

Investigating the endemic presence and persistence of HPAI H5N1 virus on Java, Indonesia

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Abstract

HPAI is endemic across parts of Indonesia, but the mechanisms of viral persistence in the poultry production system have not been well investigated. This mixed methods research conducted in Purbalingga District, Java characterised poultry populations and trade and contact networks and performed risk-based sampling for the active detection of HPAI virus in live bird markets, collector yards, backyard poultry, nomadic ducks and commercial farms. Approximately 60% of households kept birds, about half of which contributed towards household income. Traders tended to use multiple collector yards and live bird markets, and poultry might be presented at multiple markets before sale. Only the commercial farm sector implemented biosecurity practices and vaccination. Samples were screened for avian influenza virus (AIV) and positive samples were tested for the H5 and H9 sub-types. H5 virus was detected in all enterprise types, although there were few positive results in commercial farms, the backyard sector and nomadic duck flocks. The highest numbers of AIV, H5 and H9 viruses were found in the live bird markets and collector yards. The odds of detection of H5 in live bird markets and collector yards were similar; however, these were 3½ to 4 times higher than in backyard birds and nomadic ducks and 25 to 30 times higher than in commercial poultry. This suggests that transmission of infection in backyard poultry and duck production was likely to be driven more strongly by the value chain than by direct or indirect contacts at source. We could not determine whether the value chain concentrates or amplifies virus along its length, or whether AIV persists and actively circulates in live bird markets and collector yards. H5 and H9 viruses were detected year-round and were co-circulating in the different enterprise types, although no inference can be drawn regarding interactions between these HPAI and LPAI viruses.

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Summary

HPAI is endemic across parts of Indonesia, but the mechanisms of viral persistence in the poultry production system have not been well investigated. This mixed methods research conducted in Purbalingga District, Java characterised poultry populations and trade and contact networks and performed risk-based sampling for the active detection of HPAI virus in live bird markets, collector yards, backyard poultry, nomadic ducks and commercial farms. Approximately 60% of households kept birds, about half of which contributed towards supplementary household income. Traders tended to use multiple collector yards and live bird markets, and poultry might be presented at multiple markets before sale. Only the commercial farm sector implemented biosecurity practices and vaccination. Samples were screened for avian influenza virus (AIV) and positive samples were tested for the H5 and H9 sub-types. H5 virus was detected in all enterprise types, although there were few positive results in commercial farms, the backyard sector and nomadic duck flocks. The highest numbers of AIV, H5 and H9 viruses were found in the live bird markets and collector yards. The odds of detection of H5 in live bird markets and collector yards were similar; however, these were $3\frac{1}{2}$ to 4 times higher than in backyard birds and nomadic ducks and 25 to 30 times higher than in commercial poultry. This suggests that transmission of infection in backyard poultry and duck production was likely to be driven more strongly by the value chain than by direct or indirect contacts at source. We could not determine whether the value chain concentrates or amplifies virus along its length, or whether AIV persists and actively circulates in live bird markets and collector yards. H5 and H9 viruses were detected year-round and were co-circulating in the different enterprise types, although no inference can be drawn regarding interactions between these HPAI and LPAI viruses.

Keywords

Highly Pathogenic Avian Influenza (HPAI), endemicity, epidemiology, Social Network Analysis (SNA), mixed methods research

Introduction

The circulation of avian influenza viruses (AIV) across Asia is complex, involving the interplay of numerous risk factors. Reviewing the scientific literature, Gilbert & Pfeiffer (2012) identified three classes of variables associated with the incidence and spatio-temporal trends of HPAI H5N1 across studies and regions: domestic waterfowl, anthropogenic variables including factors such as human population density and distance to roads, and indicators of water presence. Other investigators reported associations with poultry population density (chicken as well as ducks), human population and rice cropping intensity (Pfeiffer et al., 2007; M. Gilbert et al., 2008; Loth et al., 2010, 2011). Once established, HPAI H5N1 virus spread is believed to be influenced primarily by local trade patterns, density and practices in wet markets or live bird markets (LBMs), the poultry production structure and value chain, and disease prevention and control systems (M. Gilbert et al., 2008). H5N1 HPAI outbreaks are highly seasonal in areas of South and South East Asia. This observed seasonality has been associated with higher temperature, poultry species, product movements and trade, and other anthropogenic factors (Pfeiffer et al., 2007; Minh et al., 2009; Tran et al., 2013).

Like many viral pathogens, HPAI H5N1 persists in multiple avian host species. Domestic ducks have been implicated as an important virus reservoir. Previous studies on Java reported them to be more than ten times as likely as chicken flocks to have seropositive birds, with scavenging being identified as a specific risk factor (Joerg Henning et al., 2010, 2016; J. Henning et al., 2013). A consequence of such a multihost system is that the identification reservoir hosts of infection, and knowledge of how these hosts enable and maintain persistence, is essential to inform targeted interventions (Haydon et al., 2002; Viana et al., 2014). The investigation of multihost systems is complex because they incorporate a number of interacting species, populations, and production systems (Viana et al., 2014). Moreover, they are influenced by a range of management practices, human behaviours and trade patterns. Very little is known about the year-round

persistence of the H5N1 HPAI virus.

In Indonesia, Java has the highest density of both human and poultry (Anon., 2019). HPAI is endemic across many parts of the country. Central and West Java have been identified as hotspots for HPAI (Farnsworth et al., 2011). The poultry production system in Indonesia is highly diverse and is classified into four sectors. Sector 1 is comprised of vertically integrated commercial poultry farms that implement high-level biosecurity measures and always market the bird/products commercially. Sector 2 represents somewhat smaller-scale commercial poultry farms with moderate to high biosecurity; birds/products are sold through slaughterhouses or poultry markets. Sector 3 consists of small commercial poultry businesses which typically have lower levels of biosecurity, with birds that are usually sold through live bird markets. Sector 4 is classified as backyard poultry farms, often a subsistence or side-business enterprise, with minimal biosecurity and products mainly consumed locally (Azhar et al., 2010; Farnsworth et al., 2011; Hendra Wibawa et al., 2018).

Trade, the value chain and human behaviours have been recognised as influential drivers for the transmission of HPAI. In Indonesia, Indrawan et al. (2018) applied value chain analysis (VCA) to investigate factors related to biosecurity and HPAI control in Western Java. They identified the co-existence of four different poultry value chains, ranging from high levels of coordination, hierarchical governance, and regulated marketing and distribution mechanisms (Sectors 1 and 2) to informal, smaller-scale chains (Sectors 3 and 4) which had weaker HPAI prevention and biosecurity measures. Sector 3 and Sector 4 production often occur in close geographic proximity (Sims et al., 2005; Azhar et al., 2010), and birds are likely to move between these two systems. It is therefore suspected that HPAI is transmitted between these sectors, contributing to the difficulty in controlling the disease (Farnsworth et al., 2011; Indrawan et al., 2018). In a questionnaire-based study of poultry traders at LBMs in Bali and Lombok, Kurscheid et al. (2015) found that knowledge of viral transmission and biosecurity was generally low. Two thirds of respondents were reluctant to report sudden or suspicious bird deaths to authorities. The investigators established that there was a correlation between knowledge of HPAI transmission and prevention and the number of birds sold; education was strongly associated with better knowledge but did not influence positive reporting behaviour. Elsewhere in South East Asia, Fournié et al. (2012) recorded traders' practices in Vietnam and Cambodia likely to influence the virus circulation in LBMs. These included factors related to trade patterns (e.g. the number of days during which traders were active, the length of time they spent at market in a day, the number of poultry sold within a day, the type of poultry etc.) as well as factors related to supply management (purchase and sale volumes, frequency and quantity of the surplus at the end of trading days, management of unsold poultry including sale to traders operating in other LBMs). They showed that poultry traders with high surplus frequency and volume represented a risk group for perpetuating HPAI H5N1 in LBMs.

For a complex and multi-faceted disease such as HPAI H5N1, investigative approaches focusing on a discrete area of interest are unlikely to effectively generate conclusive insights. Hence, an integration of methodologies incorporating elements including population studies, surveillance techniques and data, pathogen genetics and an understanding of production systems (including high risk points for transmission) presents the best scope to further develop understanding. A consequence of such an approach is that multiple sources of data and information need to be combined. These may be extant, or such data may be collected for the specific purpose of the investigation; these data may be qualitative, quantitative or both. Integration of findings can be performed using techniques such as meta-analysis, mathematical modelling or mixed methods research. Triangulation of multiple sources is performed to assess validity, synthesize findings and generalise inferences (Farmer et al., 2006). Such methodologies are increasingly being applied in the field of animal health. Commonly, qualitative approaches include stakeholder interviews, questionnaires or focus group discussions whereas quantitative techniques include data generated by observational studies, animal health surveillance or disease control programmes (e.g. Limon et al., 2017, 2014; Mosimann et al., 2017; Walker et al., 2015; Wu, Kagoli, Kaasbøll, & Bjune, 2018).

The overall objectives of this study were to determine the drivers of H5N1 HPAI persistence in endemic areas in Indonesia. Specific objectives were to determine where H5N1 HPAI is maintained in poultry populations in Indonesia, and how contact patterns between and within poultry production systems and enterprise types

contribute to the maintenance of virus, including interactions between these systems and the populations of birds. We also aimed to investigate the co-circulation of HPAI virus with H9 LPAI viruses. Finally, the study aimed to profile and describe the value chain and marketing systems of live bird marketing, including collector yards and live bird markets. The ultimate objective of this work was to provide the relevant authorities with information that could be applied to develop recommendations for effective disease control.

Methods

Study location

A desktop review was conducted to identify the most appropriate study area on Java. Data of HPAI outbreaks between 2008 and 2014 and poultry population statistics were gathered from the Participatory Disease Surveillance and Response (PDSR) database in the Department of Animal Health of the Indonesian Ministry of Agriculture (Azhar et al., 2010b; Farnsworth et al., 2011). Based on HPAI disease reports, Purbalingga District (Figure 1) was eventually selected because the PDSR data showed evidence of historical outbreaks of HPAI H5N1; the presence of a range of poultry production systems and species spanning all four Sectors, based on livestock statistics data (Anon., 2019); a suitable level of commitment from the local animal health authorities, plus personnel in the livestock division who had received training in PDSR; and proximity to the Disease Investigation Centre (DIC) laboratory at which sample testing was performed. Purbalingga District is located in the Province of Central Java, with an area of approximately 700 square kilometres and elevation ranging between 25 and 2815 metres above sea level. It consists of 18 sub-districts and 239 villages. The human population in Purbalingga is 848,952 with varying density across the District. Based on these demographic characteristics, Purbalingga was considered to be representative of Java Island, both in terms of its demographics as well as the range of poultry enterprise types and trade patterns.

Study design and implementation

The design of this study differed from conventional epidemiological observational studies in that it did not aim to obtain a representative sample of the population. Rather, the focus was on active detection of field virus and disease, elucidation of contact patterns and increasing understanding of potential high-risk points and practices for transmission of infection within and between different avian populations, production systems as well as enterprise types (chicken production Sectors and ducks). Consequently, this research consisted of a combination of complementary research activities, rather than a single study. Such a mixed methods research approach, which integrates quantitative and qualitative research methods, may help to improve understanding of potential biases of the observed data (Kristensen et al., 2008), and allow for a deeper exploration of the topic under investigation than can be achieved by either approach alone (Limon et al., 2014). It also provides a framework for integrating data from disparate sources (Vink et al., 2016).

Figure 2 provides a schematic overview of the study activities. An initial expert consultation meeting bringing together international experts in different fields of relevance to H5N1 HPAI was convened in November 2015 to elicit hypotheses related to reservoirs of persistence of the virus, risk factors for transmission of infection, production systems and linkages between systems. Potential methods to test and investigate these hypotheses were subsequently discussed. A subset of eight hypotheses which could feasibly be investigated in Indonesia (Figure 3) were utilised to inform the design of this endemicity study.

A stratification of enterprise types was applied, grouping Sector 1-3 commercial poultry farms, collector yards and live bird markets on the one hand, and Sector 4 backyard and non-commercial producers and nomadic duck flocks on the other. The rationale for this was that the first group was less numerous, so that a census of all properties in Purbalingga District could be included, whereas a sample needed to be taken of the second group. The sampling designs for the subsequent active sampling differed between these two strata, too.

The first part of the study aimed to characterise poultry populations and trade and contact networks; it was conducted from April to July 2016. All Sector 1-3 commercial poultry farms (n=204), collector yards (n=129) and LBMs (n=18) were visited and questionnaires were conducted by 22 trained enumerators and

25 Government of Indonesia District Animal Health Officers of Purbalingga District. If information arose about locations that had not been included, these were also visited. In addition, visits to all 239 villages in Purbalingga District were performed and questionnaire surveys were conducted in a minimum of 54 households. As there was no list of households, these were randomly selected. A total of 14,368 households were interviewed. The questionnaires included signalment information, flock location, details of numbers and species of poultry kept, information on biosecurity practices and flock management (including vaccination), purchase and sales of live poultry over the previous 12 months, information on the incidence of disease. All data were entered into a Microsoft Access database.

The second part of the study aimed to detect AIV in the different enterprise types and populations by implementing a series of surveys in two sampling rounds (October 2016 – February 2017 and August – November 2017). For Sector 1-3 commercial farms, collector yards and LBMs, risk-based, stratified sampling was performed. Following geographical stratification into high, medium and low backyard poultry population density areas, further stratification into Sector 1-3 commercial farms, collector yards and live bird markets was done. Subsequently, random selection was carried out to identify properties, assuming a design prevalence of 2% with a confidence level of 95% that at least one HPAI H5 virus would be detected. All sampling was performed by 25 District Animal Health Officers.

Thirty-two commercial farms were selected. No broiler farms were included in the study, but all commercial duck farms (n=6) were included in this selection, with the remainder consisting of layer farms. Oropharyngeal swabs were taken from a fixed number of 30 birds per property, pooling six swabs into one viral transport medium (VTM) sample. The sampling was risk-based insofar as possible, that is, any birds that showed suspect clinical signs as well as dead birds were preferentially selected. Farms were sampled once during the first round (high season) and twice during the second round (low season).

A selection of 41 collector yards was made. Twelve environmental swab samples were pooled in two VTM samples. If any birds were observed with suspect clinical signs, rapid testing and individual sampling were performed. The collector yards were sampled three times during the first round and four times during the second round.

All 18 LBMs in the District were sampled. The same sampling protocol was based on a protocol developed for previous studies in markets in Indonesia (Indriani et al., 2010). LBMs were also sampled three times during the first round and four times during the second round.

An enhanced passive surveillance scheme was initiated by offering incentive payments to District Animal Health Officers for reporting sudden mortality events in poultry, with at least one positive AIV rapid test result of sick and dead birds. If these birds had died or been removed, environmental samples were taken.

In addition to the enhanced passive surveillance in Sector 4 poultry, it was decided to expand the sampling to include nomadic duck flocks. Twelve ducks per flock were sampled and the samples were pooled into two VTMs. In the first round, all 20 flocks that were included in the questionnaire survey were sampled. In the second round, 196 flocks were sampled and the number of sampled ducks per flock was increased to 30, pooled into 5 VTM samples.

As only four reports were received using the incentive scheme, it was decided to actively sample backyard poultry in the second sampling round. As the frequency of mortality events in backyard poultry during the low season was likely to be low, in addition to which such outbreaks were unlikely to be reported by owners of such birds, a purposive sampling technique was used. Snowball sampling (also known as chain-referral sampling) is a non-probability sampling technique where initial study subjects are asked to identify future subjects from among their acquaintances; the technique is utilised for the investigation of ‘hidden’ populations which are difficult for researchers to access, or for rare conditions or events which are unlikely to be detected through randomised sampling techniques (using community knowledge or ‘rumour’). Following a sample size calculation specifying a design prevalence of 5% and a confidence level of 95%, 187 villages in Purbalingga were visited. An initial household keeping backyard poultry was randomly selected, and a questionnaire interview was conducted. In the interview, the respondents were asked if they experienced any mortality

in their poultry during the previous week and if not, if they could identify any acquaintances who may have had. If they could, this acquaintance was requested to be interviewed. If not, the respondents' closest neighbour owning backyard poultry was asked to be interviewed. Using this methodology, 54 households keeping chickens were sampled in each village. In the interviews, numbers and type of poultry were also recorded. If sick or dead bird were identified, individual swab samples were taken; if the carcasses could not be identified, environmental swabs were collected. Following this methodology, 127 households keeping backyard chickens were sampled.

Laboratory testing

All samples were transported to the Disease Investigation Centre (DIC) Wates laboratory and testing was carried out using real-time (RT) PCR to detect Influenza A viruses using primers and probes that had been validated previously (H Wibawa et al., 2017). Positive results were subsequently tested using specific primers and probes for the H5 sub-type (Heine et al., 2007), as well as the H9 sub-type. The latter, which represents LPAI viruses, was performed to investigate co-circulation of different AIV.

Analysis of data generated

Descriptive analysis was performed using the questionnaire data from the first phase, to describe the populations and pertinent husbandry and management practices. The data generated were used to perform Social Network Analysis (SNA). Contact networks were defined for each enterprise type on the basis of information provided by the respondents on poultry movements between enterprises. Centrality scores examined included in-degree, out-degree and betweenness. The properties of each network were assessed.

The results of sample testing were analysed descriptively to show differences between the enterprise types and the sampling rounds. Hypothesis tests (Fisher's Exact test) were done to investigate whether the differences between the proportions of positive H5 test results differed between the enterprise types. The results of the testing for H9 AIV were investigated to provide information on presence, persistence and co-circulation with H5 virus.

Results

Figure 3 shows a simplified output of the expert consultation workshop. A larger number of hypotheses and approaches to investigate these were identified and discussed, but these were reduced to the set of eight hypotheses which it was felt could be investigated by this study. These were applied to inform the study design, as described above.

Questionnaire data: census and surveys of enterprise types

Tables 1 and 2 present some characteristics of the poultry production sector in Purbalingga District. Figure 4 shows overview maps of the District. Of the 14,368 households surveyed, 8,925 (62.1%) kept poultry as a small-scale activity, the predominant breed being the local breed (kampong) chicken. Of these households, nearly half (3,866, 43.5%) primarily raised poultry for sale and 2,215 (24.8%) mainly kept poultry for personal consumption. The distribution of the numbers owned was skewed with over half of households (4,657, 52.2%) owning 5 chickens or less; few households (253, 2.8%) kept more than 30 chickens. 30% of households reported having had sick or dead poultry in the previous 12-month period.

Poultry density was highest in the more populated areas around Purbalingga town; the locations of collector yards appeared to be more or less correlated with village poultry density, but the live bird markets were more commonly found in proximity to access roads (with one large market in Purbalingga town). Poultry trading was carried out by approximately 60 traders and 100 collectors, about half of whom did business in more than one yard or market. These traders purchased poultry mainly from households in villages in Purbalingga. Traders aimed to not have any surplus of birds at the end of the trading day, but if there were unsold birds, these would be taken home to be sold subsequently or slaughtered. In some cases, birds would be left overnight at the market. Although some markets operated daily, the majority operated 2-3 times per week or less. Because many traders used multiple outlets for selling their poultry (depending on

days of operation and convenience), some birds would be presented at multiple markets before being sold. Slaughtering facilities were present in just under half of the collector yards, but the sale of live birds was more common.

Approximately 80% of commercial enterprises consisted of mid- to large scale layer and broiler farms, owned independently or in partnership. The rate of AI vaccination exceeded 90%, but the types of vaccine used and the vaccination schedules followed were not queried. Vaccination was not commonly practised on broiler farms due to the short production cycle. Commercial farms were spatially somewhat removed from population-dense areas.

Active disease finding: sampling and testing for HPAI

Tables 3 and 4 and Figure 5 show the numbers of pooled samples tested for AIV, H5 and H9, as well as their results. Proportionally, most AIV was found in LBMs (45 out of 249 samples tested, 18.1%). Similar quantities were found in collector yards (73 out of 607 samples), nomadic duck flocks (118 out of 904 samples) and backyard poultry (19 out of 146); all prevalences range from 12 to 13%. However, significantly more H5 viruses were found in LBMs and collector yards (13 out of 249 samples, 5.2% and 29 out of 607 samples, 4.8%, respectively). The pattern of H9 was somewhat different, with 19 out of 249 (7.6%) of samples from LBMs testing positive; similar quantities were detected in collector yards and backyard poultry (23 out of 607 samples, 3.8% and 5 out of 146 samples, 3.4%). While the prevalence of AIV was lower in Sector 3 commercial poultry, one sample tested positive for H5. About half of the AIV detected in commercial poultry were H9, the other half being non-H5, non-H9 AIV.

Overall, H9 were identified in 10/41 collector yards (25%), 10/18 LBMs (55%) and 5/31 commercial farms (16%) over the duration of the study. A number of collector yards as well as LBMs repeatedly tested positive. One LBM in particular (M-12) was positive at every sampling. No commercial farm tested positive more than once; however, these farms were only sampled half as frequently. As the sampling no or limited repeat sampling was carried out in backyard and nomadic duck flocks, we were unable to assess this in these sectors.

Table 5 shows the pairwise statistical tests for significant differences between enterprise types of detection of H5. The odds ratio between LBMs and collector yards was about 1 and the difference was not statistically significant. However, the odds between both of these enterprise types and commercial farms was in the order of 25 to 30 (and highly significant); nomadic duck flocks, 3.5 to 4 (and highly significant); and backyard poultry, 3.5 to 4 (and marginally significant). Both nomadic duck flocks and backyard poultry had approximately 7 times higher odds than commercial farms, but the association between backyard poultry and commercial farms was not significant, probably due to the small numbers of detections.

Social Network Analysis

Results are shown in Table 6 and Figure 6. 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).

Discussion

A key assumption of this study was that HPAI H5N1 is endemic in Purbalingga District. The concept of endemicity assumes firstly that the virus is permanently present. H5 virus was detected throughout the period of active sampling, and in all enterprise types (albeit at very low levels in backyard and commercial poultry). Therefore, we conclude that H5N1 HPAI may be assumed to be endemic in Purbalingga District, and that all poultry enterprise types have at least the potential to contribute to HPAI spread and persistence. Extrapolating this, if the conditions in Purbalingga District can be considered representative of a wider area (e.g. Java), it is likely that H5N1 HPAI is endemic in this wider area (although it may not be endemic in all areas, all year round).

Study design

The expert elicitation workshop integrated current knowledge, structured the thinking and enabled a coherent set of hypotheses to be defined which could feasibly be investigated. Eight of these hypotheses were subsequently identified for investigation by this study. These hypotheses effectively clustered into two areas: population reservoirs of AIV (commercial and backyard chickens and ducks) and system linkages (environmental sampling in LBMs and collector yards; traders as links between enterprise types; movements and contacts between poultry populations). Due to the complexity of designing one study which could accommodate all these aspects, in combination with the challenge of investigating a disease agent which is maintained at low levels in the source poultry populations, the design was complex, with multiple strands of investigation being incorporated. The Phase 1 surveys led to a better description and understanding of the structure of the poultry production system as well as the value chain. Social Network Analysis provided further evidence on contact structures which may influence transmission, although it is unfortunate that the nomadic duck contact structure could not be determined. Subsequently, the Phase 2 active disease sampling aimed to determine if, when and where AIV could be detected in an endemic context. An approach combining these methodologies in a mixed methods framework allowed these strands of investigation to be drawn together, enabled several of the study hypotheses to be refined, and could inform further discussion. Triangulation strengthened the external validity of the combined results and enabled a synthesis of these disparate outputs to form a coherent narrative.

Part 1: Census and survey

Poultry husbandry and production is a ubiquitous and heterogeneous activity in Purbalingga District. Small-scale poultry keeping is common (approximately 60% of households kept small numbers of birds, nearly half of which contributed towards supplementary household income). Although 30% of households reported having had sick or dead poultry in the previous 12-month period, these mortalities were not reported to any surveillance system. The distribution of backyard poultry density (highest in higher-populated areas), collector yards (correlated with backyard poultry density), live bird markets (in urban centres and in proximity to roads) and commercial farms (somewhat removed from human population dense areas) was much as would be expected. Nomadic duck flocks consisted of relatively large flocks which were grazed on rice paddy fields post-harvest, often on contract basis (consistent with the description of Henning et al., 2010). Approximately 20 flocks were found to move from village to village per 2 week period, within Purbalingga but also to and from neighbouring Districts. Although biosecurity was not consistently practised, H5N1 vaccination was carried out. However, the type of vaccination and vaccine schedules could not be ascertained.

Commercial production units mostly consisted of Sector 3 layer and broiler chickens, with a smaller number of duck farms, and a small number of Sector 2 and Sector 1 farms. AI vaccination was most commonly performed in the layer sector, and to intents and purposes not at all in the backyard sector. Nomadic duck farmers reported using vaccine, but the type of vaccine and vaccination schedule could not be reliably recorded.

It was common practice for live birds at collector yards to be taken to other villages (by buyers) after sale. Traders at LBMs often did not sell all of their birds (collected from different sources); surplus birds were taken back to their homes. These traders reported attending multiple markets to sell their birds. Although the commercial sector was not entirely closed, there were less opportunities for transmission of infection than between backyard poultry and ducks. Moreover, transmission between these smallholder populations was likely to be driven more strongly by the value chain and by the husbandry system than by direct or indirect contacts in the source populations. As a consequence, clinical outbreaks are more likely to manifest as ‘jumps’ between villages or locations than showing a pattern of local spread.

Part 2: Active sampling

As the focus was on active detection of HPAI virus, sampling was targeted and risk-based, and did not aim to achieve a representative sample. Nomadic ducks were undersampled during the first round, and proportionally oversampled during the second round. No village poultry were actively sampled during the

first round; the incentivised passive surveillance was unsuccessful in locating cases or focal outbreaks and was discontinued. Modified snowball sampling was used to perform risk-based sampling in the second round, but the number of samples taken was comparatively low.

Overall, the number of HPAI virus isolates identified was relatively small. H5 virus was detected in all production systems. The differences in detection of AIV and H5 virus between enterprise types and between the two rounds were found to be statistically significant, with significantly more AIV detections during the second round. It could not be determined whether this was a result of differences in the sensitivities of detection between the two rounds, or the consequence of an underlying causal trend. As a consequence of the study design, the scope for model-based analytic approaches was limited and the analysis was mostly descriptive; statistical testing was restricted to hypothesis testing to investigate strengths of association and differences between the enterprise types.

Only the commercial farm sector implemented biosecurity practices and vaccination; however, the extent of implementation was variable between farms and there was evidence that H5 virus was present, albeit at an extremely low level. “Silent spread” and persistence of HPAIV in sub-optimally vaccinated commercial flocks is a concern. In a comparative trial, Tarigan et al. (2018) used seven different commercial vaccines produced in Indonesia mostly based on the H5N1 subtype; vaccination led to highly variable outcomes, including vaccination failures, and was largely ineffective in providing long-lasting protective immunity. Poetri et al. (2014) performed an inoculation trial and showed that a single vaccination applied under field conditions induced clinical protection but was insufficient to induce protection against virus transmission. Given reported movements of birds between Sectors 3 and 4, there is a potential risk of ongoing introduction of H5N1 in commercial flocks. Furthermore, in contrast to the essentially local nature of poultry trade patterns of LBMs and collector yards, the commercial farm network was characterised by longer range movements, which had the potential to spread virus furthest geographically. However, as such movements were mainly to slaughter, this would be unlikely to result in long-distance spatial spread of HPAI.

Snowball sampling was performed to maximise the likelihood of detection of H5 in backyard and nomadic ducks flocks. Such approaches have been successfully used in Cambodia (Poolkhet et al., 2016) and Vietnam (Delabougliise et al., 2016). Despite this, very few H5 samples were detected in the backyard sector and in nomadic ducks. It was evident that underreporting of clinical cases is an issue. Our results indicated that although present in smallholder production systems, the prevalence of AIV appears to be low. Consequently, control measures focusing on this sector may target reporting behaviours, but any direct disease control measures are not indicated.

It was apparent that the largest numbers as well as the highest prevalences of AI, H5 as well as H9 viruses were found in the value and marketing chain – at LBMs and collector yards. This was supported by the pairwise statistical tests for significance between enterprise types. These showed that the odds of detecting H5 were similar in LBMs and collector yards and not statistically different; however, the likelihood of detection at these locations was $3\frac{1}{2}$ to 4 times higher than for backyard birds and nomadic ducks (these differences were marginally to highly significant) and 25 to 30 times higher than for commercial poultry (these differences were highly significant). This finding is consistent with results elsewhere in South and South East Asia. It could not be determined from our results whether the value chain concentrates or even amplifies virus along its length, or whether the LBMs and collector yards are locations in which AIV persists and actively circulates (or, indeed, a combination of both). Our SNA suggested that LBMs represented high-risk enterprises due to their small world network properties which would enable disease spread more rapidly. Collector yards played a role as a bridge between enterprises spreading the virus. Commercial farms had a possible role in the long-range spread of HPAI virus.

The pattern for H9 was somewhat different. More samples from commercial farms tested positive than would be expected; approximately half of all the AIV detected were H9. Equally surprisingly, relatively few H9 were identified from nomadic ducks. Overall, H9 were identified in 10/41 collector yards (25%), 10/18 LBMs (55%) and 5/31 commercial farms (16%) over the duration of the study. A number of collector yards as well as LBMs repeatedly tested positive. One LBM in particular (M-12) was positive at every sampling. No

commercial farm tested positive more than once; however, these farms were only sampled half as frequently. As the sampling no or limited repeat sampling was carried out in backyard and nomadic duck flocks, we were unable to assess this in these sectors. Therefore, although there was some variation, the H5 and H9 viruses were present year-round and were co-circulating, although no inference can be drawn regarding any interactions between these HPAI and LPAI viruses.

It is likely that this higher rate of detections represents a potential public health risk. For example, in one study (Shimizu et al., 2016) 85 of 101 sera that were collected from workers at a LBM in East Java tested positive for AIV antibody; over a two-year period, the average HI titre nearly trebled, and seroconversion occurred in 11 of 25 sera (44%). However, a questionnaire indicated that no workers had experienced an episode of severe acute respiratory illness.

Contact patterns between enterprise types

Small world networks have similar average path lengths, but greater clustering than an equivalent random network. The LBM network exhibited small world network properties, implying that particular members of this network were well-connected and linked, so that in the event of HPAI outbreaks, local disease spread would likely take place to poultry enterprises connected with the LBM. These markets should be targeted for surveillance and control. The collector yard network had the highest betweenness score and had a high number of daily operations. This implies that they acted as important connectors within the poultry trade system. Our results indicated that there was unrestricted flow of poultry movements between collector yards. HPAI control measures could be targeted at these collector yards to prevent disease transmission to commercial farms and LBMs.

Commercial farms sold poultry within the existing trade network as well as to markets as distant as Jakarta, hence the commercial farm network had the largest number of nodes and spanned 10 Districts, including long-range movements. Wibawa et al. (2018) investigated the rate of contacts between production Sectors. They found that most enterprises were not closed, and that visits to and from other poultry farms were common. Hence, transmission of HPAIV between sectors was possible. While we were unable to investigate the nomadic duck contact network, other investigators have consistently shown that presence of nomadic duck flocks is an important risk factor for highly pathogenic avian influenza outbreaks. For example, Henning et al. (2016) described the movement of flocks and characterised the network in Districts neighbouring Purbalingga. They found that transporters of nomadic duck flocks, hatcheries and rice paddy owners were likely to play an important role in the spread of the HPAI virus. Meyer et al. (2017) performed a similar study describing the long-distance free-grazing duck production system in South Vietnam, and showed that both direct and indirect contacts between free-grazing duck flocks were frequent and diverse.

The information generated by the different elements of the study were generally consistent with the scientific literature and the mixed methods approach was useful to draw together the evidence into a coherent narrative. Although the study design and the data generated by the study could not easily be applied for statistical models, the information and data can be utilised to inform exploratory transmission models.

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Conflict of Interest Statement

The authors declare that there are no potential sources of conflict of interest related to publication of the work described in this article.

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