ , respectively) than in control groups. Those results indicate that nutrient precursors were used more for milk production than in erythropoiesis. Mild anemia in lactating dairy sheep can be result of intensive metabolic utilisation of essential aminoacids in milk protein synthesis, rather than hemoglobin embedding. Other hematology parameters, such as MCV (fl), MCH (pg), follow the trend of lactating stage and were significantly lower ( $p < 0.05$ ) than in control animals. There was no significant difference for MCHC (g/dl) as well as WBC ( $10^9/L$ ) and LYM ( $10^9/L$ ) values between lactating and control animals. Lymphocytes are predominant population in white blood cell in sheep, for producing circulating antibodies in blood stream. Good immunological response can be favourable factor for successful adaptation and acclimatisation ability of Assaf breed, in the intensive farm breeding. Number of MON ( $10^9/L$ ) ( $1.02 \pm 0.37$ ) and GRAN ( $10^9/L$ ) ( $5.96 \pm 2.75$ ) were significantly increased ( $p < 0.001$ , respectively) only in EA compared to control animals, probably as a result of local postpartal infection, such as metritis, mastitis, retain placenta and other.

**Conclusion:** As a conclusion, hemogram in Assaf dairy sheep can be useful parameter for estimation of health status.



**P42****Giant mediastinal thymoma in a goat**

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**Introduction:** A 6-year-old, mixed-breed, female goat from a small dairy farm was barren since 3 years. During ultrasound examination a large space-occupying mass in the cranial thoracic cavity was detected and confirmed by x-ray and computer tomography (CT). Cytological examination performed after an ultrasound-guided biopsy suggested thymoma. Because of poor prognosis the animal was euthanized. Main lesion observed during autopsy was a large, irregular tumor localized in the cranial mediastinum. Based on histopathological examination thymoma was confirmed.

**Material and method:** Ultrasound examination was performed without any anesthetic drug. X-ray and CT scan examination was performed under general anesthesia. Autopsy was performed in usual fashion with direct external and internal inspection. Samples of macroscopically altered organs for histopathological examination were fixed in 10 % buffered formalin. For immunohistochemistry, tissue samples were processed in the same routine way, and immunostaining was performed using commercially available antibodies (Dako® Denmark): anti-cytokeratin (Monoclonal Mouse Anti-Human, clone AE1/AE3; concentration 1:100), anti-vimentin (Monoclonal Mouse Anti-Human, clone V9; concentration 1:100).

**Results:** *Ultrasound, X-ray and CT scan examination:* giant mediastinal mass 10cm in diameter.

*Autopsy findings:* The main significant pathology observed during autopsy was a large irregular tumor localized in the cranial mediastinum. The lesion consisted of a large mass with numerous smaller nodules on its surface. The tumor was firmly fixed to the cranial part of the pericardial sac. Several smaller satellite masses were present also at the epicardial apex. On the cut surface the tumor was heterogeneous with numerous cavities filled with serous or sero-hemorrhagic fluid.

*Histopathology:* The tumor consisted of mixed population of lymphoid cells (predominantly small mature lymphocytes, with some of blast cells) mixed with less numerous neoplastic epithelial cells. In the neoplastic cells a strong cytoplasmic immunoreexpression for cytokeratin was found, but they were negative for vimentin.

**Discussion:** Accidentally discovered mass in ultrasonography examination was confirmed on X-ray and CT scan and the dimensions of the tumor in this studies were comparable.

Basing on histopathology and immunochemistry a thymoma was confirmed in this goat. Thymoma is a tumor originating from the epithelial cells of the thymus. Typically, thymomas in goats are benign neoplasms reaching the size from 2 to 10cm in diameter (Löhr, 2013). Most animals with thymoma have no clinical symptoms at all. It was also the case in our patient, where the only clinical abnormality was reproduction failure, but it is hardly to say whether there was any connection between the thymoma and infertility or this was just a coincidence. Löhr C.: One hundred two tumors in 100 goats (1987-2011). Vet Pathol. 2013, 50(4), 668-75.

**P43**

**An outbreak of purulent meningoencephalitis caused by *Trueperella pyogenes* in a goat herd**

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**Introduction:** *Trueperella pyogenes* is Gram-positive microaerophilic non-sporulating facultative pathogen present on the skin and mucosal membranes of many animal species. Clinically apparent infection is usually a result of a mechanical injury. Many disease cases caused by *T. pyogenes* infection have already been reported in cattle and pigs whereas only a few in goats. The study presents an outbreak of the central nervous system infection caused by *T. pyogenes* along with epidemiological investigation of its origins.

**Material and methods:** Between March and May 2015 four goat kids were admitted to the hospital of the Faculty of Veterinary Medicine in Warsaw due to sudden onset of weakness and neurological signs consisting of head tilt and circling. They were 4-6 week-old on admission and had been disbudded 2-5 weeks earlier. They came from a professional dairy goat herd counting approx. 50 does. Just after birth they had been weaned and reared on a high-quality milk replacer as a part of the caprine arthritis-encephalitis (CAE) control program. They all had been serologically screened for CAEV weekly since their

birth and tested negative. An ID Screen® MVV-CAEV Indirect Screening ELISA was used. In all diseased kids antibiotic (third-generation cephalosporin) and supportive therapy was maintained for 5 days which resulted in the disappearance of clinical symptoms. The kids returned to the herd, however three weeks later the symptoms recurred in two of them and they immediately died. The autopsies revealed purulent meningoencephalitis. Moreover, epidemiological investigation was launched to track down a potential origin of the infection. Swabs were collected from all visible wounds and abscesses in goats as well as from the external ear and nasal cavity of kids in the herd.

**Results:** *T. pyogenes* was cultured from the pus and samples of brain and internal organs of two dead kids. It was also isolated from two mandibular abscesses – one in a peer of the dead kids, another in an adult male. Neither nasal nor ear swabs harbored *T. pyogenes*.

**Conclusions:** *T. pyogenes* is a potential cause of an outbreak of purulent meningoencephalitis in goats irrespective of the quality of environmental conditions in which they are kept. Neither external ear nor nasal cavity seem to be the reservoir of the pathogen. *T. pyogenes* infection doesn't seem to be linked to CAEV infection.

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#### P44

### The prevalence of *Pasteurella multocida* from farm pigs in Serbia

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**Introduction:** Respiratory disease belongs to the most important problems in intensive pig production worldwide. The development of intensive swine farming is characterized by the forming of big agglomerations for the production of swine, which has resulted in the concentration of large numbers of animals in a small space. Such a manner of production favors the incidence of respiratory infections in swine. The objectives of the presented investigation were to determine the incidence and to identify the bacteria that cause pneumonia in pigs with an accent on the incidence of *P. multocida*.

**Material and methods:** The investigations covered a total of 234 pathoanatomical altered lung tissue of dead pigs, from 6 farrow-to-finish pig farms during 2013. and 2014 years. The samples were inoculated on corresponding culture media (blood agar, MacConkey agar, Columbia agar) and incubated at a temperature of 37°C during 24-48 h. The physiological and biochemical characteristics were examined in the pure cultures

obtained in that way. Bacterial isolates were identified using standard bacteriological methods. The identification was confirmed using the BBL Crystal, E/N, G/P ID Kit (Becton Dickinson). For determination of type of *P. multocida* PCR method was used.

**Results:** From the total of 234 examined lung samples, bacteria strains were isolated from 202 samples (86%). *P. multocida* in pure culture was isolated from 71 (35 %) samples. Furthermore, mixed cultures were established in 29 (14 %) samples, where, in addition to *P. multocida*, *Streptococcus* spp was isolated from 9 (31%) samples, *Arcanobacterium pyogenes* 8 (27%), *Actinobacillus pleuropneumoniae* 7 (24%) and *Haemophilus parasuis* 5 (17%). The PCR method confirm that all 15 investigated strains of *P. multocida* belong to type A.

**Conclusions:** The high percentage (86 %) of identified bacteria from pig lung indicated their importance in ethio-pathogenesis of the pig respiratory infections. These results have shown a high incidence and importance of *P. multocida* in genesis of the pig respiratory disease. The control of respiratory disease requires an understanding of the complexities and interaction between the organisms that are present, the pig and the management of the environment. The prevalence of respiratory disease is affected by the presence of respiratory pathogenic agents, the virulence of the pathogens present, the level of the pathogens in the house environment and the immunity of the pig.

#### P45

### Distribution of the PCV2 antigen in different tissues and the histopathological changes in the same tissues from pigs with PMWS

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**Introduction:** The Circovirus disease in pigs is caused by a small, spherical DNA virus which is spread in the pig production industry worldwide. Most important of the Circovirus diseases is the Postweaning multisystemic wasting syndrome (PMWS) which causes big economical losses in the pig industry.

**Material and methods:** Samples of lung, liver, kidney, spleen and lymph nodes from 40 pigs with Postweaning multisystemic wasting syndrome from different parts of the Republic of Macedonia were collected. Samples of each organ were fixed in 10% formalin, dehydrated, embedded in paraffin and cut on 3 µm thick slides. The histopathology slides were stained with hematoxylin-eosine and the immunohistochemistry slides were treated with F217 2C6-H9-A2 monoclonal antibody using LSAB method.

**Results:** We examined 40 pigs from different herds with signs of wasting disease. The clinicopathological symptoms were: weight loss, dyspnea, skin pallor and occasionally icterus, interstitial pneumonia and lung oedema, lymphadenopathy, hepatitis and nephritis.

The histopathological investigations in the lymphatic organs showed lymphocyte depletion and necrosis in the cortex and the par cortex of the lymph nodes, as well as the presence of giant cells in the same areas, lymphohistiocytic to granulomatous interstitial pneumonia and at the same time infiltration by histiocytes. Lymphohistiocytic infiltration was also found in the liver, kidneys, the intestines and also in most of the other tissues.

The immunohistochemical examination revealed the presence of PCV2 antigen in almost half of the examined pigs. Low to moderate amounts of PCV2 antigen were detected in a wider range of tissues of lung, lymphoid organs, liver and lesser degree in kidney. The antigen is mostly present in the necrotic areas of the lymph follicles, and less present in the giant cells and the mononucleated phagocytes of the lymph follicles.

**Conclusion:** Beside the detection of the PCV2 antigen, the immunohistochemical method also allowed us to observe the distribution of the virus antigen in the tissue and recognize the pathogenesis of the disease. From the presented histopathological and immunohistochemical diagnoses it is evident that the finding of the PCV2 antigen is a closely correlated with the degree of the tissue lesions.

#### P46

### Effect of gestation on haematological and biochemical parameters in donkeys (*Equus asinus*)

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**Introduction:** In the middle Europe donkeys are more and more bred and kept as hobby animals. It is well known that during the pregnancy in female some metabolic changes occur that may alter physiological range of blood constituents. Therefore, we have hypothesized the influence of the pregnancy on haematological and biochemical parameters in blood of pregnant jennies.

**Material and methods:** Blood samples were collected in Czech and Slovak Republic between 2010 and 2011. In one group there were pregnant animals (n=11) and in second group non-pregnant animals (n=29). All donkey females were from 2 to 11 years old, in good health status without any pathological findings and their body condition score (1-9) was 5 or 6. Blood samples were collected from each animal by jugular vein puncture, transported to laboratory and examined. Examined haematological parameters were: red blood cells count, MCV, MCH, MCHC, hemoglobin, hematocrit, leukocyte, segmented neutrophil, band, lymphocyte, eosinophil, basophil and thrombocyte number. Examined biochemical parameters were: total protein, albumin, creatinine, ALP, ALT, AST, CK, GGT, LDH, cholesterol, lactate, Ca, Na, K,

P. Collected data has been analyzed by the multivariate analysis (GLM) in software Statistica for Windows.

**Results:** The significant difference ( $P < 0.05$ ) between non-pregnant and pregnant animals was in number of eosinophils and basophils and the level of creatinine and CK. On the border of statistically significant difference was the number of bands. Eosinophil, basophil and band number and also CK level were higher in pregnant animals, while the level of creatinine was higher in non-pregnant animals.

**Conclusion:** Pregnancy has a significant influence on basophil and eosinophil number, creatinine and CK levels and probably effects also number of bands. The decreased levels of MCH, creatinine, lactate and ALP and increased band, eosinophil and basophil number during pregnancy could be considered as a physiological condition related to pregnancy in jennies. It is important to know about these biochemical and haematological changes in pregnant jennies, contributing to a better understanding of processes that occur in pregnant jennies and providing practical help for the diagnosis of diseases during pregnancy.

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#### P47

### Allergen-specific IgE in horses with equine recurrent airway obstruction (RAO) and healthy controls, assessed by in vitro allergy testing

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**Introduction:** Equine recurrent airway obstruction (RAO), also known as 'heaves', is one of the most common respiratory problems in older horses. It is characterized by airway obstruction, excessive mucus accumulation and influx of neutrophils into the bronchial tree. Clinical signs develop as a result of exposure to and inhalation of aeroallergens, such as hay mold and barn dust. Many studies have been carried out in horses with regard to specific IgE in the serum and fluid obtained during bronchoalveolar lavage (BALF) and has documented *Aspergillus fumigatus*, *Faenia rectigula*, *Alternaria* spp and *Mucor* spp as possible etiological agents of RAO. Although the mentioned molds listed are the most common pathogens involved in the development of RAO, the role of mite sensitization in this disease is still unclear. The aim of our study was to investigate and compare concentrations of common allergen-specific IgE using an ELISA method in the serum of Polish Konik horses with equine recurrent airway obstruction and healthy controls.



**Material and methods:** The study was conducted on a group of 14 adult Polish Konik horses, kept in a uniform environment. The horses were divided into two groups: seven horses which did not have any respiratory problems comprised the control group and seven horses with a history of recurrent airway obstruction (RAO) constituted the study group. A clinical and laboratory evaluation, endoscopic examination and bronchoalveolar lavage (BAL) were performed in all horses. Sera of all horses were tested blind against nine molds and three mites using Heska Allercept®.

**Results:** All horses from the study group had IgE concentrations against mold s below the cutoff point and no statistical difference between the groups was found. In the serological tests, a statistically significant difference between both groups was found with regard to specific IgE against mites, wherein *Tyrophagus putrescentia* correlated most clearly with RAO. There was no difference between groups for specific IgE against molds.

**Conclusion:** Based on our observations and results, we concluded that recurrent airway obstruction is associated with high concentrations of specific serum IgE against mites, in particular *Tyrophagus putrescentia*. Our study suggests that respiratory problems in the RAO-affected horses may be due to mite sensitization since the concentration of mite specific IgE in RAO-affected horses has been found to be statistically significant compared to the healthy controls.

#### P48

##### Evaluation of different diagnostic techniques for the diagnosis of fascioloidosis in red deer

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**Introduction:** *Fasciolodes magna* is a liver trematode, commonly found in several areas in North America and Europe. The presence of *F. magna* in Croatia was confirmed in 2000, although the fluke was probably present in that area years before. *F. magna* enter uninfected habitats by natural migration of cervids and by translocation of

animals. As was recently confirmed genetically, records of fluke presence in deer populations followed the path of the Danube River, which offer suitable conditions for sustaining large deer populations and for completing of parasite life cycle. High prevalence of fascioloidosis in Croatia makes it an economically important disease in wild ruminant and different diagnostic methods are being investigated in order to control the disease.

The objective of the present study was to evaluate and compare following available diagnostic techniques for detection of *F. magna* infection in red deer: 1 coprological test (sedimentation-flotation technique on 5g of faeces-SF), 2. serological test (indirect serum ELISA technique with excretory-secretory antigens obtained from adult flukes-E/S ELISA) and 3. serum biochemistry (aspartate aminotransferase-AST, lactate dehydrogenase-LDH, glutamate dehydrogenase-GDH, glucose and globulin value and albumin/globulin ratio).

**Materials and methods:** The presence of *F. magna* was confirmed at necropsy. Samples of serum, faeces and liver of 38 red deer from the *F. magna*-endemic area, were used to estimate sensitivity of tests. Similar samples collected from 10 red deer outside the *F. magna*-endemic area were used to estimate specificity. To evaluate diagnostic value of each test, Receiver Operating Characteristic (ROC) curve analysis was performed. Sensitivity, specificity and likelihood ratio (positive and negative) were used as indices of test accuracy. Summary statistic of overall diagnostic accuracy of each test was displayed like area under the ROC curve (AUC) index with 95% confidence intervals (95% CI).

**Results:** According to reference test, the true prevalence was estimated at 66.7%. Coprological test showed an AUC index of 0.894 (95% CI 0.781-0.961), E/S ELISA 0.960 (95% CI 0.858 – 0.994), AST 0.889 (95% CI 0.580 – 0.994), LDH 0.792 (95% CI 0.637 – 0.903), GDH 0.742 (95% CI 0.586 – 0.863), glucose 0.900 (95% CI 0.771 – 0.969), globulin 0.700 (95% CI 0.549 – 0.825) and albumin/globulin ratio 0.839 (95% CI 0.697 – 0.932).

**Conclusion:** These results demonstrate that the E/S ELISA technique showed higher accuracy in diagnostics of fascioloidosis with clear discrimination between negative and positive populations. Also, E/S ELISA were highly sensitive and specific when compared to diagnosing *F. magna* by coprological means and serum biochemistry values and might be useful diagnostic test for epidemiological studies.

**P49****Prevalence of *Trichinella* larvae in Macedonian red foxes (*Vulpes vulpes*)**

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**Introduction:** The *Trichinella* nematodes are cosmopolitan zoonotic parasites found in a wide range of mammals, birds and reptiles. Despite this broad host spectrum, members of the Carnivora are the most common hosts, and in carnivorous wildlife, the main reservoir is the red fox (*Vulpes vulpes*). The red fox is the main indicator for infections in European wildlife and many studies have been carried out to determine the *Trichinella* prevalence in red fox populations. There is no active/passive surveillance of red foxes for *Trichinella* in Macedonia, so the present study was initiated to assess the prevalence of *Trichinella* larvae in the Macedonian red fox population.

**Material and methods:** Tissue samples (masseter muscle, tongue and forelimb) from 404 red fox carcasses were collected throughout the country in the hunting seasons 2012-2013, 2013-2014 and 2014-2015 as part of the national surveillance program for efficacy of the oral vaccination of foxes against rabies. All samples were tested by trichinoscopy and standard artificial digestion method as recommended by the International Commission on Trichinellosis.

**Results:** *Trichinella* larvae were found in 87 foxes, indicating an overall prevalence of 21.5% (95% confidence interval 17.5-25.5%) for *Trichinella* infection in Macedonian red foxes. The prevalence per hunting season was highest in 2012-2013 [24.2% (71/293)], followed by 2013-2014 [19.0% (12/63)] and 2014-2015 [8.3% (4/48)]. The positive foxes were mostly distributed in the Southwestern and the Northeastern region. The infection level of the positive foxes ranged from 1 to 15 larvae per gram of tissue.

**Conclusion:** This study shows that *Trichinella* is widespread in red foxes across Macedonia with a significantly decreasing prevalence. Although decreasing, the prevalence indicates the presence of an active cycle of sylvatic trichinellosis in Macedonia and that the red fox has an important role in maintaining and spreading the disease. In this manner, the identification of the *Trichinella* larvae to species level, how the foxes become infected and how is the parasite cycle maintained needs additional investigation.

**P50****Simultaneous occurrence of sebaceous and modified sebaceous gland carcinomas in a dog**

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**Introduction:** Sebaceous and modified sebaceous gland tumors are uncommon cutaneous tumours with the potential to arise from any sebaceous gland in the body of the dog. These tumors include sebaceous adenoma, sebaceous epithelioma, sebaceous carcinoma, meibomian adenoma, meibomian epithelioma, meibomian carcinoma, hepatoid gland adenoma, hepatoid gland epithelioma and hepatoid gland carcinoma.

**Material and methods:** An 13-year-old, Doberman, male dog presented to Mehmet Akif Ersoy University Veterinary Teaching Hospital, Department of Surgery for examination of the masses in the subcutis of right forearm, on the left upper eyelid and in the left perianal region. Since the masses were not infiltrate to the adjacent tissues could be removed easily.

**Results:** These masses were measured as 1.5x1x0.7 cm, 1x1x0.8 cm and 2.5x1.5x1 cm respectively. At the microscopical examination, these tumors were diagnosed as sebaceous carcinoma, meibomian carcinoma and hepatoid gland carcinoma due to the characteristic histological appearances.

**Conclusion:** Herein, we report a case with a simultaneous sebaceous carcinoma, meibomian carcinoma and hepatoid gland carcinoma in the same dog. To our knowledge, this is the very rare case report of these tumors occurring simultaneously in the same dog.

**P51****Fourth branchial arch defect (4-BAD) – Case report**

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**Introduction:** Fourth branchial arch defect (4-BAD) is a term describing a syndrome of congenital defects, affecting that external structure of the larynx, which have a basis in the fourth branchial arch.

**Material and methods:** a four-year old welsh pony mare was admitted to The Equine Clinic in Brno because of the stridor audible during exercise and coughing during feeding. The physical examination was without

any pathological findings, only laryngeal palpation was abnormal. Resting airway endoscopy revealed right arytenoid paresis, rostral palatopharyngeal arch displacement and feed aspiration in rostral part of trachea. The high-speed treadmill video endoscopy (trot (speed 4 m/s) and continued in gallop) of upper airways revealed loss of right sided arytenoid cartilage abduction, and axial deviation of the right vocal cord. During the examination inspiratory stridor was audible. X rays of the larynx showed hypoplasia of laryngeal cartilages (*cartilago thyroidea* and *cartilago cricoidea*) and presence of air in the rostral part of oesophagus. To enhance the efficacy ventilation laser ventriculocordectomy was chosen as a treatment. The intervention was performed in sedation (0,012 mg/kg IV detomidin/ 0,025 mg/kg IV butorphanol) and local anaesthesia of nasal, pharyngeal and laryngeal mucosa (2% lidocain). Before right laser cordectomy and ventriculotomy tracheostomy was performed. Cordectomy was made by two cuts. First cut was performed with endoscope inserted through left nostril. Before second cut nostrils were changed, the endoscope was inserted through right nostril and the grasping forceps was inserted through the left nostril into nasopharynx by the assistant. The vocal ligament was grasped under visual control and second cut was performed. Ventriculotomy was performed consecutively after grasping Morgan ventricle by forceps. We used a 600 µm bare-fibre (25 W). The post-surgical treatment consisted of dexametasone (0,1mg/kg PO) for three days, flunixin-meglumine (1.1 mg/kg IV; PO), sulfadiazin, trimetoprim (25 mg/kg IV; PO) administration for 14 days and rest for one month.

**Results and Conclusion:** The follow up examinations were performed three times during the two weeks long hospitalization after surgery. Control endoscopy revealed a gradual reduction of *rima glottides* edema. After the discharge to home care, owner stated that the mare has no problems during exercise, no stridor is audible. The horse swallowed feed without any problems.

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## P52

### Canine streptococcal mastitis with skin lesion-case report

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**Introduction:** *Streptococcus canis* is opportunistic pathogen that is zoonotic in animals and humans and cause to necrotizing fasciitis which is rapidly progressing, leading to multiple organ failure.

**Material and methods:** A six year old Kurzhaar which is suffering from physical wounds and deep skin lesions on udders is brought to Mehmet Akif Ersoy University Faculty of Veterinary Medicine- Obstetrics and Gynecology Clinic. In physical examination, necrotic wounds and fragility in the epithelial tissue from abdominal and inguinal region of the dog were observed. On the different location of body of the dog, especially at the decubitis location, ulcer like wounds and inflammation were seen. Also milk was stinking and mucoid. The body temperature was 40°C, breathing rate was 29/min and pulsation was 114/min. The swab samples taken from wounds and the milk samples of the dog were sent to Department of the Microbiology. The samples were spread onto 10 % sheep blood agar. Antibiotic susceptibilities of the bacteria isolated were determined by a disc diffusion method on a Mueller Hinton agar plate. The dog which died on the fourth day of hospitalization was sent to Pathology Laboratory of Veterinary Faculty for necropsy.

**Results:** After incubation of the samples, pure culture of *Streptococcus canis* was recovered and identified on the basis of cultural, morphological and biochemical characteristics. *S. canis* was found to be susceptible to amoxicillin, amoxicillin+ clavulanic acid, ampicillin, penicillin, cefoperazone and florfenicol; and to be resistant to trimethoprim+sulphamethoxazole, danofloxacin, enrofloxacin, gentamicin, erythromycin, lincomycin, ciprofloxacin, ceftiofur, and rifamycin. Severe ulceration areas, necrosis and granulation tissue proliferations were observed during the histopathological examination of udder tissue. In the histopathological examination of other tissues, lung edema, neutrophil leukocyte infiltration in heart, bile stasis in liver, hepatocyte degeneration, and mononuclear cell infiltration in kidneys were observed. Patient was diagnosed with necrotic mastitis, septicemia and toxemia considering histopathological lesions.

**Conclusion:** *S. canis* is associated with necrosis, septicemia and toxemia that cause pathologic differentiations in organs and even death.

## P53

### Chronic ehrlichiosis canis in Miniature Pinscher - clinical case

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**Introduction:** Ehrlichia canis is an obligatory intracellular pathogen causing canine ehrlichiosis, potentially fatal disease. Rapid and accurate diagnosis



leads to favorable prognosis. Three stages characterize the disease. The first, acute stage (8–20 days following transmission by infected tick) lasts 2–4 weeks manifested by fever, depression, dyspnoea, anorexia, laboratory findings: thrombocytopenia, leucopenia, mild anaemia. The second stage is subclinical, lasts 40–120 days or even years, in which the patient remains persistently infected without clinical signs except mild thrombocytopenia. The chronic stage is characterized by haemorrhages, epistaxis and edema; the results of laboratory study resemble the first phase of the disease. The course of this phase may often be complicated by superinfections by other microorganisms which can lead to bone marrow hypoplasia with negative prognosis. Dogs infected with *E. canis* remain infected even after antibiotic treatment.

**Material and methods:** Two years old female Miniature pinscher was admitted in the University veterinary hospital in Skopje for emergency cesarean section. Breathing difficulties, nausea and vomiting followed by ocular and nasal discharge, weakness, head ticks and ascites were observed few days after the surgery. Clinical examination revealed low body temperature, tachycardia and tachypnea. Laboratory findings showed severe anemia, thrombocytopenia and renal failure (urea 71.5 mmol/l and creatinine 607  $\mu$ mol/l). Antigen rapid *E. canis* test was positive and patient was treated for the renal failure with intense fluid therapy and vitamins (B12, Vit C) and doxycycline (5mg/kg BID p.o, 3 weeks). Control CBC and serum urea and creatinine were performed one and two weeks after the initial treatment. Two weeks after the treatment, the patient was improving and was discharged with doxycycline treatment for one more week with advices for special low protein diet due to the impaired kidney function.

**Results and Conclusion:** One year later, the patient was admitted again in the hospital with the similar clinical signs as previous (anorexia, nausea and vomiting, bad mouth odor). The owner did not follow diet recommendations. CBC revealed anemia (RBC  $2.821 \times 10^{12}/l$ , PCV 17.6%, Hb 7.8g/dl), serum urea and creatinine were increased (51.1mmol/l and 537.0  $\mu$ mol/l, respectively). Despite the aggressive treatment, one month later, the patient did not respond and human euthanasia was performed. Pathohistology findings showed severe organ damage: hemosiderosis hepatis, nephritis interstitialis gravis partum necroticans, pneumonia interstitialis et oedema, lymphopenia et hemosiderosis gravis lienis. Dogs in the chronic stage of the disease have guarded prognosis due to the multiple organ failure. Prompt and accurate diagnose followed by adequate therapy are crucial for long term prognosis. Recurrence can occur months to years after primary infection. The general conclusion is that relapsing renal failure, despite intensive symptomatic treatment, usually results with “*infaust*” prognosis.

## P54

### Presence of *Dirofilaria repens* at dogs in Belgrade area

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**Introduction:** *Dirofilaria repens* is mosquito-borne zoonotic filaria. It is presence in several regions of the world, including western Balkan, southern Europe, Africa and southern Asia. Parasite live in the subcutaneous tissue of domestic and wild carnivores (dogs, cats, foxes, wolves etc.) and they are the reservoir for these parasites. *D. repens* also causes a zoonotic disease called human dirofilariosis. Humans are accidental “dead end” hosts in which the life cycle is not completed. Many European countries are considered enzootic for this type of zoonosis. In Serbia first occurrence of human ocular dirofilariosis are established 1995. and after that increasing number of human infections are reported.

*D. repens* are smaller than *D. immitis*. The parasite has a complex life cycle, and mosquitoes from the genera *Aedes* and *Culex* serving as intermediate hosts. Adults reside in the subcutaneous connective tissues where females produce larvae (microfilaria) in the natural host organism and release them into the circulation. Like those of *D. immitis*, *D. repens* microfilariae reside in the blood stream. Adult filaria in dogs is usually found during castration and surgical intervention in the abdominal area, in the connective tissue, by subcutaneous tissue and at the omentum. Infections are usually passed asymptomatic. Only in a one case are observed skin changes and lesions.

**Material and methods:** Determination of adult filaria performed by histology studying the morphology after their removal and identified by their thick laminated cuticle, broad lateral ends and large muscle cells. Microfilaria which established using modified Knott test are determined by morphological characteristic (measuring between 350 and 385  $\mu$ m in length and 7 to 8  $\mu$ m in diameter, with a curved tail and rounded cephalic extremity). Our result was confirmed by IDEXX 4DX test.

**Results and Conclusion:** In Belgrade area in period 1996-2005 *D. repens* is found at 5.3% and in period 2006-2009 at 19.2%. The latest research determined the increase of infections to 27.6%. This rapid spread of

*D. repens* can be explained by the global climate change, the high number of mosquitoes in urban settings and their generalist feeding behavior.

## P55

### Squamous cell carcinoma in tongue in a cat with unilateral kidney hypoplasia

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**Introduction:** Squamous cell carcinoma, originated from epidermal keratinocytes and indicating various degree of squamous differentiation is a malignant neoplasm. This tumor is one of the most common oral and skin tumors in cats.

**Material and methods:** Invasive squamous cell carcinoma on the tongue and unilateral renal hypoplasia cases were diagnosed in a 10-year old, Persian cat that brought to the clinic with complaints of eating difficulty were evaluated by clinical and pathological findings.

**Results and Conclusion:** Clinical examination revealed numerous biting wound and ulcers on tongue. The tongue was hard and multiple nodules diagnosed at palpation. Because of the poor prognosis and owners wants the cat euthanatized. At necropsy the tongue was observed to be bitten and torn from various places. The tongue was hard consistency and could not move easily. When the abdominal cavity opened, the right kidney was hypoplastic and an infarct found at the left kidney. Other organs appeared normal. At the histopathological examination of the language, various sized foci that composed of squamous epithelial cells under a layer of tongue epithelium were noted. These cells were anaplastic and pleomorphic appearance and of necrotic cells groups were found in the center of the foci. The tumor cells invades the muscle around the tongue was observed. Hystopathological examination of hypoplastic kidney revealed that cystic dilatation and calcification of tubules. Interstitial connective tissue proliferation and infiltration were found in the interstitial tissue of infarcted area of the other kidney. Degeneration was observed in the liver. This is the first squamous cell cancer case in tongue in a cat with renal hypoplasia in Turkey.

## P56

### Colonotomy in Sharplanina mountain dog - clinical case

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**Introduction:** Intestinal obstructions are the most frequent indication for intestinal surgery in companion animals. Foreign bodies (linear or nonlinear), pyloroantral hypertrophic disease, polyps, intussusception, neoplasia, strangulation, volvulus are the most common causes of bowel obstruction. Solitary foreign bodies usually obstruct the small intestines while retention of feces due to various pathological conditions results with obstipation where surgery is highly indicated. This condition is often diagnosed in older pets with limited activity and inadequate feeding. The clinical signs are variable, ranging from infrequent defecation, straining to defecate with small or no fecal volume, hard and dry feces, hard colon, fecal impaction, anorexia, depression, weight loss and dehydration. Physical examination, laboratory data, plain and contrast radiography are necessary for establishing adequate diagnosis.

**Material and methods:** A five year old female, Sharplanina Mountain dog was admitted at the University Veterinary Hospital within Faculty of Veterinary Medicine in Skopje with symptoms of constipation for almost 10 days. Abdominal pain during palpation, bent position without signs of vomiting was diagnosed during the clinical examination. Plain abdominal radiography and abdominal ultrasonography confirmed colonic constipation. Hematology revealed normal hemogram (RBC=5,86; HCT=34,7; HGB=15,6; WBC=11,9). After unsuccessful treatment with laxatives and warm water enemas, standard celiotomy was performed. Preoperative treatment of thyetilperazinum 0,1 mg/kg, ranitidine 2mg/kg, 0,3mg/kg hioscin-butylbromid along with 20 mg/kg cefuroxime was administrated. Premedication was achieved by i/m administration of 0,02 mg/kg acepromazine maleate, followed by s/c application of 0,28 mg/kg morphine hydrochloride and i/v 0,1mg/kg Diazepam. General anesthesia was achieved by i/v induction of 4mg/kg Propofol and maintained with Isoflurane (1,5-2%).

**Results and Conclusion:** Colonotomy was performed and hard, solid content of grayish color weighting nearly 1,5 kg was removed from the colon. The colon was closed with two layers of inverted sutures using PGA 2/0. Secondary finding of pyometra was unexpected due to non-specific clinical symptoms and additional ovariohysterectomy was performed. The incision line was closed in a standard manner. Intra-operative analgesia was achieved with 1 bolus of 0,25 mg/kg Ketamine. The patient was discharged with adequate postoperative

analgesia and antibiotic treatment along with prescribed diet. Abdominal ultrasound, control radiography and hematology (RBC=4,97; HCT=30.0; HGB=13.0; WBC=14,6) were performed on the 10<sup>th</sup> day after the surgery. The colonic obstruction was diagnosed using clinical investigation and visual imaging; however those findings were not corroborated with laboratory findings. Additionally, pyometra was visually concealed by the present colon enlargement and also not confirmed by hematology data.

## P57

### Polyomavirus and *Macrorhabdus ornithogaster* combination as a cause of high mortality in a commercial budgerigar aviary

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**Introduction:** Polyomavirus infection is a very contagious disease that caused high mortality especially in young budgerigars. *Macrorhabdus ornithogaster* is a yeast organism containing a eukaryotic nucleus. *M. ornithogaster* colonizes the proventriculi and ventriculi of birds and cause mortality.

**Materials and methods:** In a commercial aviary of 360 budgerigars (*Melopsittacus undulatus*) more than 100 chicks died over a 5-month period with clinical symptoms of feather abnormalities, restarted growth, abdominal swelling, vomitus, diarrhea and high mortality. Owner stated that almost all chicks died before their 2 weeks of age. Twenty six of them presented to department of pathology for diagnosis.

**Results:** At necropsy, diarrhea, weakness and feather abnormality were the most common findings. Hemorrhage in proventriculus was observed in most of the birds. Direct microscopy of unstained and Giemsa stained impression smears of proventricular mucosa, revealed numerous *M. ornithogaster* agents. Microscopic examination of the proventriculus revealed numerous agents on the mucosa. At the histopathological examination of the visceral organs large clear or basophilic intranuclear inclusion bodies that characteristic for polyomavirus infection in the kidney, spleen, lung, liver and heart were observed. Avian polyomavirus and *M. ornithogaster* infection was diagnosed based on the clinical and histopathological examination.

**Conclusion:** This study showed that simultaneous infection can cause high mortality than single ones.

## P58

### The effects of different doses of vitamin C supplementation on blood and egg cholesterol

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**Introduction:** Egg yellow contains protein, ash and lipid that consist of triglycerides 65%, phospholipids 28,3 % and cholesterol 5,2 %. American Heart Association recommends cholesterol intake should be limited to 300 mg daily. An egg yellow includes 190-210 mg cholesterol. It is clear that egg is also a great source of nutrients like choline, lutein, vitamin D, calcium etc. Cholesterol is a requirement for cell membrane, growth and many of the hormones. Some researches still continue to try to reduce cholesterol of egg yolk. This study was carried out to determine the effects of layer rations containing 50, 100 and 200 mg/kg vitamin C on blood cholesterol and egg cholesterol.

**Material and methods:** A total of 96 commercial hens (28 weeks of age) were used for observing the effects of vitamin C. There were a control group and three treatment groups (50, 100, 200 mg/kg added to the ration) in each experiment, each containing 24 hens. The experimental period lasted 16 weeks. Serum and egg cholesterol level were determined at six weekly intervals (at 30<sup>th</sup>, 36<sup>th</sup> and 42<sup>th</sup> weeks).

**Results:** Although blood cholesterol level decreased in 30<sup>th</sup> and 36<sup>th</sup> week, this decrease was not statistically different. In 42<sup>th</sup> week, 100 and 200 mg/kg vitamin C supplementation caused a reduction in blood cholesterol ( $p < 0.05$ ). Egg cholesterol decreased in 36<sup>th</sup> week (over 15% in 50 mg/kg and 100 mg/kg vitamin C supplemented group and approximately 25% in 200 mg/kg vitamin C supplemented group) and 42<sup>th</sup> week (around 30% in all groups) ( $p < 0.05$ ).

**Conclusion:** Some researchers reported that excess vitamin C induce to decrease liver cytochrome P450 that detoxifying enzyme and it may have a negative effect on liver cholesterol metabolism. In addition, high doses of vitamin C may cause hyperthyroidism that degrade cholesterol to bile salts. Blood cholesterol may decrease as a result of these processes. However, egg cholesterol may decrease as a result of the reduction of blood cholesterol. High levels of vitamin C may be used for supplying the increasing demands of low-cholesterol eggs.



**P59****First record of *Atractolytocestus huronensis* in open waters in Croatia**

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**Introduction:** For more than ten years Laboratory for Aquaculture and Pathology of Aquatic Organisms has been monitoring the River Kupa basin in Croatia. Fish health status is important part of this monitoring and in the year 2014 a total of 80 fishes (10 fishes from each locality) have been checked for health status during which 12 different parasite species have been found.

**Material and methods:** Native preparations of gills and skin were made and checked for ectoparasites under the light microscope. After dissection, internal organs were macroscopically examined in order to check the presence of endoparasites. The digestive tract was separated from the other organs and different intestinal parasites were visible inside of the different parts of the intestine. Parasites were placed in 70% or 99% ethyl alcohol. For the determination of morphological properties, the individual intestinal parasites were observed under the light microscope and were determined according to the previous description in the literature.

**Results:** Parasitological examination of fish revealed six types of ectoparasites (*Dactylogyrus* sp., *Gyrodactylus* sp., *Trichodina* sp., *Ichthyophthirius multifiliis*, *Epistylis* sp., *Diplozoon* sp.) and six types of endoparasites. Regarding endoparasites parasitological examination revealed two from the Acanthocephala phylum - *Pomphorhynchus laevis* and *Acanthocephalus anguillae*/ *Acanthocephalus* sp.; three from Cestoda class - *Khawia sinensis*, *Atractolytocestus huronensis* and *Triaenophorus nodulosus*, and one from Trematoda class. Each kind of endoparasite was characteristic for the fish host: *K. sinensis* and *A. huronensis* were found in common carp; Acanthocephala in chub, bream, prussian carp, brown trout, danubian roach, bleak and schneider; *T. nodulosus* in pike and trematodes in pike and loach.

**Conclusion:** Finding of *A. huronensis* in common carp is the first record in open waters in Croatia. Investigated parasites could due to the great abundance and other stressful moments have an effect on fish health.

**P60****Development and pathogenicity of *Eimeria dicentrarchi* (Apicomplexa: Eimeriidae) in farmed European sea bass**

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**Introduction:** To our knowledge, so far only 2 coccidia of the genus *Eimeria* have been reported in European sea bass (*Dicentrarchus labrax*), namely *Eimeria dicentrarchi* Daoudi et Marquès, 1987 and *Eimeria bouixi* Daoudi et Marquès, 1987. Originally, *E. dicentrarchi* was described as a species that develops in the pyloric caeca epithelium. Following the first report in France, *E. dicentrarchi* has been reported in several other Mediterranean countries. Although infections are common, little is known about this coccidian species.

**Material and methods:** To gain further insight into its site(s) of infection, development and pathogenicity, the present study analysed 57 European sea bass (16-27 cm long) from a commercial fish farm in the Adriatic Sea for the presence of intestinal coccidia. Fresh preparations of mucus and intestinal scrapings were analysed for infection; when oocysts were found, histology was performed to determine site(s) of infection. Samples of pyloric caeca, anterior intestine and posterior intestine were fixed in 10% neutral buffered formalin. Fixed material was embedded in paraffin and 7 µm serial sections were prepared. Sections were stained with haematoxylin and eosin, toluidine blue and periodic acid-Schiff stain.

**Results:** Oocysts of *E. dicentrarchi* were found in 7 of 57 (12.3%) European sea bass. Most oocysts in mucus and intestinal scrapings were in the sporulated stage, with smaller numbers of unsporulated and semisporulated oocysts also observed. Histological examination revealed *E. dicentrarchi* at different developmental stages in all three sections of digestive tract, though most parasites were found in the anterior intestine. Oocysts were localised in the epithelium and only occasionally beneath the epithelium, in the lamina propria. Most meronts and macrogamonts were located in the basal part of enterocytes, while most microgamonts were in the supranuclear region. No gross pathological changes were detected in any of the European sea bass infected with *E. dicentrarchi*. Most histopathological changes were localised and restricted to individual epithelial cells. Infected epithelial cells were partially destroyed, leading to necrotic changes. The cell membrane of infected cells

was damaged. Release of oocysts into the intestinal lumen was associated with complete destruction of microvilli and rupture of the host cell membrane. Vacuolisation of epithelial cells and detachment of the lamina epithelialis from the lamina propria were occasionally observed in infected areas.

**Conclusion:** The results of this study provide more information on this eimerian species.

## P61

### ***Thelohanellus hovorkai* (Myxosporea) in farmed common carp (*Cyprinus carpio*): two cases from Croatia**

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**Introduction:** *Thelohanellus hovorkai* Akhmerov, 1960 is a non-native myxosporean parasite that was originally described in *Cyprinus carpio haematopterus* in eastern Russia. Today it is widely distributed among European fish farms.

**Material and methods:** This study describes two cases of thelohanellosis caused by *T. hovorkai* in farmed common carp in Croatia.

**Results:** *Thelohanellus* infection was found in a one-year-old common carp collected from a fish farm in Croatia, as well as in a two-year-old common carp imported from Slovenia. The infection was characterised by the presence of whitish plasmodia (2.3-2.9 mm) in mesenteries. The parasite was tentatively identified as *T. hovorkai* based on spore morphology and specific localisation of plasmodia.

**Conclusion:** Import of live common carp into Croatia will likely facilitate the further spread of this highly pathogenic *Thelohanellus* species.

## P62

### **The role of communication in the successfully management of veterinary ambulance**

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**Introduction:** More than human medicine, veterinary medicine is a field that requires the most updated information and scientific technology for success in treating patients. However, it is also an art in which communication and effective listening are vital components necessary for effective care, especially because vets have not communication with patients direct, but with his owners. Veterinarians treat a variety of animals, ranging from dogs and horses to cattle and iguanas. However, with every animal that practitioners treat, they must also communicate with the animal's owner and caregiver.

**Material and methods:** It was done structured inquiry to the clients of university veterinary clinics during the several months, and it was collected more than 50 questionnaires during this time. The purpose of this study was to investigate client perceptions of communication behaviors between client and veterinarians during the veterinary medical interview. Fifty small animal veterinary clients participated, identifying communication behaviors used during the medical encounter and rating their level of satisfaction with various aspects of the medical interview.

**Results and conclusion:** Pets are increasingly being considered a part of the Macedonian family. People are willing to devote substantial financial resources to their care of their pets, which is why small animal private practice is the fastest growing sector of veterinary medicine. As client expectations rise, veterinarians are facing increased pressure to understand and enrich the client-veterinarian relationship. This is especially important for the growing awareness that the major cause of client dissatisfaction is inadequate communication.

Results showed various communication behaviors used during the encounter as well as various behaviors that clients prefer their veterinarian to employ. Stepwise regression was used to show that clients are most satisfied when they have their veterinarians undivided attention as well as when they are provided with practical information about their pet's health. Drawing from uncertainty reduction theory, results show that the employment of effective communication behaviors by both veterinarian and client could result in improved veterinary medical encounters.

**P63****Marketing mix for veterinary services in the Republic of Macedonia**

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In the Republic of Macedonia in past years, the number of graduates from veterinary schools is constantly increasing and thus increasing private practitioner competition for a limited Macedonian market of veterinary services. Competition has also increased from other organizations in the agricultural sector. In same time, reforms in Macedonian legislation, has reduced the traditional mark-up veterinarians can charge for their services, especially on agriculture sector drugs, reducing the profit veterinary clinics obtain from their pharmacy. Furthermore, costs of maintaining a veterinary clinic in the Republic of Macedonia have increased, driving up fees charged for veterinary services, especially those for large animals. In this situation, Macedonian private practitioners are forced to seriously consider applying marketing mix for promotion of veterinary services they are offering. In this paper, we are discussing opportunities for applying marketing mix for veterinary services in the Republic of Macedonia. Main objective is to identify marketing tools that beneficiaries of marketing of veterinary services are considering most relevant when choosing veterinary services providers. This paper attempts to advance knowledge on marketing mix for veterinary services, as well as to address the need of veterinarian practitioners to apply them and to successfully deal with the increased market competition.

**P64****Clinical use of stem cells in veterinary medicine**Ozren Smolec<sup>1\*</sup>, Plamen Trojačanec<sup>2</sup>,  
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Nina Brkljača Bottegaro<sup>1</sup>,  
Damjan Gračner<sup>4</sup>, Marko Pećin<sup>1</sup>, Josip Kos<sup>1</sup>*<sup>1</sup>Clinic of Surgery, Orthopaedics and Ophthalmology, Faculty of Veterinary Medicine University of Zagreb**<sup>2</sup>Clinic of Surgery, Orthopaedics and Ophthalmology, Faculty of Veterinary Medicine-Skopje**Ss. Cyril and Methodius University, Skopje, Macedonia**<sup>3</sup>Department of Surgery, Orthopaedics and Ophthalmology, University of Novi Sad, Faculty of Agriculture**<sup>4</sup>Clinic of Internal Diseases, Faculty of Veterinary Medicine, University of Zagreb*

Stem cells have long been regarded as undifferentiated cells capable of proliferation, self-renewal, production and tissue regeneration. Stem cells can be broadly classified as embryonic or adult, depending on the developmental stage from which they were obtained. Research on stem cells continues to advance knowledge about how an organism develops from a single cell and how healthy cells replace damaged cells in adult organisms.

The application of stem cells that has received the most attention in recent years is as a novel source of cells for cell replacement therapy for the treatment of a wide range of diseases. The clinical use of stem cells in veterinary medicine is clearly in its early stages. Applications of stem cells in the treatment of musculoskeletal pathologies are currently in use in several species.

Adult stem cells have been used for treatment in osteoarthritis, cartilage defects, spinal cord injuries, chronic wounds, ligaments and tissue repair, liver disease and bone defects in animals. Stem cells are very promising to improve animal health due to new techniques.

Small and large animal species serve as valuable models for preclinical evaluation of stem cell applications in human medicine.

In veterinary medicine stem cells are mainly used for treating in equine and canine musculoskeletal diseases. Controlled, well-designed studies are needed to move this new research field forward. Generally, stem cells have a great potential in veterinary clinical usage

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If several authors and institutions are listed, they should be clearly indicated with the department and institution each author is affiliated with. In separate paragraph, address for correspondence, including the name of corresponding author, academic degree, address (institutional affiliation, city, zip-code and country), telephone and fax number, and e-mail address, should be given.

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- Titles should be brief and informative.
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- This is a brief background. It is not necessary to include all of the background literature. Brief reference to the most pertinent data generally is enough to inform readers with findings of others in the field. The specific questions to be addressed the study should also be described.
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1. Bunger, L., & Hill, W. G. (2005). Genetics of body composition and metabolic rate. In E. J. Eisen (Ed.), *The Mouse in Animal Genetics and Breeding Research* (pp. 131–160). London: Imperial College Press.
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