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Highly Pathogenic Avian Influenza (HPAI) Endemicity Study in Indonesia

Technical report

August 2019

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This technical report summarizes results from the study analysis and compilation of the study discussion with experts during endemicity study workshop in Bangkok, Vietnam, Indonesia and Bangladesh in 2015−2019.

Executive summary

H5N1 Highly Pathogenic Avian Influenza (HPAI) has been endemic in Indonesia for more than a decade. Control measures have been taken but the cases are still being reported, indicating a persistence of the virus. However, mechanism of the persistence and factors contributing the H5N1 HPAI endemicity in Indonesia remain unclear. In this study, we identified where the virus is circulating during off-season, including the species affected and enterprises involved, as well as contact patterns between enterprises, also identification of sub-type of the Avian Influenza (AI) virus with virology and molecular sequencing of isolates.

The study design was cross-sectional with survey and sample collection. The samples were taken in two rounds in study area in Purbalingga. This district is representative for other districts in Java where HPAI is endemic.. The first round was conducted between October 2016 and January 2017. The second round was in August to November 2017. The study confirmed that H5N1 HPAI still persist during these months. The virus was found in all poultry enterprises included commercial poultry farms, live bird markets, poultry collector yards, nomadic duck flocks, and poultry backyard flocks in villages. The HPAI H5N1 virus was also detected in all poultry types including layer, broiler, ducks, and kampong chickens. Environmental samples in live bird markets and collector yards also tested positive for the HPAI H5N1 virus. The H5 HPAI virus which circulated was clade 2.3.2.1, while no clade 2.1.3 was detected.

Co-circulation with sub-type H9 clade Y-280 was found in all poultry enterprises. However, there were high percentages of samples which tested positive for Avian Influenza but were H5 and H9 were negative, especially in ducks. This indicates that other H subtypes of the Avian Influenza virus were also circulating in the study area.

Results from social network analysis (SNA) confirmed that live bird markets, poultry collector yards, and poultry commercial farms have higher contact rates and network. This means that these are important enterprises if contaminated with the HPAI virus.

Considering the above findings, recommendation to improve HPAI control in Indonesia are suggested, consisting both of short and long term plans. Short term plans include focus HPAI control in collector yards and live bird markets, continue surveillance for HPAI contamination in live bird markets, test for HxNy, and implement vaccine stewardship in commercial farms. Long term plans include conduct duck study, develop regulation for check point to control poultry movement, perform post-vaccination monitoring in commercial farms, and conduct evaluation to the existing national HPAI surveillance.

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List of acronyms

AI	: Avian influenza
BMKG	: Badan Meterologi Klimatologi dan Geofisika (Meterological, Climatological and Geophysical Agency)
BY	: Backyard poultry
CF	: Commercial farm
CI	: Confident Interval
CY	: Collector yard
DAH	: Directorate of Animal Health
DIC	: Disease Investigation Center
FAO	: Food and Agriculture Organizations
FGS	: Full Genome Sequencing
GIS	: Geographic Information Systems
HPAI	: Highly Pathogenic Avian Influenza
ILRI	: International Livestock Research Institute
LBM	: Live bird market
LPAI	: Low Pathogenic Avian Influenza
ND	: Nomadic duck
OR	: Operational Research
PCR	: Polymerase Chain Reaction
PDSR	: Participatory Disease Surveillance and Response
RT-PCR	: Real-Time Polymerase Chain Reaction
RAP	: Regional and Asia Pacific
SNA	: Social Network Analysis
VTM	: Viral Transport Media

1. Background

H5N1 HPAI outbreaks are highly seasonal in areas of South and South East Asia. The mechanism for this observed seasonality has been associated with weather, poultry product movement, increased demands for poultry products and associated increase in movements due to trade, amongst others (Gilbert, 2008).

In Indonesia, Java has the highest density of both human and poultry populations. This is reflected by the highest incidence rate of outbreaks reported to the Participatory Disease Surveillance and Response (PDSR) database, and Central and West Java have been identified as the hotspots for HPAI (Azhar, 2010).

On Java, the low or off-season for HPAI is the period between July and October when rainfall is low and very few reports of outbreaks are observed. Very little is known about the activity of the H5N1 HPAI virus during this period. Despite a low incidence rate, HPAI cases are still being reported. The recurrent outbreaks that occur in successive high seasons signify that the virus persists during this inter-epidemic period, allowing for sustained transmission of infection between influenza seasons.

Influenza virus circulation is complex, involving the interplay of factors including climate, virus movement, population movements and aspects of population immunity and susceptibility (Farnsworth, 2011). A number of possible hypotheses as to where the virus may be hiding during the off-season and factors contributing to persistence were discussed during an FAO expert meeting in Bangkok in November 2015. Factors considered include type of production system, type of birds, backyard poultry density, use of vaccination, and presence of atypical species (See Table 1). To answer a number of these hypotheses, a study method of cross-sectional field study was conducted. The overall objectives were to determine the drivers of H5N1 HPAI persistence during the off-season or inter-epidemic period in an endemic area in Indonesia. Specific objectives were as follows, to:

1. To determine where H5N1 HPAI is maintained / persists in poultry populations in Indonesia during the low season, and the contact pattern

2. To investigate the co-circulation of HPAI virus with H9 LPAI viruses.
3. To profile and describe the value chain and marketing systems
4. To provide the relevant authorities with information that could be applied to develop recommendations for effective disease control.

Table 1 Hypothesis and method of the study evaluation

Reservoir hypothesis	Methods of evaluation
Atypical species	Map distribution and compare to known virus distribution Rat/rodent trappings in live bird markets (LBMs), Collector yard (CY), and rice fields
Survival in the environment during the summer	Sample in various environments Find evidence of sharing between systems
Wild bird reservoirs	Map distribution of wild and compare to known virus distribution
Traders as links between systems and sectors	Sample traders in endemic areas
LBMs as virus hotels	Sample LBM environment and birds arriving at LBMs
Density-dependent reservoir (chickens, ducks)	Survey of poultry population Sample based on poultry density strata
Metapopulation – interaction between systems	Confirm more than one significant factor Contact structure of various sectors to identify linkages between sectors Link connectivity to virus finding Deterministic model to evaluate the likelihood of persistence in system of interest
Continuous circulation in commercial poultry	Obtain finer resolution population data (layers/broilers) Sample commercial farms to determine levels of virus circulation Improve sensitivity of passive surveillance in unvaccinated flocks Monitor mortality, dead bird testing, sentinels (ducks) Sample farms that vaccinate
Vaccination enables endemicity	Captive control study Sample commercial farms to determine level of virus circulation
Backyard chickens as reservoir	Update backyard population Sample and testing backyard chickens in prevalence areas during low season Participatory search Map linkages between sectors
Ducks as reservoir	Sample ducks for virus Monitor production indicators and mortality

2. Material and Methods

2.1. Study Location

A desk review was carried out to identify the most appropriate study area. Data of HPAI outbreaks between 2008 and 2014 and poultry population statistics were gathered from PDSR database in the Directorate of Animal Health (DAH). Based on HPAI reports, 10 representative districts were selected. From these, the district of Kulon Progo, Purbalingga, Semarang, and Bantul were shortlisted and visited. Purbalingga was eventually selected because it had:

1. Historical occurrence of HPAI H5N1 outbreaks during the off-season as evidence by the PDSR data;
2. Different types of poultry species based on livestock statistics data;
3. A suitable level of commitment from the local animal health authorities, plus personnel in the livestock division who had received training in PDSR by FAO and Operational Research (OR) by International Livestock research Institute (ILRI);
4. Proximity to the Disease Investigation center (DIC) Wates laboratory.

Purbalingga is located in Central Java, consists of 18 sub-districts and 239 villages (See Figure 1). Although the area has sub-administratively more segregated compare to other districts such as Kulon Progo that only has 88 villages, this district is recognized as the second smallest district in Central Java. With this segregation means that there were more animal health personnel in lower administration level. This was a benefit for our study as we included them in our survey implementation. Purbalingga is geographically representative for the rest of Java in general complete with housing complex, low and high lands. It also has urban-like area in the southern-western side and rural area in the eastern and northern part.

Poultry statistics in Purbalingga was around 733,000 layers, 1,894,000 native chickens, and 4,158,000 broilers (Badan Pusat Statistik Propinsi Jawa Tengah, 2017).

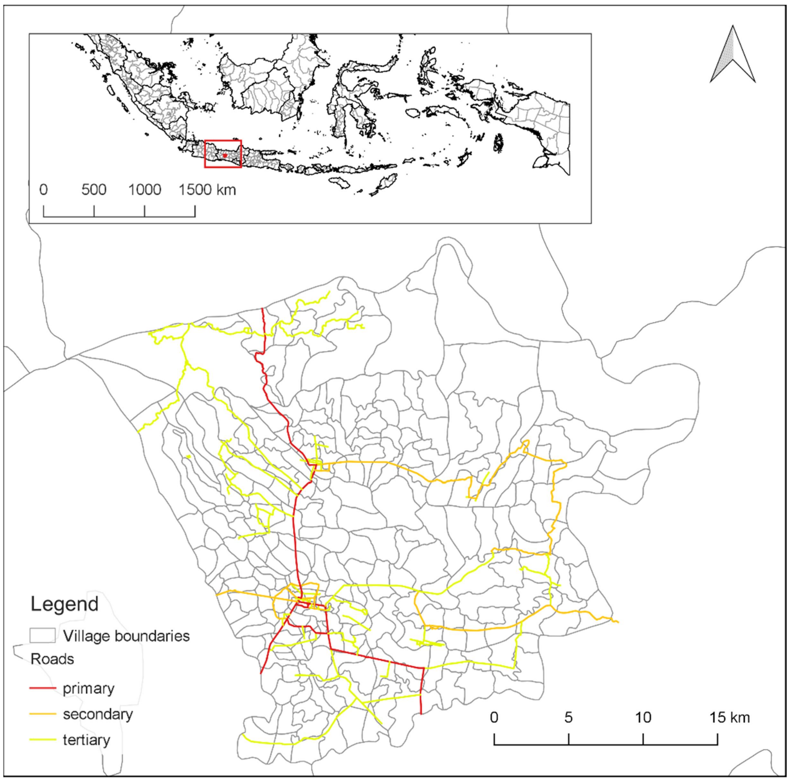


Figure 1 Map of Purbalingga district

2.2. Study design

Although this was designed as a cross-sectional study, it differs from most observational field studies in that it did not aim to obtain a representative sample of population. Rather, the focus was on active detection of field virus in different avian population production systems and enterprises types during what was considered to be lowest period of the year for virus circulation.

The study comprised of the two successive phases:

Phase 1: Characterization of the poultry population and trade and contact networks through the following:

- A census of commercial poultry farms;
- A survey of poultry trading enterprises like live bird markets (LBMs) and collector yards (CYs);
- A survey of nomadic duck flocks;
- A survey of households keeping backyard poultry.

Phase 2: Detection of the virus through

- Active sampling to detect virus:
    - Sampling of commercial poultry farms (layer and duck farms)
    - Sampling of live bird markets and collector yards
    - Sampling of nomadic duck flocks
- Passive reports of all enterprise types in the study area with particular focus on commercial broiler farms and backyard poultry
- Sampling in sick or dead chickens or environment if the sick or dead chickens were disposed, in village through village surveillance. This was only done in study year 2.

2.3. Timeline

Study was intended to be carried out between July and October 2016. However due to operational and logistical complications in a first sampling round in 2016, a second sampling round was added in 2017. See Table 2.

Table 2 Study timeline

Activities	2016				2017				2018		
	Jan-Mar	Apr-Jun	Jul-Sep	Oct-Dec	Jan-Mar	Apr-Jun	Jul-Sep	Oct-Dec	Jan-Mar	Apr-Jun	Jul-Sep
Desk review	■										
Census & Survey		■									
Sampling round 1				■	■						
Sampling round 2							■	■			
Laboratory testing				■	■	■	■	■	■		
Analysis			■	■	■	■	■	■	■	■	
Reporting											■

The census in commercial poultry farms, live bird markets, chicken collectors and the survey in villages was completed in June 2016.

The sampling for detection of AI virus was intended to be conducted in July 2016 but this was delayed to October 2016 due to requirements for obtaining approval for sampling. Subsequently, the sampling was slower than expected due to personnel changes in the local government of Purbalingga, so it extended into February 2017.

2016 was an unusually wet year due to the La Niña[1] effect. As a consequence, the condition of sampling in a low dry season was not met. It was therefore decided to conduct a second round of sampling during the low season of 2017.

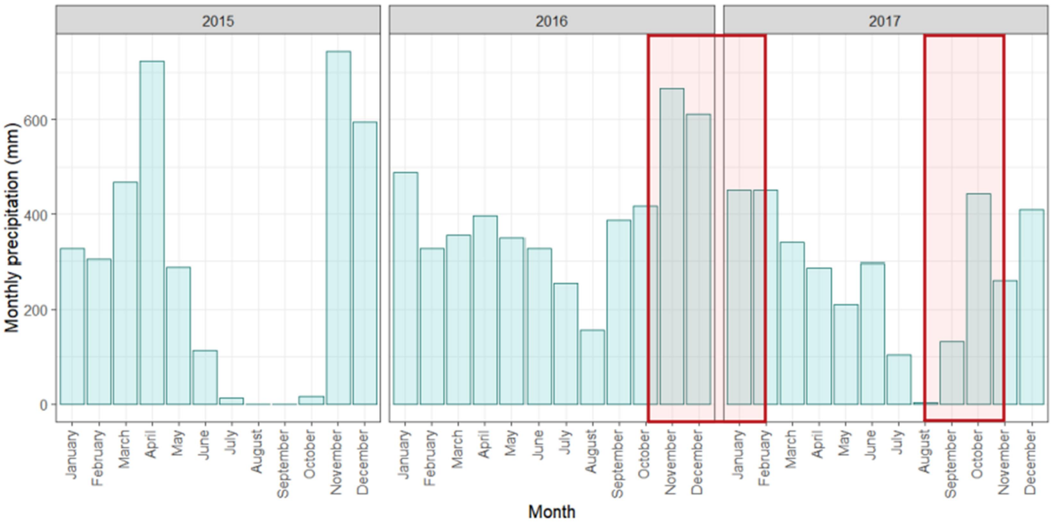


Figure 2 Precipitation data for Purbalingga District, 2015-2017.

See Figure 2, in 2015 was a typical year; 2016 did not have a clear dry season and 2017 was somewhat dryer. The red oblongs reflect the duration of the two survey rounds.

[1] http://asmc.asean.org/asmc-el-nino/

3. Implementation

3.1. Phase 1: Characterization of the poultry production system and value chain

Prior to Phase 1, data were obtained on the location and number of commercial farms (CFs), CYs and LBMs. 204 CFs, 129 CYs and 18 LBMs were identified. Detailed information about poultry enterprises is described in Table 3.

Table 3 Details of poultry enterprises in Purbalingga District

Enterprise type	No. (units)	No. (birds)	Median (Min - Max)
Commercial poultry farm	204	949	2.900 (0 – 70,000)
Poultry collector yard	129	23	40 (5-4.000)
Live bird market	18	11	(10-9,000)
Nomadic duck	20	9	175(13-500)

All these locations were visited and questionnaires were conducted by 22 hired enumerators and 25 Agricultural District Officers from Purbalingga.

Visits to all 239 villages were conducted; as there was no list of households, these were randomly selected. A systematic questionnaire survey was conducted by visiting to a minimum of 54 houses in each village. In total 14,368 households were interviewed.

All data were entered into database by the enumerators, casual data encoders and national data encoder in FAO.

3.2. Phase 2: Active sampling to detect AI virus

Details of the sample size calculations are given in Appendices.

3.2.1. Sampling schedule

There were 204 poultry commercial farms surveyed and 81% (166) were willing to participate in the sampling phase. In terms of selection unit, we selected 32 commercial farms and ensured that all ducks farms were included. For collector yards and LBMs, 41 collector yards were selected randomly and all 18 live bird markets were included. Sampling was conducted by 25 animal health services in the Agriculture District Office in Purbalingga. Information on schedule visit is presented in Table 4.

Detail of study implementation in first study year per location category and frequency of sampling and unit of sampling is summarized in Table 5.

Table 4 Sampling units and schedule round-1

Location	No of unit	Aug-16	Sep-16	Oct-16
Commercial farm	32	1x	-	-
Live bird market	18	1x	1x	1x
Collector yard	41	1x	1x	1x
Nomadic duck	30 ducks/flock	During study period		
Passive reports	Only in sick/dead birds	During study period		

Table 5 Implementation of the HPAI endemicity study round-1

Location	No of unit	Oct 2016 – Feb 2017
Commercial farm	31 (1 farm was double sampling)	1x
Live bird market	18	3x
Collector yard	42 (1 extra collector yard)	3x
Nomadic duck	12 ducks/flock	sample 20 flocks
Passive reports	Only in sick/dead birds	sample 7 locations

Nomadic ducks are usually found in rice fields during post-harvesting seasons. However, we only obtained data from 20 nomadic duck flocks during the census in round 1. Survey and sampling nomadic ducks was not part of the initial design, but a limited number of nomadic duck flocks were sampled in the 1st round. As the enumerators were not given a proper training on survey in nomadic duck and duck-handling technique, they only managed to sample 12 ducks per flocks, less than a half from the number (30 ducks) we expected.

The second year sampling started in August 2017. Samples were collected from the same locations but with a higher frequency (See Table 6). Live bird markets and collector yards were sampled 4 times in 4 months, which was only 3 times in the first round. Sampling in commercial farms was conducted twice, which was only once in the first round. In round 2, nomadic duck flocks were sampled once over a longer period between August and November 2017. As results in this we managed to collect more samples from nomadic duck farms. In total 196 flocks and 30 ducks per flock pooled in 5 pools were collected. For commercial farms, data regarding farming characteristics, biosecurity, and health and vaccination status were recorded.

To increase the chance of detecting HPAI virus in backyard chickens in second round, a structured village survey was conducted. For the design we used 5% prevalence, an absolute error of 3% and a 95% confidence interval. Using randomly selection, 187 out of total 239 villages in Purbalingga were visited and 54 households who kept chickens were interviewed. In the interview, villagers were asked about HPAI compatible events in their household and immediate neighbors (snowballing sampling). Then if the sick or dead birds were identified, swab samples from the birds were taken. If carcasses were not available, environment swabs were then collected. In these visits, poultry data were recorded.

Details of number of units surveyed are described in Table 6 and Table 7.

Table 6 Sampling units and schedule round-2

Location	No of unit	Aug-17	Sep-17	Oct-17	Nov-17
Commercial farm	32 (same farms as designed study year 1)	1x	-	-	1x
Live bird market	18	1x	1x	1x	1x
Collector yard	41 (same collector as designed study y1)	1x	1x	1x	1x
Backyard/villages	Only in sick/dead bird	1x during study period			
Nomadic duck	30 ducks/flock	1x during study period			
Passive reports	Only in sick/dead bird	1x during study period			

Table 7 Implementation of the HPAI endemicity study round-2

Location	No of unit	Aug-17	Sep-17	Oct-17	Nov-17
Commercial farm	33 (5 diff farms from study y-1)	1x	-	-	1x
Live bird market	18	1x	1x	1x	1x
Collector yard	41 (except 1 CY was sampled in 4th Dec)	1x	1x	1x	1x
Backyard /villages	Only in sick/dead bird	Sample 127 households			
Nomadic duck	30 ducks/flock	Sample 196 flocks			
Passive reports	Sample was also collected from environment when no bird was found	sample 4 locations			

3.2.2. Sampling protocol

The type of sample in each unit is depending on the locations. In commercial farms and nomadic duck flocks, the swab sample was taken from birds. We took environment samples in collector yards and live bird markets. However if the enumerator found any sick or dead birds in these enterprises, rapid testing was performed and swab samples were also collected from these birds to be further tested in laboratory. Passive reports were also followed-up and samples were collected from sick or dead birds. This included rapid testing and swab samples collection. Environment samples were also taken if carcasses not present

Table 8 Type of samples taken

Enterprise type	Type of samples
Commercial farms	Live birds (oropharyngeal swab)
Nomadic duck flocks	Live birds (oropharyngeal swab)
Collector yards	Environment; and sick/dead birds (if any)
Live bird markets	Environment; and sick/dead birds (if any)

3.2.3. Sample testing

Laboratory testing was conducted by DIC in Wates and Subang. Each pooled sample was tested using Real-Time Polymerase Chain Reaction (RT-PCR) to detect Influenza-A viruses using primer specific to matrix gene and if positive continued for H5 test. We run additional testing for RT-PCR testing for H9 and performed Full Genome Sequencing (FGS) on virus isolates in DIC Wates.

3.3. Data analysis

Exploratory analysis was performed to identify and visualize pertinent features of the data. Descriptive analysis was done to summarize and identify trends. Hypothesis testing was subsequently performed where possible to determine statistical strengths of association.

Social Network Analysis (SNA) was performed to explore linkages between village poultry and different enterprise types, and to investigate movements and contacts.

Given the study design, the scope for developing statistical models to investigate strengths of association with risk factors was limited. However, the study data will be useful to inform the design and help to parameterize predictive transmission models. This is out of scope for the current work.

3.3.1. Descriptive analysis

Utilizing the coordinate data of all interviews during the Phase 1 questionnaire work, some exploratory spatial analysis could be performed to visualize locations of the different enterprise types, as well as the density of the backyard poultry population. Population distributions of the different enterprise types could also be visualized using histograms and boxplots.

SNA visualize and estimated various network parameters between the enterprise types.

The Polymerase Chain Reaction (PCR) test results of the samples taken during both rounds of Phase 2 were summarized in tabular format and displayed visually. Trends and patterns related to potential seasonality and differences in virus distribution between the enterprise types were similarly visualized. Summary measures of frequency of AI virus detection were calculated.

3.3.2. Hypothesis testing

The frequency (number or counts) of Matrix and H5 PCR positive test results could be expressed as a proportion of the total numbers of sample tested, for both of the sampling rounds and stratified by enterprise type. As the Matrix positive results signify the presence of the undifferentiated AI virus, it was assumed that the difference between the Matrix positive results and the H5 positive subset of these represented Low Pathogenic Avian Influenza (LPAI) positive samples.

Using these different levels, statistical testing for the differences between multiple proportions was then carried out. Pearson's Chi-square test of independence is appropriate to use the identified sample proportions in count data to test hypotheses about the unobserved population proportions. For i counts the observed frequencies, O are used to calculate the expected frequencies, E, and this is expressed as the Chi-square statistic:

$$\chi^2 = \sum \frac{(O_i - E_i)^2}{E_i}$$

The statistical probability of obtaining this statistic is then returned. Hence, this test assesses whether the variables (sampling round and / or enterprise types) are associated or not; the null hypothesis is that this is not the case, i.e. the variables are independent.

This test has a number of assumptions, specified by McHugh (2013) as follows:

1. The study groups must be independent, i.e. the data should not consist of paired samples. In practice this implies that the data were obtained through random selection.
2. The data in the cells should be frequencies or counts rather than percentages or some other transformation of the data.
3. The levels (or categories) of the variables are mutually exclusive.
4. Each subject contributes data only one cell in the χ^2.
5. The variables are measured as categories, usually at the nominal or ordinal level. While there is no limit to the number of cells (i.e. the table can have any number of variables and levels), a number over 20 can make it difficult to meet the assumption below, and to interpret the meaning of the results.
6. The values of the cell expected frequencies should be 5 or more in at least 80% of the cells, and no cell should have an expected of less than one. Essentially, this is related to the sample size.

All but the final assumption are met. Aggregation of the data (e.g. by collapsing enterprise type) may allow the assumptions to be met, but limit the scope for hypothesis testing. However, where the assumptions for this test are not met, the Fisher's Exact test can be used instead.

This enabled various hypotheses to be formally tested. These are defined in Table 9. Contingency tables were specified in the R statistical software, and all hypothesis tests carried out.

Table 9 Definitions of null hypotheses, data to test these and hypothesis tests used

Null hypothesis	Data	Test
1. There is no difference in the **numbers of AI viruses detected in the first and second sampling round, within enterprise types.** As these rounds represent a wet and dry (low) season, this approximates the hypothesis that there is no seasonality in the detection of AI viruses.	Counts of AI negative, LPAI positive and H5 positive PCR test results from the first and second round, stratified by enterprise type.	Fisher's Exact test.
2. There is no difference in the **numbers of H5 viruses detected in the first and second sampling round**. As these rounds represent a wet and dry season, this approximates the hypothesis that there is no seasonality in detection of H5 viruses.	Aggregated counts of H5 negative and H5 positive PCR test results from the first and second round.	Pearson's Chi-square test of independence.
3. There is no difference in the **numbers of H5 viruses detected in the different enterprise types.**	Counts and H5 negative and H5 positive PCR test results from the second round, stratifies by enterprise type.	Fisher's Exact test – overall (single statistic) as well as pairwise comparisons.

3.3.3. Regression analysis

Using the PCR test result as the outcome, logistic regression (binomial if only the H5 result is considered, multinomial if the differentiation between LPAI and H5 is observed) can be performed. Explanatory variables may include the sampling round, enterprise type and additional variables that could be identified. This has not yet been performed, and extreme caution is recommended given the constraints in the study design, which introduce sources of error and bias which may invalidate the outputs of such models.

4. Results

4.1. Phase 1: Characterization of the poultry production system and value chains

4.1.1. Household poultry

Survey in 239 villages with interviewing respondent in at least 54 households per village was conducted. At this survey, of 14,368 households (HH) had been visited, there were 60% houses kept poultry. Figure 4 presents relevant spatial features of the poultry demographics and enterprise types included in the study. Proportion of households keeping chicken per village is spatially described in Figure 3. Number of poultry kept described in Figure 5.

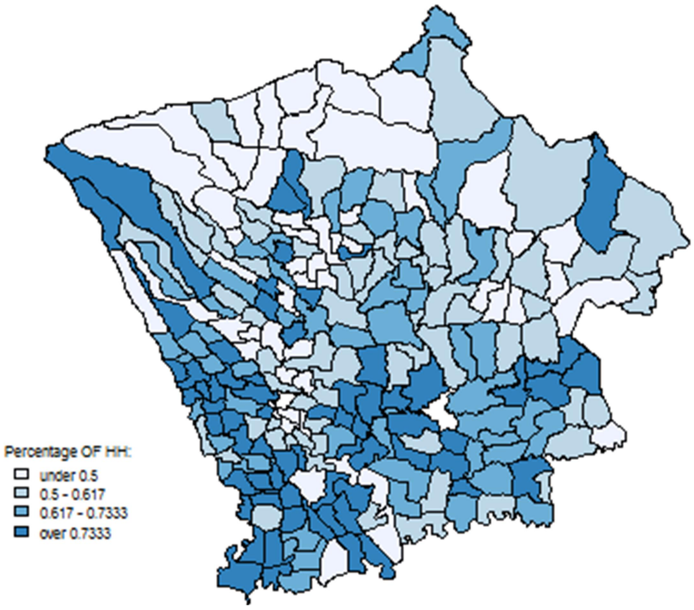


Figure 3 Proportion of households kept poultry by village

Table 10 shows type of poultry kept by households. In this study also revealed that from 14,368 respondents, 6% confined their chicken all day, 14% confined and releases at home's yard, 34% confined and released outside home's yard, 8% fully unconfined, and 38% did not answer.

Table 10 Types of poultry kept by households

Type of poultry	Percentage	average (birds)
Kampong chicken	86	8
Duck	5	17
Muscovy duck	15	7
Song bird	13	4

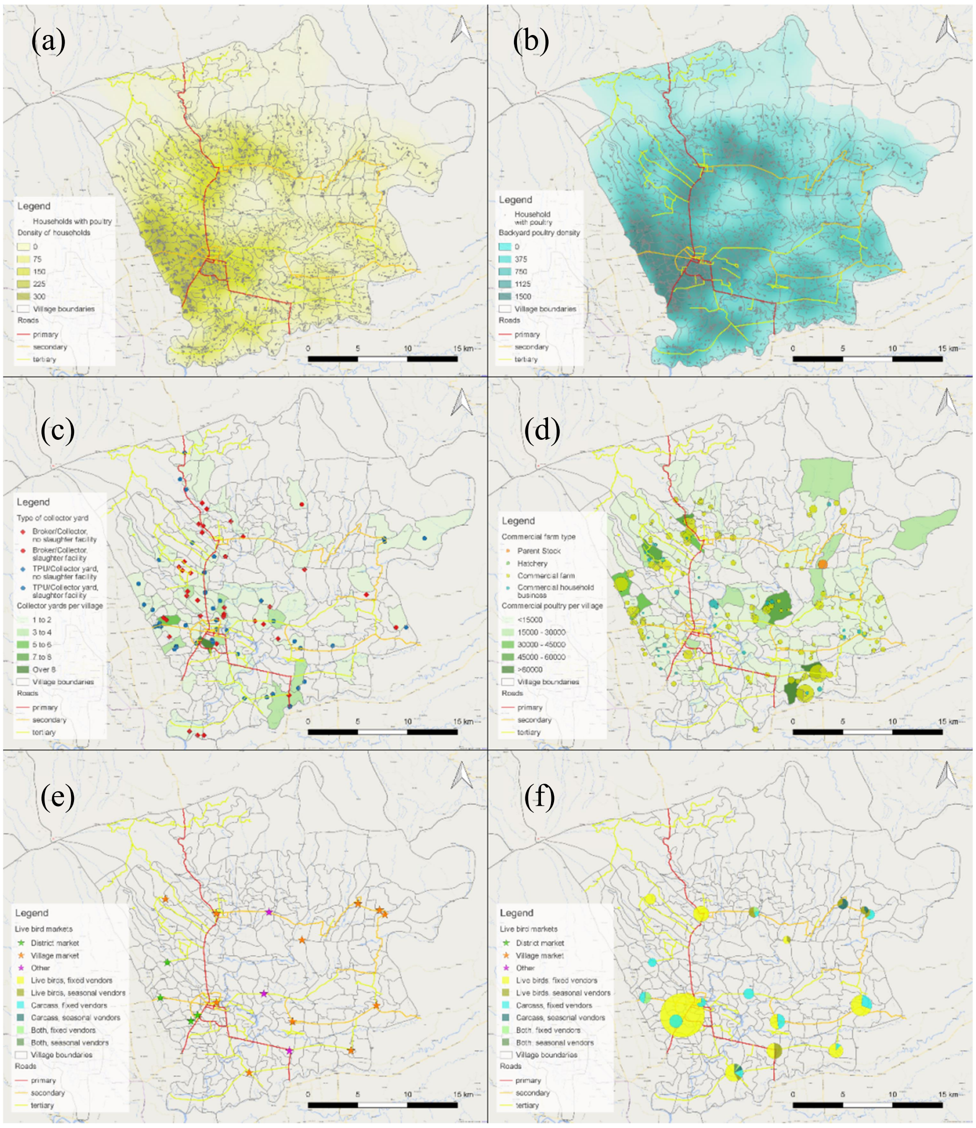
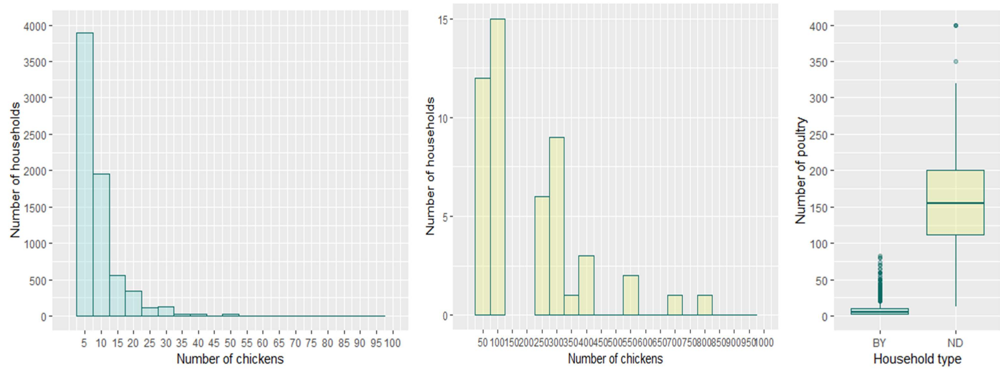


Figure 4 Maps showing pertinent demographics and features of Purbalingga District.

Figure above showed (a) location and density of surveyed households with poultry (n=8,789); (b) density of backyard poultry; (c) locations of collector yards (n=129), stratified by type, with number per village; (d) locations of commercial farms (n=204), stratified by type and scaled by size, with number of poultry per village; (e) locations of live bird markets (n=18) stratifies by type and (f) by production type, scaled by volume of sales.



(a) Backyard poultry **(b) Nomadic ducks** **(c) Boxplots**

Figure 5 Poultry population distribution in the village

Of the 8,925 respondents who kept poultry, almost half kept poultry for sale, a quarter kept chickens as pet and personal consumption. Few kept chickens as hobby. See details in Table 11.

Table 11 Main reason of the villagers keeping poultry

Main reason for keeping poultry	Number (%) of households	Number of birds kept		
		Total	Median	Mean
Hobby	714 (8.0%)	4,002	4	5.6
As a pet	2,114 (23.7%)	12,726	5	6.0
Sale	3,866 (43.3%)	35,044	7	9.1
Personal consumption	2,215 (24.8%)	15,191	5	6.9
Overall	8,925 (100.0%)	67,043	5	7.5

There were only 34 (0.002%) households that had their chicken vaccinated which included AI, ND and other non-specify vaccination. The population of vaccinated poultry was 1 to 22 birds and the types of vaccinated poultry were kampong chicken, duck, and song birds.

4.1.2. Commercial farms

There were 204 commercial farms identified and data on characteristics of farms were recorded. We included data of commercial farms with farm capacity more than 100 birds so commercial poultry in households that raise more than 100 kampong chickens were also included.

Maximum farm population of layer chickens in Purbalingga was 80,000 and broiler chickens 70,000. Descriptive analysis of current population is described in histogram in Figure 6; at the time of interview, a number of these farms were not stocked to capacity. Details of species in commercial farms are described in Table 12 and Figure 4 (d).

These 204 commercial farms were located in 15 out of 18 sub-districts and 97 out of 239 villages in Purbalingga which also include 1 hatchery and 1 parent stock.

Table 12 Farm population and capacity by species

Species	unit	Current population			Farm capacity		
		Average	Min	Max	Average	Min	Max
Broiler	125	5,093	0	70,000	5,992	100	70,000
Layer	49	5,063	194	45,000	7,420	200	80,000
Duck	11	472	4	3,000	584	4	4,000
DOC	1	7,000	7,000	7,000	7,000	7,000	7,000
Quail	11	1,248	185	3,000	1,550	200	5,000
Mix	7	1,969	0	4,500	3,469	1,000	6,000

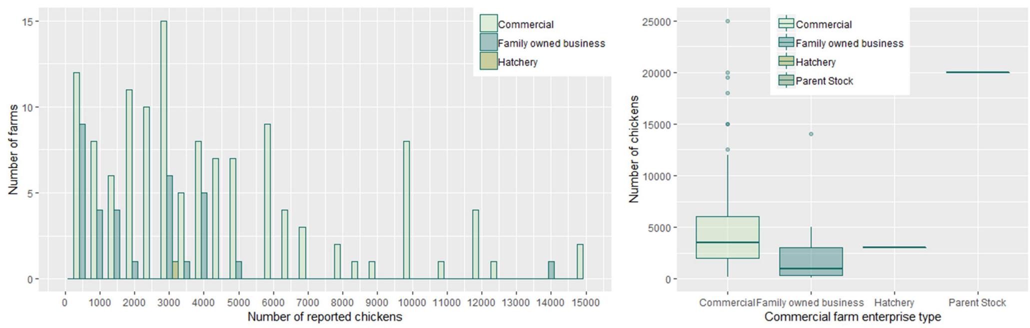


Figure 6 Poultry population distribution in commercial enterprises

Based on commercial poultry ownership, there were 3 categories identified that include partnership (56.4%), independent business (42.2%) and run by company (1.5%). Many farmers conducted vaccination (93.6%), but the detail information on the type of vaccine was not recorded. See Table 13.

Before sampling, we asked respondents if they are willing to participate in the sampling phase and allowed us to sample their chickens. As answer, there were 166 (81%) farmers willing to participate in the sampling session, but 31 farmers (15%) declined, and 7 poultry farmers were (3%) unsure. We only included all farmers who were willing to participate in the sampling phase.

Table 13 Status, business type, and vaccination in commercial poultry farms in Purbalingga

Variable	number	%
Status:		
Independent	86	42.2
Partnership	115	56.4
Company	3	1.5
Total	204	100
Business type:		
Parent Stock	1	0.5
Hatchery	1	0.5
Mid-large scale	161	78.9
Small scale	41	20.1
Total	204	100
Vaccine use (vaccine type is not specified):		
Yes	191	93.6
No	13	6.4
Total	204	100

4.1.3. Live bird markets

We identified 18 LBMs and 67 poultry traders in 13 sub-districts of the Purbalingga. See the map in Figure 4 (e) and (f).

Many live bird markets were located in 4 sub-districts, including Klagung (16.4%), Panican (13.4%), Kutawis (11.9%) and Bukateja (10.4%). Of the 18 markets, 61.1 % (11 of 18) were categorized as village markets, 22.2 % (4 of 18) as district markets and 16.7 % (3 of 18) as other type of live bird markets.

More than half of live bird markets in Purbalingga operates daily (55.6%, 10 out of 18). Some markets are only open 2 (22.2%) or 3 (5.6%) days a week and the others (16.6%) operate at specific days based on Javanese and Islamic calendar.

The percentage of markets selling live chickens was 62.7 %, while 32 % sold carcasses and 4.5 % sold both live chickens and carcasses. Most markets were normally visited by 10 to 30 buyers each week (data not shown). Some of these traders (52%) also sold chickens in other live bird markets.

Table 14 Type and day operation of live bird market in Purbalingga district

Variable (n=18)	No of LBM	%
Type of LBMs:		
Village market	11	61.1
District market	4	22.2
Other	3	16.7
Days operation		
2 days a week	4	22.2
3 days a week	1	5.6
Everyday	10	55.6
Other	3	16.7

Respondents were asked about poultry death (or mortality) over last 12 months. Only one market had the mortality case and the carcass was thrown in fish pond.

Table 15 Number of trader selling poultry and poultry products in live bird market per day

Product sold	No of traders		
	Mean	Min	Max
Live poultry			
Regular	21	0	160
Non-regular	3	0	10
Carcass			
Regular	6	0	14
Non-regular	1	0	6
Live poultry and carcass			
Regular	2	0	6
Non-regular	0	0	2

Traders in live bird markets

Following the census in LBM, we interviewed 67 traders from all 17 live bird markets in Purbalingga. This number was less than number of traders identified on the census of live bird markets because some of the traders interviewed during the census in live bird markets were actually the same person.

We identified trading patterns using descriptive analysis of LBM's connectivity. Based on the data it showed that traders operated in more than one places or live bird markets. There were 33 traders who sold chickens in more than one live bird markets. The description is shown in Figure 7.

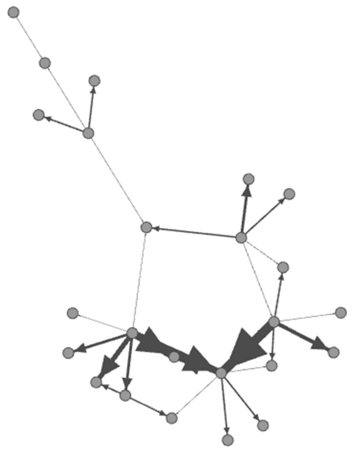


Figure 7 Connectivity of poultry trade between live bird markets

Out of the 67 traders, 42 sold live chickens and 25 sold carcasses. Detail of amount of product sold by the 67 traders is presented in Table 16.

Table 16 Detail of poultry sold by poultry traders

Poultry sold (n=67)	No of traders	Current amount			Daily in normal day		
		Average	Min	Max	Average	Min	Max
Live poultry (birds)	42	19	3	60	22	5	50
Carcass (kg)	25	41	3	150	35	8	150

It shows that out of 67poultry traders, 16 had poultry sold every day, and 51 did not complete selling poultry in one day. For the unsold live chickens, 45 traders brought them home and the other sold it to other markets and/or slaughtered.

Out of the 67 traders, 15 (22.4%) worked in 6 live bird markets that has slaughtering facilities. Also out of 15 traders, 11 (73.3%) slaughtered the chickens outside the market while the others slaughtered the chickens inside the markets.

We recorded data of average buyers visiting chicken traders. Fifty traders (74.63%) were visited by 10-30 buyers in a day, 11 traders (16.42%) had less than 10 buyers a day, 2 traders (2.99%) had 30-50 buyers, and 4 traders (5.97%) has more than 50 buyers. See detail in Table 17.

Table 17 Average number of buyers

No of buyers	No of traders	%
<10	11	16.42
10-30	50	74.63
30-50	2	2.99
>50	4	5.97
Total	67	100

4.1.4. Collector yards

A total of 129 collector yards were interviewed in 17 sub-districts and 70 villages in Purbalingga. Many of the collectors operated in Kalimanah (22%) and Bojongsari sub-district (13.1%). Of the 129, 58 (45 %) collectors have poultry slaughtering facilities and 71 (55%) did not provide slaughtering facility. Most poultry collector operate daily (111 collectors, 86%) and some (4 collectors, 3.1%) open business only 2-3 times a week, while the other (14 collector, 10.9%) have working days based on Javanese and Islamic calendar.

The species that were traded by collectors were mainly kampong chicken; spent layer and broiler (see Table 18). There were 15 collectors specialized in more than one poultry species.

Table 18 Number of poultry trade by collectors

Species	No of collector	Current number			Capacity		
		Mean	Min	Max	Mean	Min	Max
Kampong chicken	72	37	1	600	128	5	3,000
Spent layer	2	40	30	50	2,050	100	4,000
Broiler	54	81	0	1,000	178	10	1,500
Duck	6	4	2	8	16	5	30
DOC	2	35	35	35	55	50	60
Fighting cock	21	2	2	2	10	10	10
Muscovy duck	27	5	1	13	14	5	30
Song bird	1	3	3	3	5	5	5

We collected movement data of the poultry collector yards. Based on the data it was identified that the collectors supplied chickens from places around Purbalingga (82%) and outside (18%) not far from the district. See Figure 8.

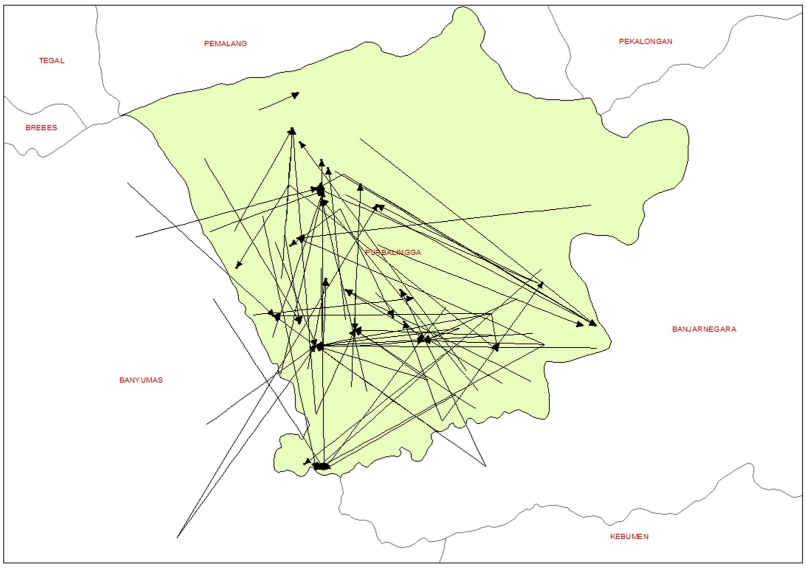


Figure 8 Poultry source traded by collector yards (arrow showing direction)

We also recorded where the poultry were sent by the collectors. Data shows that the collectors sent the poultry mainly to villages in Purbalingga (83%), and the rest to outside the district but still nearby Purbalingga (16%) and outside the Jawa Tengah province (1%). The farthest destinations were Jakarta. See Figure 9.

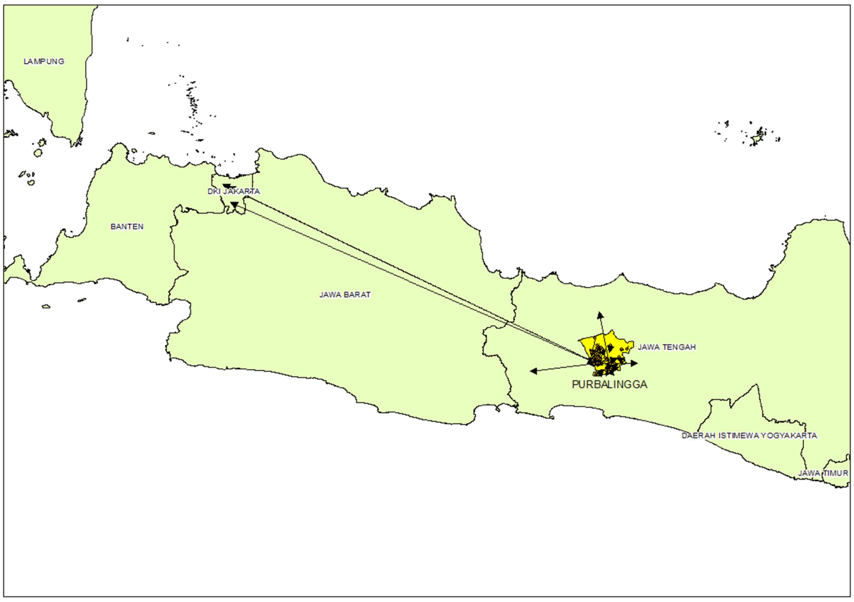


Figure 9 Poultry destination traded by poultry collectors

Number of live poultry traded per day changes over-time depending on factors for example festival and market change. Data regarding location and poultry traded over last 12 months were recorded. We had 178 movement data from 101 respondents. Numbers of delivery per week and poultry size were estimated with maximum, average, and minimum number. See Table 19

Table 19 Univariate analysis number of poultry traded per week from Purbalingga

Size	n	Mean	S.D.	Quantiles			
				Min	0.25	0.75	Max
Maximum	178	296	522.83	0	40	350	3500
Average	178	150	264.87	0	12	175	2310
Minimum	178	85	207.17	0	0	60	1750

Poultry were traded from various locations including village, farm, market, slaughter house, and collector and different poultry species. See detail in Figure 10- Figure 13. It shows that collectors of spent layer were few but they traded it in large numbers. In this data also shows that collector mainly received poultry from slaughter house. The slaughter house was meant Segamas market that has been well-known as the largest live market for poultry and cattle in Purbalingga and center for poultry slaughter houses. Many collectors stand-by at this location.

The graph also shows that poultry collectors mainly sold poultry to farms. This might be ambivalent if poultry farm stock the day old chicks from poultry shop, breeder, or other farms; or it might be true if farms supplied chicks from them but through middleman or broker. In the survey, brokers were included in collector yards category. So in this case collector or broker was only as an indirect actor. See that also data in Table 18 that there were high number of collector for broiler and kampong chickens which possibly brokers for farms.

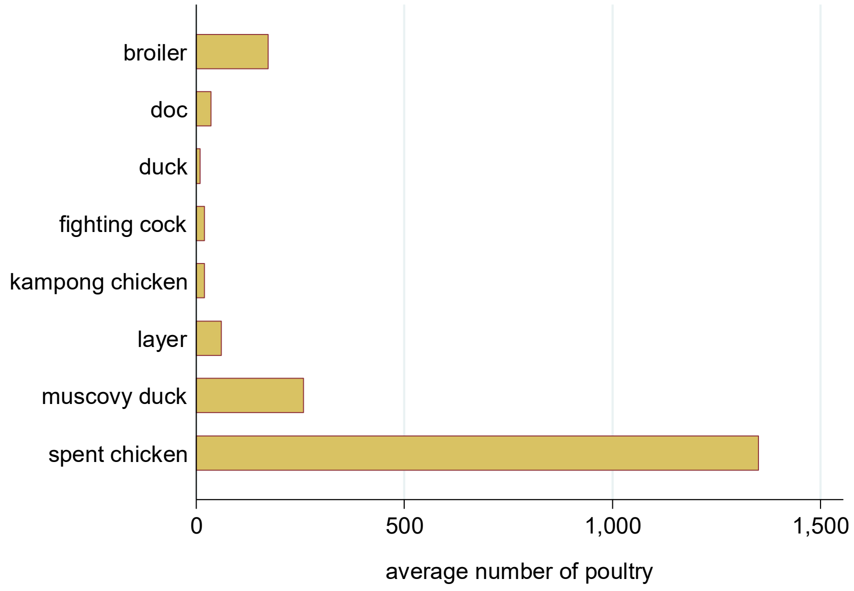


Figure 10 Poultry species delivered to collector (origin)

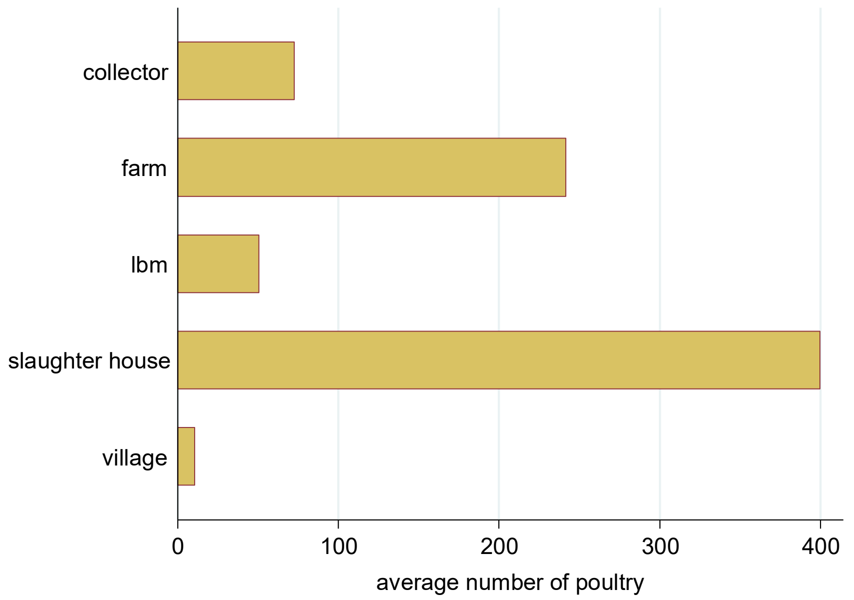


Figure 11 Poultry source delivered to collector (origin)

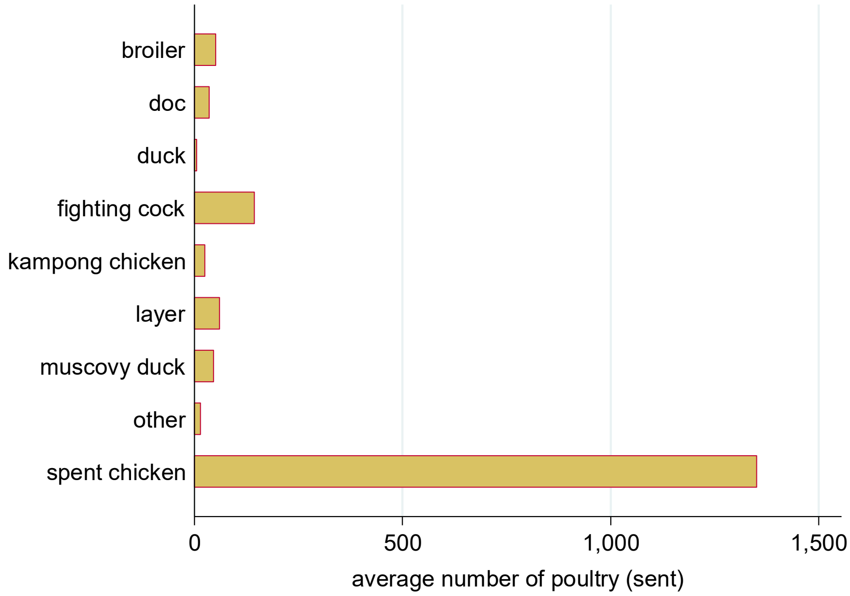


Figure 12 Poultry species sold by the collector (destination)

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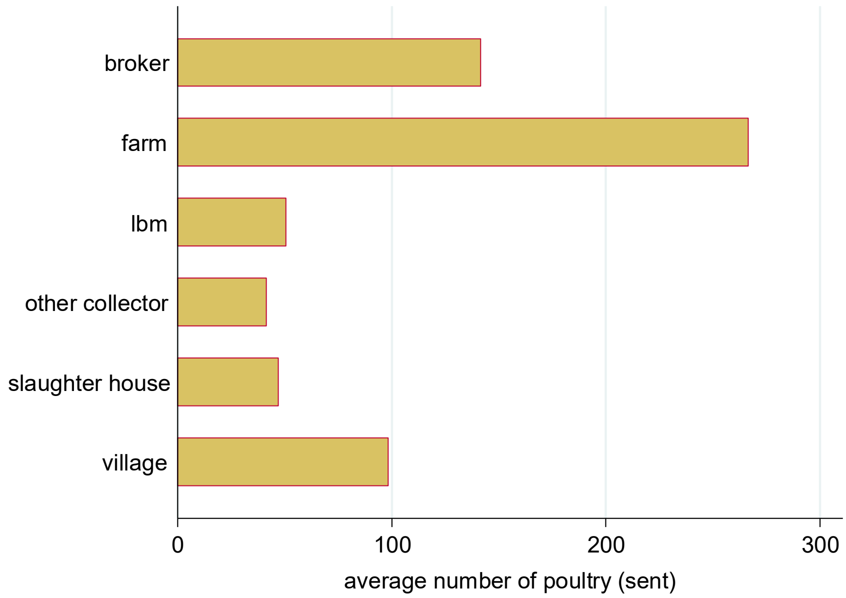

Figure 13 Poultry destination by the collector

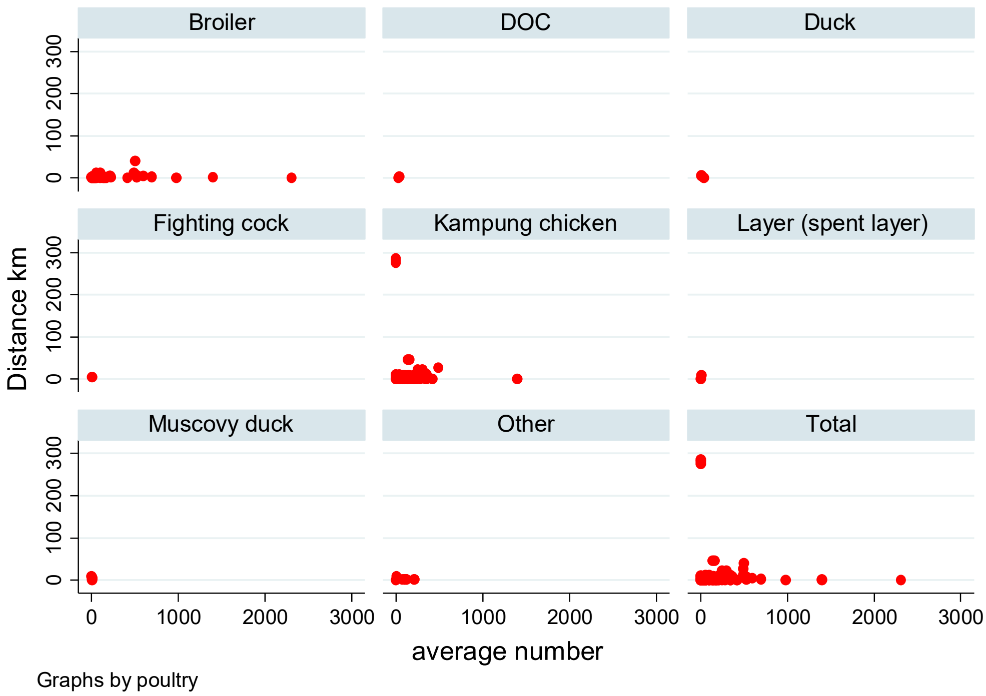

Figure 14 Estimated distance (km) and average number of poultry traded per week from Purbalingga

Graph above illustrates average number of poultry traded per week and the distance in kilometers (km). However there were some missing data regarding location. Mainly respondents gave information on province and district name of where the poultry were traded. So the distance was calculated at least using district location if sub-district or villages name was not disclosed.

Overall the graphs show that there was no correlation between numbers of poultry sold and poultry destination.

We also recorded historical events of poultry mortality in collector yards over the last 12 months. Figure 15 shows sum of mortality and number of reports. There is slight similar trend to mortality in backyard flocks which peak in May or June. However this data might contain recall bias.

Special for collector yard, data of survey that consisted poultry mortality event was not fully recorded. This was due to respondents complained and refused for the repeated interview. They still allowed us to take samples.

We also investigated risk factors for confirmed AI and H5 test with simple contingency 2x2 tables of odd ratio (OR) as presented in Table 20 and Table 21. The results of analysis suggest that CYs with slaughter facility as well as selling broiler were factors that correlated to the HPAI infection (OR ≥1).

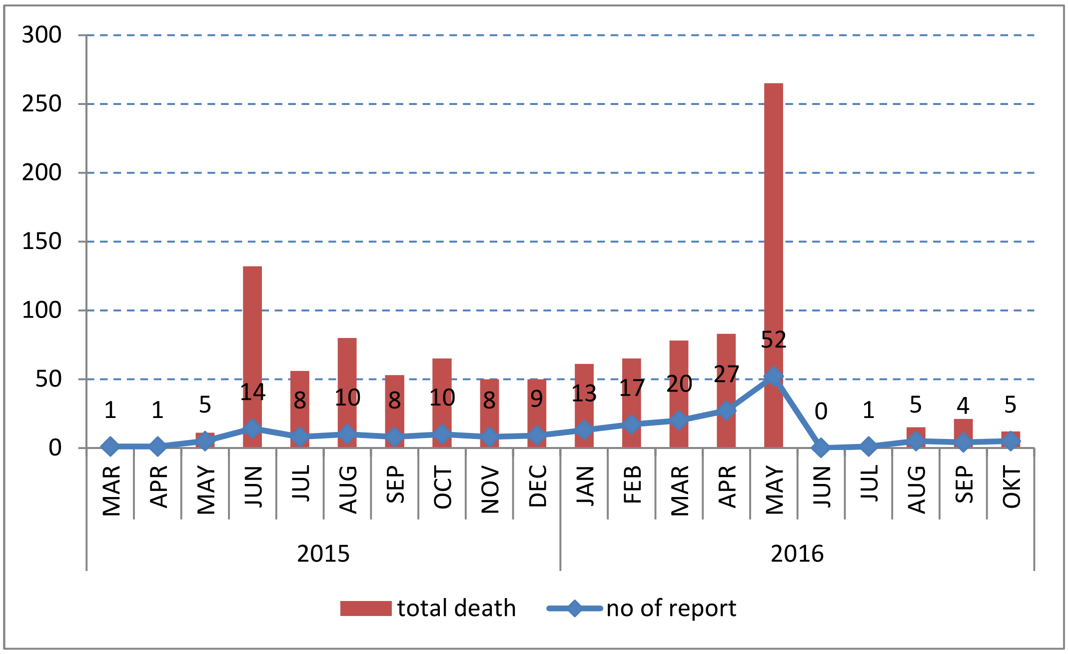

Figure 15 Historical cases of poultry mortality in collector yards

Table 20 Odd ratio for Avian Influenza (Matrix pos) in CYs

Test	slaughter facility		sell broiler	
	yes	no	yes	no
Matrix positive	52	19	47	24
Matrix negative	268	268	261	275
Odd ratio	2.73		1.19	

Table 21 Odd ratio for H5 in CYs

Test	slaughter facility		sell broiler	
	yes	no	yes	no
H5 positive	21	8	20	9
H5 negative	30	11	27	14
Odd ratio	1		1.15	

4.1.5. Nomadic duck flocks

In the first study year, 20 migrating duck flocks were surveyed in Purbalingga. Analysis showed that all ducks were layer type and kept extensively. The age of migrating started after 1-2 month (61.1%) of age. Most of the flocks (75%) did not have direct contact with other poultry. Less than half of the flocks (41.2%) were vaccinated and vaccination was mainly conducted by the farmer themselves. The farmers could not say what vaccine was used. Information regarding how farmer conducted cold chain vaccine was not disclosed. See details in Table 22 and Table 23.

Table 22 General information surveyed nomadic duck flocks in the first round of the study

Variable	Category	#	%
Respondent	male	18	90
	female	2	10
	total	20	100
Production type	egg	20	100
	total	20	100
Age	<1 month	3	18.8
	1-2 month	7	43.8
	> 3 month	6	37.5
	Total	16	100
Age before nomad	<1 month	6	33.3
	> 2 month	1	5.6
	1-2 month	11	61.1
	Total	18	100
Contact with other poultry	Yes	5	25
	No	15	75
	Total	20	100
Vaccination	No	10	58.8
	Yes	7	41.2
	Total	17	100
Vaccinator	Other	3	30
	Farmer	7	70
	Total	10	100

Table 23 Summary of profile of nomadic ducks (1st round)

Variable	#	median	min	max	Not recorded
Age (weeks)	18	52	35	80	2
Age brooder	3	8	8	8	17
Age growers	6	18	10	52	14
Age layers	15	52	24	120	5
Production cycle	16	71	4	96	4
Number of egg	15	250	5	465	5
Average age	2	395	70	720	18
Average weight	2	1.6	1.3	2	18
% egg hatched	2	2.5	0	5	18
% egg sold	7	100	0	100	13
Number of duck	20	175	13	500	0

In the second study year, more data on nomadic duck flocks were obtained. The movements showed that the flocks came from Cirebon, Pemalang, Demak, Cilacap and Banjarnegara districts. The flocks in Purbalingga were moved to surrounding district in Central Java and long distances such as Yogyakarta, Surakarta, and Tegal. See map on Figure 16 and Figure 17.

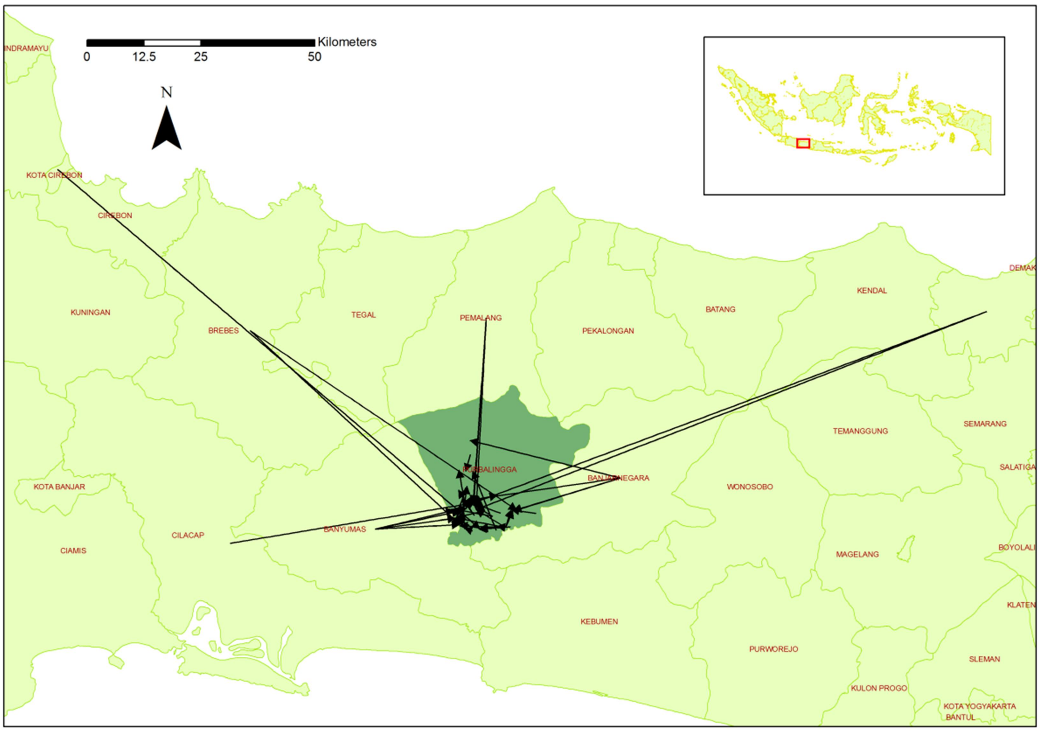

Figure 16 Origins of nomadic duck in the second study round

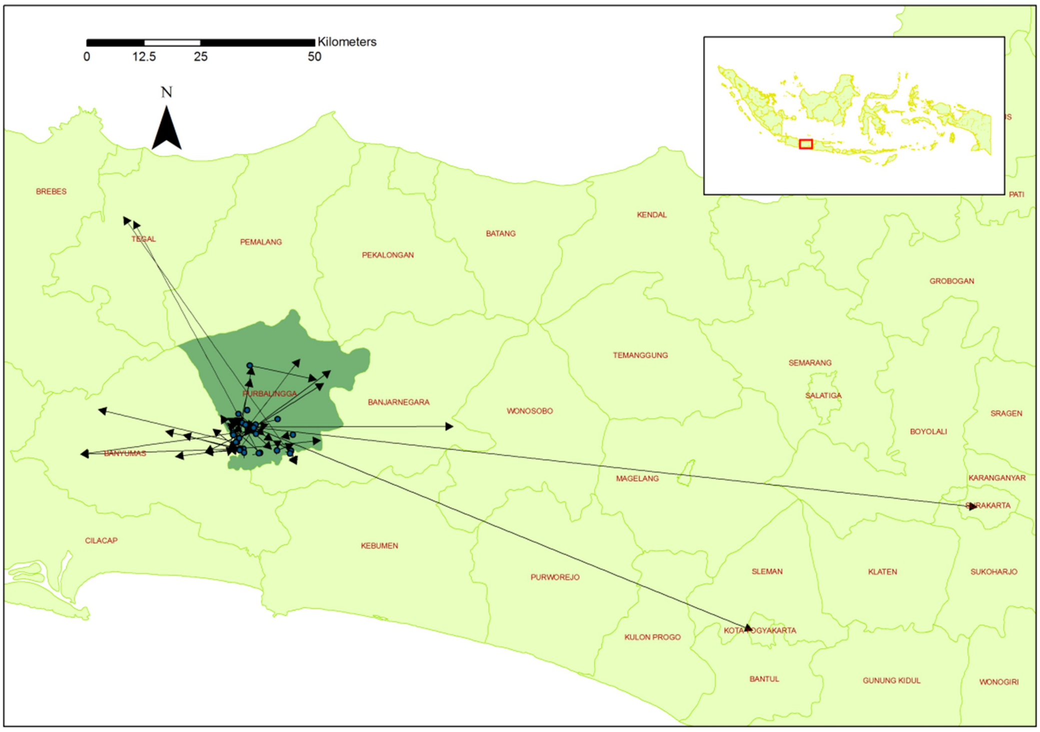

Figure 17 Destinations of nomadic ducks in the second study round

4.2. Mortality events in village poultry

Respondent were asked about HPAI compatible events in their flock over last 12 months. Data showed that 30% households had dead chickens but none of the cases were reported to local authority. The mortality increased from December, peaking in April and mortality was low in September - November. However this data still might contain recall bias (See Figure 18).

We also analyzed factors or predictors that associated with the chicken mortality. The analysis includes testing statistical assumption of logistic regression. Inclusion criteria were ownership more than 5 backyard chicken per household and mortality more than 30%. Results of odds ratio for predictor variables are presented in Table 24.

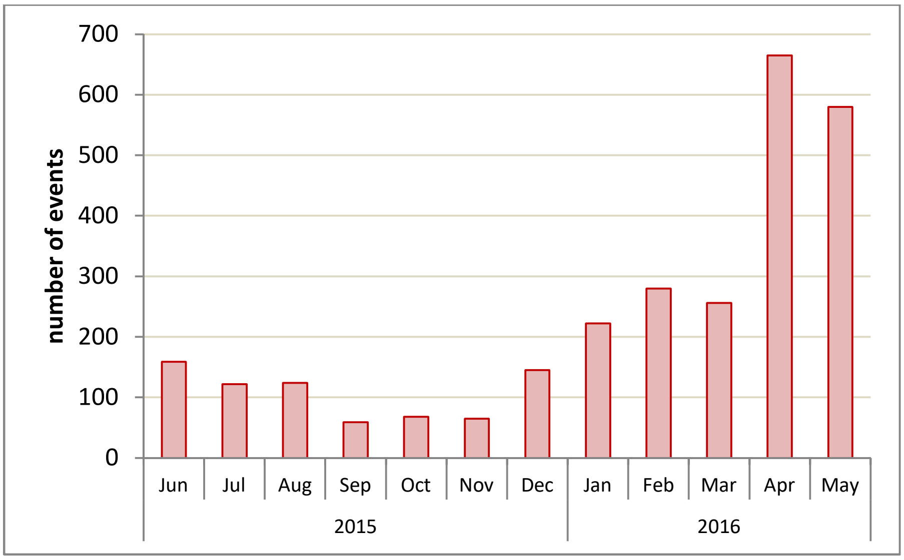

Figure 18 Timeline of mortality event in backyard poultry

From the Odds Ratio it indicated that improper carcass disposal was correlated to the poultry mortality event (OR 4.2, Confidence Interval (CI) 95% 3.08 – 5.74). It also suggests that vaccination prevents mortality events (OR 0.6, Confidence Interval 95% 0.39 – 0.99). However this actually did not assume causation.

Table 24 Correlation between poultry mortality and predictors

Predictor variables	OR	95% CI
Chickens roaming	1.3	1.21 – 1.47
Carcass is not properly disposed	4.2	3.08 – 5.74
Join cock fighting	1.6	1.04 – 2.50
Visit places with poultry	1.3	1.16 – 1.53
Vaccination in birds	0.6	0.39 – 0.99
Ponds in surrounding	1.3	1.17 – 1.50

4.3. Phase 2: Active sampling to detect AI virus

In the first round 41 collectors, 32 commercial poultry farms, 18 live bird markets, and 20 nomadic duck flocks were sampled. In the second round samples were collected from the same locations, 196 nomadic duck flocks with additional sampling of backyard poultry in villages. Since broiler farms were left out in round 1, some broiler farms were included in the village surveillance in round 2.

4.3.1. Backyard poultry sampling

In the round-2, 10,172 households with chickens were visited. Out of 10,172, there were 89 (0.87%) households which had current sick or dead birds, and 125 (1.23%) houses experienced sick or dead chickens in the previous week before the visit. The enumerators conducted rapid test on 32 remaining chickens at houses where there were sick birds or mortality in the current or previous week. The rapid test confirmed 1 positive case. Swab samples were also collected for laboratory testing at the DIC in Wates.

4.3.2. Sample test results

A total of 2,414 pooled samples were tested using the Matrix (AI) and H5 PCR test. H9 PCR test was also performed to see if any co-circulation in the samples.

Table 25 RT-PCR test results per round

Enterprise type	Round 1				Round 2				Totals			
	Tested	AIV+	H5+	H9+	Tested	AIV+	H5+	H9+	Tested	AIV+	H5+	H9+
Commercial farms	274	1	1	-	331	26	0	14	605	27	1	14
Collector yards	277	11	8	-	330	62	21	23	607	73	29	23
LBMs	107	3	2	-	142	42	11	19	249	45	13	19
Nomadic duck flocks	40	2	1	-	864	116	12	14	904	118	13	14
Passive surveillance	12	0	0	-	4	0	0	0	16	0	0	0
Backyard poultry	-	-	-	-	146	19	2	5	146	19	2	5
Total	597	17	12	-	1,817	265	46	75	2,414	282	58	75

Numbers and percentages of the total (prevalence) are shown.

Table 26 Proportion of positive results of AIV, H5, and H9

Enterprise type	Number tested	Positive test results		
		AIV+	H5+	H9+
Commercial farms	605	27 (4.5%)	1 (0.2%)	14 (2.3%)
Collector yards	607	73 (12.0%)	29 (4.8%)	23 (3.8%)
LBMs	249	45 (18.1%)	13 (5.2%)	19 (7.6%)
Nomadic duck flocks	904	118 (13.1%)	13 (1.4%)	14 (1.5%)
Passive surveillance	16	0 (0%)	0 (0%)	0 (0%)
Backyard poultry	146	19 (13.0%)	2 (1.4%)	5 (3.4%)
Total	2,414	282 (11.7%)	58 (2.4%)	75 (3.1%)

Positive H5 samples were detected from all enterprise types. Data also shows that some places were repetitively confirmed of H5 virus. Two had been detected in collector yards that trading kampong chickens and one was in live bird market. One collector was H5 positive in January 2017, August 2018, and September 2018. Another was positive in February 2017, August 2018, and September 2018. One live bird market showed H5 positive in February 2017 and August 2018. Some samples were found to have two H sub-type H5 and low pathogenic avian influenza (LPAI) H9 in the pooled samples, indicating co-circulation in the ssame environment.

Details of distribution of positive samples per location in each sampling round are described in Figure 19 and Figure 20, and per species in Table 29. Meanwhile to see the distribution of infected locations (minimum 1 positive sample from the location) in collector yard (CY), commercial farm (CF), live bird market (LBM), nomadic duck flock (ND), passive report (P), and village chicken flock (BY), see Table 27 and Table 28.

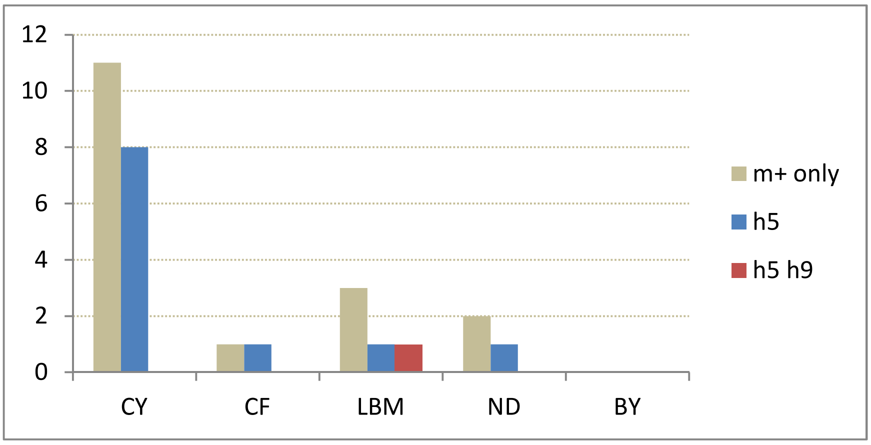

Figure 19 Positive samples in round 1

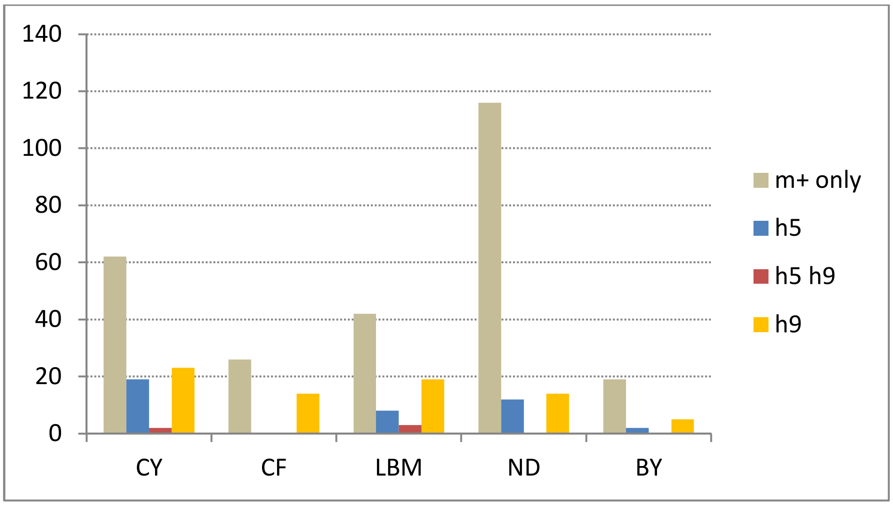

Figure 20 Positive samples in round 2

Table 27 H5 positive per unit location

	no of unit location	no of pool sample		H5 + per unit location							
		round 1	round 2	Oct-16	Dec-16	Jan-17	Feb-17	Aug-17	Sep-17	Oct-17	Nov-17
CY	42	277	329	1	1	3	2	4	6	2	4
CF	41	161	331	1	0	0	0	0	0	0	0
LBM	18	107	142	0	0	1	1	2	2	1	2
ND	216	40	863	1	0	0	0	4	0	2	0
P	4	12	4								
BY	110		146	0	0	0	0	0	1	1	0

Table 28 H9 positive per unit location

	no of unit location	no of pool sample		Matrix + per unit location							
		round 1	round 2	Oct-16	Dec-16	Jan-17	Feb-17	Aug-17	Sep-17	Oct-17	Nov-17
CY	42	277	330	3	2	3	2	16	13	7	8
CF	41	161	331	1	0	0	0	9	0	1	3
LBM	18	107	142	1	0	1	1	7	6	9	5
ND	216	40	864	2	0	0	0	16	15	21	6
P	4	12	4								
BY	110		146	0	0	0	0	9	4	1	2

Table 29 Number of positive samples per species

Sample	Round 1			Round 1 total	Round 2				Round 2 total	Grand Total
	Matrix pos				Matrix pos					
	H5 pos	H5 H9 pos	H5 H9 neg		H5	H5 H9 pos	H9 pos	H5 H9 neg		
Broiler	1			1			1	2	3	4
Duck	1		1	2	12		16	94	122	124
Kampong chk	2			2			3	2	5	7
Layer	1			1			14	8	22	23
Muscovy					1			1	2	2
Quail								3	3	3
Environment	6	1	4	11	28	5	41	34	108	119
Grand Total	11	1	5	17	41	5	75	144	265	282

Virus isolation was performed on all samples testing positive for Influenza A and full genome sequencing (FGS) was conducted on virus isolates. A total 12 influenza-A positive samples were sequenced in August 2018 and 23 influenza A positive samples were sequenced in February 2019.

On Figure 21, samples from the study are indicated in red. Phylogenetic tree is reconstructed from 1485-bp length of HA gene which about 87% of complete sequence using Neighbour Joining Tree and and TN93 nucleotide substitution mode with Gamma distributed parameter. The tree was built by using MEGA 6 bioinformatics software. As shown in Figure 1, samples from the study (N=17) belonged to H5N1 Clade 2.3.2.1c with the majority of these samples are clustered in a putative group iii of H5N1 clade 2.3.2.1c, while 3 virus samples belonged to another putative sublineage of clade 2.3.2.1c (group i).

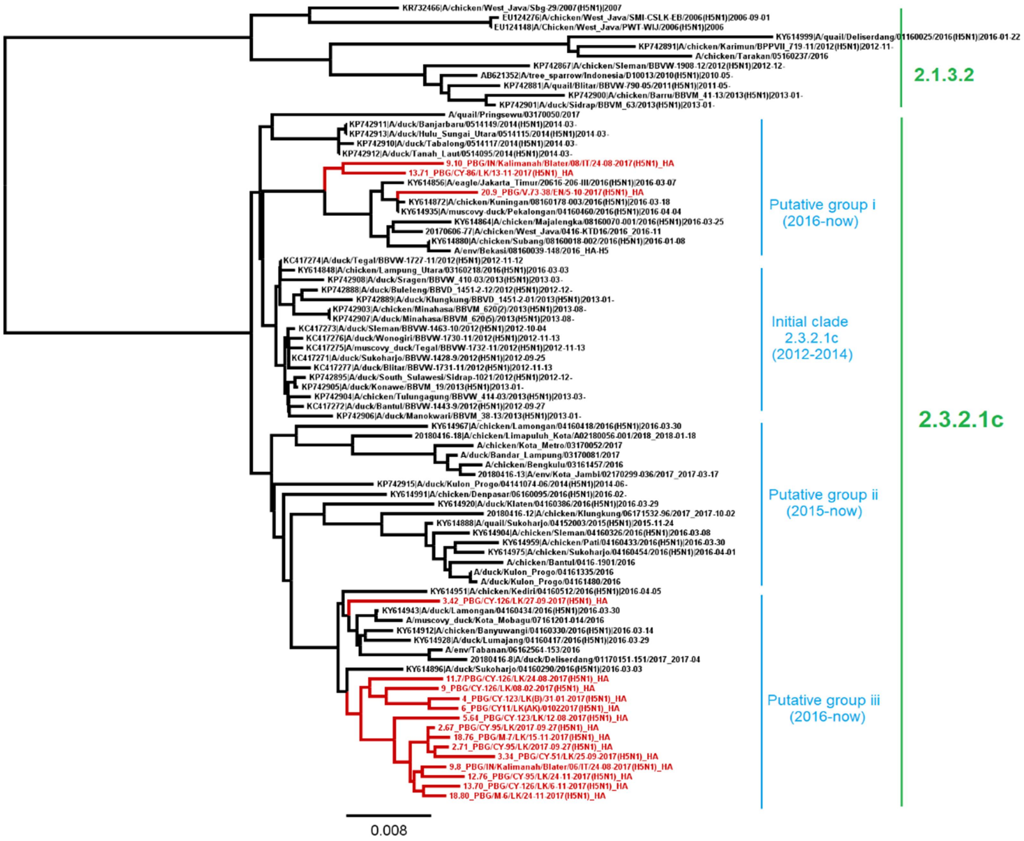

Figure 21 Phylogenetic analysis of HA gene of H5N1 virus from the samples

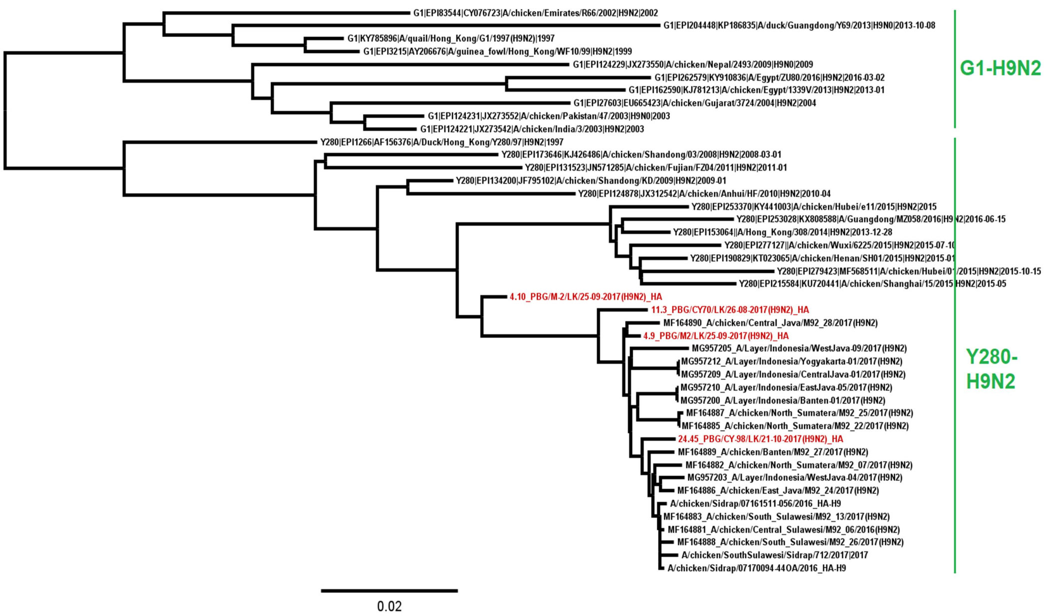

Figure 22 Phylogenetic analysis of HA gene of H9N2 virus from the samples

While in Figure 22, the samples from the study are shown in red. Phylogenetic tree is reconstructed from 1485-bp length of HA gene which about 87% of complete sequence using Neighbour Joining Tree and TN93 nucleotide substitution mode with Gamma distributed parameter. The tree was built by using MEGA 6 bioinformatics software. The samples (N=3) belonged to H9N2 Clade Y-280.

4.3.3. Hypothesis testing

The contingency table for the counts of PCR negative (0), LPAI positive/H5 negative (1) and LPAI positive/ H5 positive (2) results, stratified by round 1 and 2 (R1/R2)) and enterprise type are as follows:

Contingency table:

# result	CY	CF	LBM	ND	BY
# R1.0	266	160	104	38	0
# R1.1	3	0	1	1	0
# R1.2	8	1	2	1	0
# R2.0	270	305	103	752	127
# R2.1	39	26	28	100	17
# R2.2	21	0	11	12	2

The Fisher's Exact test estimated a p-value of 0.000498, which enables the null hypothesis that there is no difference in the number of AI viruses detected in the first and second sampling round, within enterprise types, to be rejected. The Chi-squared test returned a p-value of 2.2E-16, although the assumptions may not be valid. However, although overall significant, these results do not give any further information.

Different numbers of samples were collected in each round. Below we investigate the difference between rounds, the enterprise types were aggregated:

Contingency table:

# result	R1	R2
# 0	568	1557
# 1	5	210
# 2	12	46

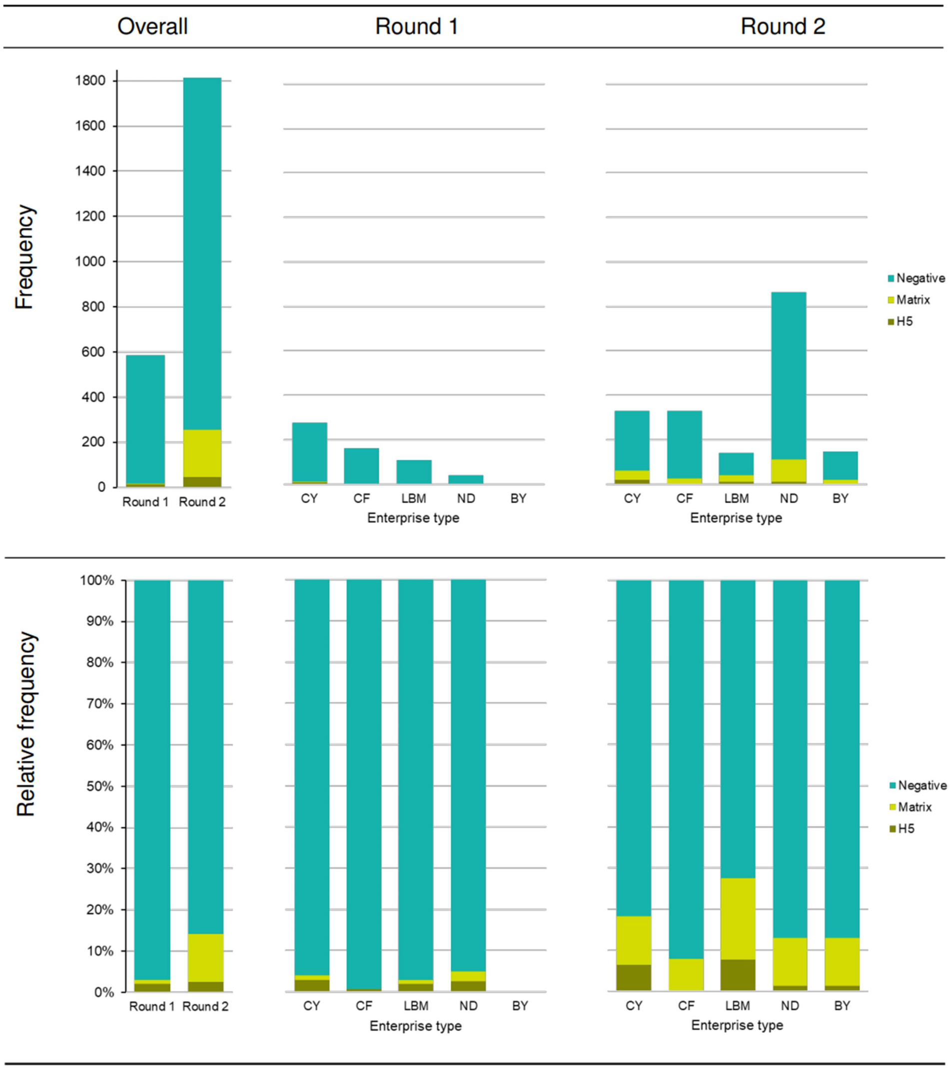

Figure 23 Frequency and relative frequency for the sample test results (H9 is not presented)

Conditions are now met to do a Chi-square test; this resulted in a p-value of 1.637E-14. This signifies that there was a significant difference between the two rounds. It is apparent that this is due to a difference in the expected numbers of LPAI counts, which were much higher in the second round. To investigate whether there was also significance for H5 virus, this test was repeated ignoring this level:

```
# Contingency table: note that LPA-pos are H5-neg
# result      R1     R2
# 0+1         573    1767
# 2           12     46
```

This resulted in a p-value of 0.6097 (the Fisher Exact test estimated this as 0.6423). In other words, the null hypothesis that there was no difference in the number of H5 viruses detected in the first and second sampling round was retained. This implies that, there was evidence of a significant difference in the amount of AI detected, but no of H5 virus.

As there was no difference in the amount of H5 virus detected during each round, it was assumed that it was valid to combine the results from these rounds. Continuing with these data, but investigating if there are differences between enterprise types:

```
# Contingency table
# result      CY    CF    LBM   ND    BY
# 0+1         578   491   236   891   144
# 2           29    1     13    13    2
```

These counts were tested using Fisher's Exact test. This again estimated an overall *p*-value of 0.0004998. To assess differences between enterprise types, pairwise test were done; the results of all pairwise combinations are shown in Table 30.

Pairwise Fisher's Exact tests for strength of association (odds ratios, OR with 95% confidence intervals) of H5 virus were detected at enterprise types. CF: Sector 1-3 commercial farms; CY: collector yards; LBM: live bird markets; BY: Sector 4 backyard farms or village chicken; ND: nomadic duck flocks.

Table 30 Pairwise Fisher's Exact test for the test results in each locations

	CF		CY		LBM		BY		ND	
	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)
CF			0.04	(0.001-0.25)	0.04	(0.001-0.25)	0.15	(0.002-2.85)	0.14	(0.003-0.94)
CY	24.40	(4.05-1,000)*			0.91	(0.45-1.95)	3.61	(0.90-31.25)	3.61	(1.72-7.25)
LBM	26.95	(4.00-1,145.2)*	1.10	(0.51-2.22)^			3.95	(0.88-37.04)	3.77	(1.59-8.93)
BY	6.79	(0.35-402.04)^	0.28	(0.03-1.12)#	0.25	(0.03-1.14)#			0.95	(0.10-4.27)
ND	7.16	(1.07-304.68)*	0.29	(0.14-0.58)*	0.27	(0.11-0.63)*	1.05	(0.23-9.71)^		

* Significant at 95% confidence level
# Marginally significant
^ Non-significant

It was shown that the largest numbers and highest prevalence of AI, H5 and H9 viruses were found in the value or marketing chain..

4.4. Social Network Analysis

Poultry movement in live bird market, collector, commercial farm and nomadic duck flock were recorded and analyzed. Detail network parameter of live bird market, collector, farm, and nomadic duck flock are provided in Table 31

Table 31 Parameter of network in live bird market, collector, commercial farm, and nomadic duck flock

Variable	Live bird market	Poultry collector	Commercial poultry farm	Nomadic duck
Number of nodes	78	267	288	50
Number of directed links	96	343	424	61
In-degree centralization	0.15	0.13	0.085	0.058
Out-degree centralization	0.075	0.08	0.064	0.058
Betweenness centralization	0.041	0.12	0.084	0.01
Average path length	4.7 (-4.3)	12.47 (-5.5)	4.5(5.07)	4.7(-4)
Cluster coefficient	0.048(0.021)	0(-0.008)	0(0.007)	0 (-0.026)

Figure 24 SNA of different poultry systems

Trade within the commercial farm network had the largest number of nodes and spanned 10 Districts. For the collector yard network, trade extended to three Provinces (Jakarta, East and West Java), and the betweenness centralisation score of this network was the highest of all networks examined at 0.12. In the live bird market network, trade of poultry was limited to the two Districts of Purbalingga and Banjarnegara. Three markets were found to have the highest centrality scores. The resulting network had small world properties (average path length = 4.7, cluster coefficient = 0.048).

5. Discussion

5.1. Study implementation and lesson learned

5.1.1. Constraints and limitations

A number of complications arose during the implementation of the study. These summarized as follows:

1. The constraints in the study design, the issues experienced while implementing it and the changes that were made to the design over the duration of the study. These are summarized as follows:
   a. During round 1 of the study, nomadic ducks were not initially considered, hence few questionnaires were performed and this sector was not as well scoped as the other enterprise types.
   b. Delays in implementation due to organizational reasons (obtaining approvals) as well as logistical reasons (changes in management of the district veterinary services, which meant that personnel were not available or had to be retained). As a consequence, sample-taking was delayed, and the number of samples taken was relatively small.
   c. As a consequence of La Niña effect, the rainfall was unusually high during the first round, and as such this round was not considered to be representative for a typical low season. The second round samples were collected during the low season months and rainfall was more representative for these months. If there is a seasonal effect, combining the data from both sampling rounds is therefore not epidemiologically defensible.
   d. Active sampling of household (backyard) poultry and nomadic ducks was not considered or limited in scope during the first round of sampling.
   e. Respondents in collector yards were not all willing to further interviewed. The monthly survey data then was not fully recorded.
   f. Sampling protocols were variable between enterprise types, and moreover, were variable between the first and second sampling rounds. This implies that the sensitivity of detection should be expected to be

variable between enterprise types, as well as between the sampling rounds. As a consequence, it is probably unwise to attempt to quantify the sensitivity of detection.

2. Inherent in the study design, the phase 2 sampling did not aim for representative population sample, but was more focused on active detection of AI viruses. A common objective of statistical analysis, i.e. estimation of population-based measures of disease frequency and measures of association of risk factors for a defined outcome, cannot be directly investigated. This limits the scope for statistical modelling and means that only direct assessment and testing for significant differences between the observed rounds and populations can be performed (insofar) as these can be justified considering the constraints noted in point 1 above). Caution is required in extrapolation to population level.
3. The small number of HPAI virus isolates identified. This was to be expected given the whole concept and design of the study, but of course it limits the power of the study to identify meaningful associations and perform inference and extrapolation.

This implies that the analytic outputs included in this report are mostly descriptive. Statistical testing has been limited to hypothesis tests based on the observed data. Advanced analysis (including statistical modelling) has been limited, and the scope for performing this is likely to be limited.

Notwithstanding this, the data reveal interesting trends which can inform further discussion, allow several of the hypotheses which determined the design of this study to be refined, and will be highly useful for determining the design of an eventual follow-up study. In addition, the data will be informative for parameter estimation, e.g. for the development of transmission models.

5.2. Characterization of poultry

Poultry farms in Purbalingga are heterogeneous, including extensive farming backyard poultry and commercial poultry farms. In the village, we visited 14,368 house and it shows more than half (60%) of households in Purbalingga keep poultry with the main types of poultry raised are kampong chicken. Mostly the ownership of each house is 5 chickens and few houses have chickens more than 30. The areas with high backyards poultry density can be found in the western, southern and few parts in the eastern part of Purbalingga. Poultry density in the north is relatively less because it is mountain area which has less human population.

A census was in 204 commercial poultry farms. The census identified one parent stock and one hatchery. The type of breeds in commercial units is mainly layer and broiler chicken and few are quail and duck. The layer chickens are mostly AI vaccinated. One important thing is that 20 flocks of nomadic ducks were identified which move from villages in Purbalingga to the neighboring district and even outside the province of Central Java. This is important because nomadic duck flocks do not implement biosecurity and it can be a potential factor for avian influenza transmission over longer distances. However they did conduct AI vaccination.

5.2.1. Poultry trading

The poultry marketing were practiced by 67 traders and 129 collectors. They bought poultry from households and farms in Purbalingga and sold them to customers in - and outside Purbalingga district. These birds were mostly sold out in a day, but there were some stayed overnight at the market. If the poultry was not sold out in one day, the trader or collector sold them to another place or slaughter them.

5.2.2. HPAI compatible event

Mortality data in village chickens shows that 30% of the households had sick or dead poultry in the last 12 months. In other words, of the 14,368 houses visited, around 4,000 households experienced sick and/or poultry mortality. But none of these cases were reported.

The sick and dead poultry over the last 12 months showed a similar trend to HPAI reports in general in Indonesia, with a decrease cases between July and November and then going up starting in December up to April. We assume that at least part of these cases was related to HPAI.

We analyzed correlation between poultry mortality event and several factors. We found that in case of mortality, carcasses were not properly disposed (OR 4.2, CI 95% 3.08-5.74). However this did not determine the cause and effect, but it is necessary to educate people on the importance of proper carcass disposal.

5.3. Active sampling to detect AI virus

Considering the results of PCR testing of both rounds of sampling, as shown in Table 25 and Figure 23, and of the statistical test results shown in Table 30, a number of discussion points can be raised. Overall, the differences in detection of AI (Matrix) and H5 virus between enterprise types and between the two rounds were found to be statistically significant. Further hypothesis testing teased out some of the differences that led to this.

5.3.1. Sample numbers in Round 1 and Round 2

Nomadic ducks were under-sampled during the first round, and oversampled during the second round. This contributes to the fact that about three times as many samples were taken during the second round. No village poultry were actively sampled during the first round, and the number of samples during the second round was comparatively low. Therefore, sampling was not performed proportional to population sizes.

5.3.2. Detection of AI virus

It may be hypothesized that the amount of AI virus that is detected by population and environmental sampling is proportional to the numbers of disease cases or outbreaks detected or reported; in other words, it is reflective of the seasonal trends of disease. There were significantly more Matrix positive test results during the second round. Although slightly more H5 viruses were detected in the second round as a proportion of the total number of samples taken, the difference was not statistically significant.

As the Round 1 samples were taken during quite a wet period and the Round 2 samples were taken during an off-season, the relationship between incidence of clinical disease and detection of H5 virus therefore does not appear to hold.

A comment has been made above about the fact that the sampling protocol was not the same for both rounds and for all enterprise types. This may have affected the detection rate, although this cannot be easily quantified. Seasonality of detection is discussed more broadly in 5.4.3 below.

5.3.3. Stratification by enterprise type

Looking at enterprise types, the overall prevalence of H5 was the lowest in CFs. As this is the only sector that consistently practices vaccination and better biosecurity, this may not be a surprise However, H5 virus was detected in commercial farms, so it must be assumed to be present, if only at a low level.

Sampling in commercial type did not include broiler. However we consider analyzing HPAI cases in broiler and for this we can use CYs data that sell broiler.

H5 prevalence was also detected a low level in the backyard sector and in nomadic ducks. As no samples were taken from village birds in the first round of sampling, the prevalence cannot be compared between the first and second round. The prevalence in nomadic ducks is comparable between the two rounds. It seems very coincidental that the prevalence of AI (Matrix) and H5 virus is exactly the same for the nomadic ducks and backyard samples in the second round

It is apparent that the largest numbers as well as the highest prevalence both of AI and H5 viruses were found in LBMs and CYs. This is specifically the case for the second sampling round. Pairwise hypothesis testing confirms these observations. The odds ratio between LBMs and CYs is about 1 and there is no statistical difference. However, the odds between these enterprise types and CFs is in the order of 25 to 30 (and highly significant); ND flocks, 3.5 to 4 (and highly significant); and Backyard poultry (BY) poultry, 3.5 to 4 (and marginally significant). There was no difference between ND flocks and BY poultry. Both of these had approximately 7 times higher odds than CFs (the association was not significant between BY and CF due to the small numbers of detections).

5.4. General discussion

5.4.1. HPAI H5N1 endemicity in Purbalingga District

A key assumption of this study is that HPAI H5N1 is endemic in Purbalingga District. The evidence of this need to be assessed before other inferences can be made and conclusions can be drawn.

The concept of endemicity assumes firstly that the virus is permanently present. This means that it can be detected even in the low season. Evidence that the virus present is the same one over the seasons, as opposed to being (re)introduced, resulting in new incursions rather than recurrence of disease, is also required; this cannot be determined on the basis of clinical disease alone.

The agent may infect a range of host species and also persist in different environmental reservoirs or compartments.

Extending this to the study area:

- H5N1 virus was identified during what is assumed to be the low season for disease (specifically, the second sampling round).
- Furthermore, it was detected in all enterprise types (albeit at very low levels in backyard and commercial poultry).
- Almost similar viruses sequence wise were detected in same enterprises and the district over time in 2017
- Although it was also found that there were high percentage of samples with Avian Influenza (Matrix) but H5 and H9 negative. Further HxNy investigation may be needed.

Further work is currently underway to assess the evidence for the virus being the same type.

Tentatively, it can therefore be stated that H5N1 HPAI is assumed to be endemic in Purbalingga District.

Endemicity at what scale?

A scale effect is at work. The larger the population under consideration that the higher the likelihood that the organism will persist. It occurs whether by local spread, direct or indirect transmission; or longer-range spread (movements). Factors including virulence, immunity and the environment of course play a role. (Hosseini, 2011)

As the population sizes are correlated with geographical area, there is also a relationship between HPAI endemicity or persistence and geographical area (Farnsworth, 2011).

Given that endemicity is therefore relative, the issue is to clearly define the scale at which it is being considered. In itself this may not be difficult – as in this case, an administrative area (District) is considered. It becomes more problematic when it is desirable to compare different areas (or populations, enterprise types etc.). If endemicity is confirmed in one District, is it meaningful to extrapolate this, for instance, to the neighbouring District?

However the virus does not stop at the district border. It is only to be discussed if it can be endemic at village, district or island level.

A similar stratification by host species can be considered. Within one District, the virus may be endemic in one host species (e.g. ducks) but not in others (e.g. backyard poultry).

5.4.2. Differences between enterprise types

The importance of the value chains and behaviours

The study results show that most AI and HPAI viruses were detected in the value and marketing chain, at collector yards and live bird markets. This indicates that these are points or nodes of higher risk. This finding is entirely consistent with results elsewhere in South and South East Asia (Fourni ´ e, 2013).

If the virus is present at a very low level in the "catchment" or source populations, the value chain effectively concentrates or even amplifies virus along its length

(Martin, 2011). Several reasons can be hypothesized. Behaviours of buyers and sellers can influence this: for instance, traders anecdotally prefer to buy sick birds due to the lower purchase price whereas farmers preferentially sell these to avoid loss due to mortality, and potentially to reduce biosecurity risk to the rest of the flock (Indrawan, 2018). Latently or sub-clinically infected birds may start excreting large amounts of virus under stress (at CYs and LBMs). Uninfected birds may become infected at these locations if there are large amounts of virus in these environments.

The numbers of LPAI viruses were also highest from CYs and LBMs. It is probably HPAI and LPAI are not behaving differently. LPAI might even easier to spread as it does not kill chicken straight away.

The Phase 1 questionnaires provided evidence that it is common for birds at CYs to be taken to other villages (by buyers) after sale. Traders at LBMs often do not sell all of their birds (collected from different sources) and they may take these back to their homes. They will attend multiple markets to sell their birds. All of these behaviours represent potential avenues for transmission of virus throughout the District.

The importance of commercial sectors

In this study, few H5 viruses were found in the commercial sector. Vaccination takes place in this sector but the effectiveness of this vaccination cannot easily be assessed. If this sector represents an entirely separate and closed production system, it can be compartmentalized. However, this is clearly not the case, with this sector selling birds directly to LBMs and conversely, buying live birds from other sources. What is perhaps less clear (and potentially quite variable) is the extent to which this sector is open.

Not all commercial poultry farms reflect to the standard profile of farming practices. There are difference between layer and broiler both on vaccination and biosecurity. For example mainly broiler includes in sector 3 and some layers are in sector 2. If vaccination is consistently performed at commercial farms and is effective, this should be sufficient to protect these farms from outbreaks. Poultry from this sector entering the value chain should pose no problem. However, commercial farms buying and introducing poultry of unknown vaccination status from the informal sector comprise a biosecurity risk. Outbreaks on commercial farms can have the greatest consequences in terms of mortality and economic losses.

The role of ducks

It is well known that ducks can function as a reservoir species. The nomadic duck sector is of particular interest, as these birds circulate widely and hence have opportunity for both acquiring and disseminating infection (potentially latently or subclinical), although this assumes there are (direct or indirect) contacts between ducks and other poultry (Henning, 2014) . It is unfortunate that this sector was not included in the Phase 1 survey by design, and sampling was only at a low level during the first sampling round.

A comparatively high number of AI viruses were isolated from duck samples, although the proportion of H5N1 and H9N2 viruses is likely to be lower (in other words, there is a greater diversity of other LPAI viruses circulating). Further Full-Genome Sequencing (FGS) will yield interesting results. Ducks may play an important role in the mixing and recombination of AI viruses.

5.4.3. Pattern of infection and disease

For a given pathogen, the host expression of disease can change over time due to determinants such as population (field) immunity, virulence factors (including re-assortment and recombination), co-infection with other organisms, vaccine-mediated immunity etc. If HPAI can be assumed to have been endemic in Purbalingga District for an extended time, questions of interest related to this may include the following:

- A high case fatality rate (CFR) is generally assumed in chickens at least. To which extent, is this the case, and has this CFR changed over time? Are there differences between species and types of poultry?
- Does disease expression vary seasonally?

- Do badly vaccinated or poorly vaccinated birds (i.e. with partial immunity) develop less severe disease, and does this play a role in the maintenance of endemicity?

These questions are difficult to answer, and the results from the endemicity study are insufficiently strong to investigate these. Also this was not part of the study objectives.

The relationship between seasonality and infection or disease

A longer study period is required than this study to investigate seasonal trends, and this should be explicitly incorporated into the study design. Nevertheless, an unintended consequence of the two sampling rounds, with the first occurring in a wetter period and the second in a more typical dry or low season, is that the results can be compared to give an indication of whether there are differences between the two. An interesting finding was that the numbers of AI and HPAI viruses found during the second sampling round were actually significantly higher (LPAI) or the same (H5) as the first sampling round. But also consider that the increased of H9 detection was contributed by the high number of nomadic ducks samples. If the assumption is that the virus is present at lower levels during the off-season, this result is counter-intuitive. This may be meaningful but may also be coincidental. Alternatively, it could be an artefact if the sensitivity of detection was higher for the second sampling round.

In the past we saw seasonality in LBMs but no longer or sample size might be too small. Yet, we did not sample over a year to have the full picture for example with weather trend over 12 months. However, rainfall might be not the only factor for seasonality. The seasonality of detection of virus at collection points (CYs and LBMs) is likely to be less; these premises operate year-round and the potentially, the virus can be maintained in the environment. Given that very low numbers of viruses were found in the backyard and commercial sectors, this cannot be investigated.

The metapopulation hypothesis

It is interesting to assess from the endemicity study results what indications there are for transmission of H5N1 between the different avian species.

In terms of mixing of these populations, although the commercial sector is not entirely closed there are probably less opportunities for transmission of infection than there are between backyard poultry and ducks (especially nomadic ducks).

Moreover, transmission between these populations is likely to be driven more strongly by the value chain (BY) and by the husbandry system (ND than by direct or indirect contacts. As a consequence of this, clinical outbreaks are more likely to manifest as 'jumps' between villages or locations than showing a pattern of local spread.

SNA may be used to clarify the contact structure between nomadic ducks and domestic poultry populations, although it is not clear if the data will enable this. This pattern of transmission can be incorporated into disease spread models (Mart´ınez-L´opez, 2009).

The relatedness of the AI viruses, as determined by FGS will inform this – see also the next point.

The viral community: interactions between strains

The sequencing of the isolated AI viruses will provide clues on which strains and clades are circulating and in which poultry species. Although the objective of the study is to investigate HPAI H5N1, there may be patterns and trends of co-infection, associations or interactions. As other LPAI strains may be more commonly found, such knowledge would be useful. H9N2 has been strongly indicated plays a role in H5 mutation towards pandemic strain. The identified Y280 lineage on H9N2 has been known for its ability to infect humans. The high prevalence of the virus in poultry in endemic countries like Indonesia, low antibody in humans, and high number of shift of poultry viruses to human-like receptor binding will enable a human pandemic threat (Alexander, 2009; Push, 2018).

Construction of phylogenetic trees and other metagenomic tools were discussed at the workshop; it would be good to follow up with this, but it is too early to speculate about this. Such work would enable comparison with other, recent work investigating genetic analysis and reassortment of H5N1 virus.

5.4.4. Extrapolation of the endemicity study findings

Ultimately, a question of fundamental interest is the degree, to which the study findings are representative for, can be extrapolated to, or can be directly compared with other areas and situations. The previous discussion addresses some relevant aspects.

- If the conditions in Purbalingga District in terms of the environment, poultry production and husbandry systems, poultry population structure, value chain and marketing etc. are considered to be representative of a wider area (e.g. Java), and we assume that H5N1 is endemic here, we may conclude that HPAI H5N1 is endemic in this wider area (although it may not be endemic in all areas). Patterns of reported and confirmed HPAI cases (through surveillance) can be further investigated if relevant, but retrospectively trends support this. The discussion on "endemicity at what scale?"
- If such extrapolation can be assumed, recommendations for interventions and control, as informed and developed on the basis of this study, can also be extrapolated.
- Caution would be advised for extrapolation to other areas which are not directly comparable e.g. islands with lower poultry and human populations and densities, with different climate, environment and / or different production systems.
- In terms of the bigger picture, some of the transmission dynamics of the virus are likely to be generic and applicable more widely. Of course, comparison of the results from this study with similar work such as is being conducted in Bangladesh will also be of interest. The value of this work is that it can inform transmission models.

6. Recommendations

6.1. Measures for disease control of HPAI H5N1

Utilising the existing HPAI policy as a starting point:

- Assess what can be improved on the basis of the endemicity study results and outcomes; review and update.
- As the existing policy provides a comprehensive framework, the most practical method is to look at each element in turn.
- Specifically assessing the following:
    i. Biosecurity
    ii. Surveillance
    iii. Vaccination
    iv. Public awareness

i. Biosecurity

- It is clear from the results that LBMs and CYs are high-risk value chain nodes. As such, interventions should target these to have the maximal impact on animal as well as public health.
- Specific recommendations would focus on:
    - Vendor behaviour: vendors with surplus birds for sale utilise multiple LBMs, so that birds are mixed and exposed in multiple locations, in stressful conditions, with potentially poor biosecurity.
    - Vendor practices at the LBM or CY.
- With reference to LBMs:
    - Cleaning and disinfection (C&D) at LBMs and CYs (where feasible).
    - Improved market practice (or where this is regulated by the Standar Nasional Indonesia (SNI), enforcement of existing regulations). These should be tailored to whether the market sells live birds only, live birds and carcasses, or carcasses only.
    - Where slaughter is carried out on-site, improvement of biosecurity through interventions such as implementing specific / dedicated slaughter areas with minimum requirements and developing standards governing:
        - Design of the facilities;

      - C&D practices;
      - Slaughtering practices.
    - A complication is that this work is the mandate of the Ministry of Trade rather than the Ministry of Agriculture.
    - Public awareness and education for consumer-driven change: an example was mentioned that the emerging urban middle class of higher-educated people represents a group that is more critical and may be prepared to spend more for a safer product. Utilising this can be instrumental in making "bottom-up" change happen, rather than relying on "top-down" enforcement or regulation.
- With reference to CYs:
    - C&D is probably the only feasible intervention in this enterprise type.
- With reference to commercial farms:
    - Efforts should focus on Sector 3 farms; Sectors 1 and 2 are well managed; resources are insufficient for effective Sector 4 interventions.
    - Activities should focus on improving biosecurity.

ii. <u>Vaccination</u>

- Efforts should focus on Sector 3 and commercial duck production.
- The vaccine produced is considered to be recent and is updated with circulating Indonesian strains (Influenza Virus Monitoring), so there are no strong concerns about vaccine quality. However, production of sufficient quantities of vaccine to ensure availability in the event of a significant outbreak is important.
- Another issue is to regulate the utilisation of unlicensed (in Indonesia) vaccines from overseas. It is known that this occurs, though it is not apparent to which degree this takes place.
- "Vaccine stewardship": ensure correct vaccination schedule, dosing and application.

iii. <u>Surveillance</u>

- Underreporting is the main issue that should be tackled.
- Consistent and coherent messaging to stakeholders (related to passive / syndromic surveillance) is necessary.

- Commitment is required at all levels to make this work (central government, local government, FAO, labs, etc.). This is very challenging.

iv. <u>Public awareness</u>

- Consumer education / awareness are important to support change, especially of middle-class, urban consumers with a little more spending power who are likely to be more discriminating in the products they choose.
- Educate farmers and producers on the benefits of vaccination, and the importance of correctly performing vaccination.

6.2. Strategy for follow-up

6.2.1. Short-term plans

Requirements for development, discussion and presentation of:

- Implementing recommendations generated by the endemicity study.
- Using these to review and possibly modify intervention measures as part of the response to confirmed outbreaks.

6.2.2 Long-term plans

1. Research focusing on ducks (nomadic and stationary)
    - Importance as a reservoir for HPAI, affecting persistence and transmission, is still not fully understood.
    - These are separate production systems that can be comparatively easily studied.
    - While husbandry practices cannot be easily changed, evidence-based studies are required to understand constraints.
    - Such work might focus not just on animal health, but also consider production-related work (e.g. nutrition and feed).
2. Poultry movements
    - Government movement checkpoints are in place but it is unclear how these are applied in the event of outbreaks (this is a general point, i.e. wider than just poultry).
    - A better understanding is needed of the transport systems, actors and behaviours between the source farms and the markets (incorporating CY). It is unclear if the virus is amplified in the environment of these facilities or whether this happens at intermediary steps in the poultry value chain.

3. Utilizing outputs from other research to inform study designs.
    - For example, the current intervention study in Bangladesh to investigate the role of middlemen and to quantify the amplification of virus in the poultry value chain.
    - Important role for longitudinal studies is to understand the dynamics of transmission.
4. Clarifying causality
    - Incomplete understanding of the complex factors that make up the "sufficient cause" for development of AI disease outbreaks. These probably include seasonal factors.
5. Vaccination studies
    - The vaccine production system in Indonesia is well-regulated and effective.
    - What is less well controlled, however, is how this vaccine is applied. "Vaccine stewardship" is important to ensure effective application and reduce the potential for "silent spread" due to poor or incomplete vaccination.
    - Post-vaccination monitoring, specifically focusing on Sector 3 producers, can give an insight into this.
    - Other vaccination related studies and activities can include:
        i. Cost-benefit studies to understand better the drivers, behaviours and motivation, as well as the economic benefits to farmers, of performing vaccination.
        ii. Strengthening relationships with the private sector (IVM, Ceva), specifically with regards to data and information sharing and analysis.
6. Poultry contact structures
    - Studying characteristics and practices relating to the mixing of birds from different sectors and systems (chickens, ducks and commercial), including stress factors and other risk factors, and how these impact transmission.
    - The design of such studies would be informed by the preliminary mathematical models that are expected to be developed before the end of EPT2. The outputs of the studies would enable better parameterization and development of models.
7. Data collection, quality and storage

- Ongoing improvement of data quality in iSIKHNAS.
- Better documentation of key gaps and requirements in terms of what data is collected and recorded (and how).
- Access to current data is limited – explore data sharing arrangements / agreements

8. Surveillance evaluation

   - Application of SET scheduled for August. This will lead to the development of a surveillance action plan.
   - Other evaluation frameworks which have been developed in recent years (e.g. RiskSUR / EVA tool, Oasis, Serval) could also be investigated for use.

9. Working collaboratively

   - It is important that there is good coordination with other stakeholders to ensure complementarily and avoid duplication of research; this is necessary to optimize the development of new knowledge.
   - This would include collaboration between FAO and academic and research institutions (e.g. national and international universities, ILRI, etc.).
   - Such activities could include academic supervision (e.g. enrollment in sandwich programmes, FETPV inputs, discrete projects / activities, etc.)

10. Disease reporting

    - Mitigating underreporting will result in a more accurate picture of the actual disease situation. Social science approaches could be applied to:

      i. Understand behaviors and drivers to reporting;

      ii. Determine how such barriers could be overcome.

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Epidemiological terms

Animal health surveillance The systematic (continuous or repeated) measurement, collection, collation, analysis, interpretation, and timely dissemination of animal-health and –welfare data from defined populations. These data are essential for describing health-hazard occurrence and to contribute to the planning, implementation, and evaluation of risk-mitigation actions.

Bias Deviation of results or inferences from the truth, or processes leading to such systematic deviation. Any trend in the collection, analysis, interpretation, publication, or review of data that can lead to conclusions that are systematically different from the truth.

Case fatality rate The proportion of animals with a particular condition (cases) who die from that condition. The denominator is the number of incident cases; the numerator is the number of cause-specific deaths among those cases.

Confidence Interval An interval estimate, computed from the statistics of the observed data that might contain the true value of an unknown population parameter; this interval has an associated confidence level that quantifies the level of statistical confidence that the parameter lies in the interval. If confidence intervals are constructed using a given confidence level from an infinite number of independent sample statistics, the proportion of those intervals that contain the true value of the parameter will be equal to the confidence level.

Endemic A disease or risk organism regularly found in a population or geographical area.

Enhanced passive surveillance Systems which rely on observer-initiated provision of animal health data with active investigator involvement (e.g. by actively encouraging producers to report certain types of disease or by active follow up of suspect disease reports).

Hypothesis A supposition arrived at from observation or reflection that leads to refutable predictions. Any conjecture cast in a form that will allow it to be tested and refuted.

Incidence rate Incidence is the rate of new (or newly diagnosed) cases of the disease. It is generally reported as the number of new cases occurring within a period of time (e.g., per month, per year). It is more meaningful when the incidence rate is reported as a fraction of the population at risk of developing the disease (e.g., per 100,000 or per million population). Obviously, the accuracy of incidence data depends upon the accuracy of diagnosis and reporting of the disease.

Odds ratio A measure of association which quantifies the relationship between an exposure and health outcome from a comparative study.

Outbreak The occurrence of cases of disease in excess of what would normally be expected in a population or geographical area. Synonymous with epidemic, but is often used for a more limited scale, in terms of numbers of cases and geographic area.

Passive surveillance Systems which rely on observer-initiated systems for provision of data. These systems have been adapted to increase the likelihood of outbreak detection, e.g. by incentivisation. The use of the term 'passive' has been viewed as misleading as it implies that surveillance investigators are inactive; the use of 'general surveillance' is increasingly used as an alternative.

Population In epidemiology, a group of individuals that are comparable in the sense of being exposed to similar risk factors: this means they share certain characteristics. In practice, these include environmental features (e.g. a geographic area), physical or physiological factors, behavioural characteristics etc.

Representative sample A sample whose characteristics correspond to those of the original population or reference population.

Seasonality Change in physiological status or in disease occurrence that conforms to a regular seasonal pattern.

Snowball sampling A non-probability sampling method where research participants recruit other participants for a test or study, using their own judgment. It is used where potential participants are hard to find. It consists of two steps: 1) Identify potential subjects in the population. Often, only one or two subjects can be found initially. 2) Ask those subjects to recruit other people (and then ask those people to recruit.

Transmission of infection Any mode or mechanism by which an infectious agent is spread through the environment or to another animal.

Social network analysis terms

Betweenness This is the measure of centrality defined as the number of times an actor connects pairs of other actors, who otherwise would not be able to reach one another. It is a measure of the potential for control as an actor who is high in "betweenness" is able to act as a gatekeeper controlling the flow of resources between the alters that he or she connects.

Centrality A numerical measurement of importance of a node. Degree is a simple example. Four types of centrality: 1) Degree Centrality – number of connections; 2) Closeness Centrality – closeness to the entire network; 3) Betweenness Centrality – to what degree a node provides a bridge to other nodes; 4) Eigenvector Centrality – connection to well-connected nodes, bridging nodes.

Clustering coefficient A measure of the likelihood that two associates of a node are associates. A higher clustering coefficient indicates a greater 'cliquishness'.

Directed link A link, edge or tie which is directional, e.g. when A sends a letter to B.

Node An individual actor, person or thing within the network.

Path All nodes and all lines are distinct. No node is connected more than once along a path. This is a direct route.

Appendices

A sample size calculation for the field survey

Number of sample is calculated based on the risk of poultry units or/and the environments between units either randomly or purposively from clinical signs and observed deaths of poultry. The number of samples is determined to detect at least one positive sample of H5 virus with calculation based on prevalence 2% and 95% confidence interval.

$$n = \frac{z_{1-\alpha/2}^{2} P(1-P)N}{d^{2}(N-1) + z_{1-\alpha/2}^{2} P(1-P)}$$

Further, sampling size was calculated and frequency of sampling was decided not only based on the representativeness but also looking at available resources. As mentioned, the study design use period of low seasons, so that sampling was scheduled between July and October 2016.

The sampling includes oropharyngeal swab from 30 birds per unit of commercial farms or 5 pools since 6 swabs are pooled in 1 Viral Transport Media (VTM) tube. The environmental swab were collected from live bird markets and collector yards which were collected in 2 pools in each units regularly per month, including swab collection of oropharyngeal of ducks in 5 pools once from nomadic duck flocks. Further if sick or dead bird case was reported then the swab sample was also collected either from the bird or if the carcass is not found, environmental sample was collected.

To measure number of unit of sampling, we require baseline data that were gathered from census.

Details of sample determination for each category are presented in Table 32 below.

Sampling strategy includes a two-staged stratified cross-sectional survey of commercial farms (commercial layer and duck farms), live bird markets, poultry collector yards, and nomadic duck flocks. The sampling also includes a random/risk-based selection of units and birds/environment within units either randomly or purposively based on clinical signs or mortality. See Table 33.

Table 34 presents number of poultry enterprises identified during the 2016 census stratified by backyard poultry density (birds per sq km) in Purbalingga district. The number of eligible farms was 59 and includes layer (n=46), duck (n=7) and mixed duck/layer (n=6) farms.

The sample size is determined to detect at least one H5 positive of bird. In commercial poultry farms, we calculate number of birds per farm to sample with expected 2% of birds in a farm would be positive and Confidence Interval of 95%. Average number of birds per farm type uses a fixed number of 30 birds was decided, giving a between farm detection prevalence of 2.25% (˜2.25% detection prevalence compared to the original 5%).

We sample H5N1 HPAI viral contamination within and between markets by collecting environmental swabs and sample from apparent sick or dead birds. The number of unit to sample was sample size determination in collector yard was calculated to detect H5N1 HPAI viral contamination within and between collector yards/collectors (environmental samples). The number of yards to sample was 41 collectors. The determination of the number of environmental samples to take within a collector yard is based on protocol developed (but mostly estimate) and used for previous studies in markets in Indonesia. This is described subsequently for each yard type (with or without slaughtering facilities) as shown in Table 35 and Table 36.

Sample size determination in nomadic duck flocks was calculated with 2% of birds in a flock would be positive and Confidence Interval of 95%; Average number of birds per flock (n=200). Then a fixed number of 30 birds was decided, giving a between farm detection prevalence of 2.25%. See Table 37.

Table 32 Sampling determination based on census data in 2016

Density category	No of villages	Enterprise type		No	Average poultry per unit	No of samples: Birds per unit	No of units	round 1	round 2	Total	No of VTMs: samples per vtm	total round 1	total round 2	total
Low	118	Commercial poultry:	Broiler	80	6864								0	0
			Mix broiler and layer	2	6750								0	0
			Hatchery	0	0								0	0
			Layer	31	8070	121	12	1452	1452	2904	6	242	242	484
			Other	1	1000								0	0
		Collector yard		20	387	93	3	279	279	558	6	47	47	94
		Broker/Collector		33	98	43	12	516	516	1032	6	86	86	172
		LBM		8	67	32	2	64	64	128	6	11	11	22
Medium	68	Commercial poultry:	Broiler	31	4409									
			Mix broiler and layer	2	1250									
			Hatchery											
			Layer	16	5778	142	7	994	994	1988	6	166	166	332
			Other							0	6	0	0	0
		Collector yard		20	161.75	61	3	183	183	366	6	31	31	62
		Broker/Collector		32	148.656	57	12	684	684	1368	6	114	114	228
		LBM		8	126.7143	51	2	102		102	6	17	17	34
High	51	Commercial poultry:	Broiler		4681.25								0	0
			Mix broiler and layer		2525								0	0
			Hatchery		3500								0	0
			Layer	11	2604.545	136	5	680	680	1360	6	113	113	226
			Other		200								0	0
		Collector yard		14	62.8571	29	3	87	87	174	6	15	15	30
		Broker/Collector		10	332.7	88	5	440	440	880		73	73	146
		LBM		2	205	69	1	69	69	138	6	12	12	24
total						922	67	5550	5448	10998	72	927	927	1854

Table 33 Stratification based on census

Enterprise type		Low(*)	Medium(*)	High(*)	Total(*)
Commercial farms:					
- Native chicken		0	2(2)	0	2(2)
- Broiler		11(8)	96(77)	17(14)	124(99)
- *Duck		0	7(5)	0	7(5)
- *Layer		7(7)	31(27)	8(8)	46(42)
- #Mixed poultry		0	5(3)	1(1)	6(4)
- Other		0	18(13)	1(1)	19(14)
	Subtotal	18(15)	159(127)	27(24)	204(166)
Collector yards/broker:					
- Slaughter facilities		51	4	3	58
- Non- slaughter facilities		66	3	2	71
	Subtotal	117	7	5	129
LBM		13	3	2	18
	Total	148	169	34	351

Table 34 Number of samples in commercial farms

Category	No of units	Average birds/ unit	Sample			
			No of farm units	No birds/unit	No of samples	No of *VTMs
Layer	46	7835	22	30	660	110
Duck	7	871	4(7)[$]	30	120 (210)	20 (35)
Mixed	5	3705	2(5)[$]	30	60(150)	10(25)
Total	58	6639	28(34)	30	780(1020)	130(170)

Table 35 Number of samples in live bird markets

Category	No of units	Sample			
		No of units	No of env/unit	No of samples(*)	No of VTMS(*)
LBM (Environment)	18	18	12(6x2)[#]	216 (432)	36(72)

Table 36 Number of samples in collector yards

Category	No of units	Sample			
		No of units	No of env/unit	No of samples(*)	No of VTMs(*)
Collector yard (environment)	129	41	12(6*2)[#]	492(984)	82(164)

Table 37 Number of samples in nomadic duck flocks

Category	No of units	Sample			
		No of farm units	No birds/unit	No of samples	No of *VTMs
Ducks	60 Actual = 20	30	30	660	110