A systematic approach to illuminate a new hot spot of avian influenza virus circulation in South Vietnam, 2016-2017

Short running title

A new AI hotspot in South Vietnam

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Summary

In South Vietnam live bird markets (LBMs) are key in the value chain of poultry products and spread of avian influenza virus (AIV) although they may not be the sole determinant of AIV prevalence. For this reason, a risk analysis of AIV prevalence was conducted accounting for all value chain factors. A cross-sectional study of poultry flock managers and poultry on backyard farms, commercial (high biosecurity) farms, LBMs and poultry delivery stations (PDSs) in four districts of Vinh Long province was conducted between December 2016 and August 2017. A total of 3,597 swab samples were collected from birds from 101 backyard farms, 50 commercial farms, 58 sellers in LBMs and 19 traders in PDSs. Swab samples were submitted for AIV isolation. At the same time a questionnaire was administered to flock managers asking them to provide details of their knowledge, attitude and practices related to avian influenza. Multiple correspondence analysis and a mixed-effects multivariable logistic regression model were developed to identify enterprise and flock manager characteristics that increased the risk of AIV positivity. A total of 274 birds were positive for AIV isolation, returning an estimated true prevalence of 7.6% (95% confidence interval [CI]: 6.8% to 8.5%). The odds of a bird being AIV positive if it was from an LBM or PDS were 45 (95% CI: 3.4 to 590) and 25 (95% CI: 1.4 to 460), respectively, times higher to the odds of a bird from a commercial poultry farm being AIV positive. The odds of birds being AIV positive for respondents with a mixed (uncertain or inconsistent) level and a low level of knowledge about AI were 5.0 (95% CI: 0.20 to 130) and 3.5 (95% CI: 0.2 to 62), respectively, times higher to the odd of birds being positive for respondents with a good knowledge of AI. LBMs and PDSs should receive specific emphasis in AI control programs in Vietnam. Our findings provide evidence to support the hypothesis that incomplete respondent knowledge of AI and AIV spread mechanism were associated with an increased risk of AIV positivity. Delivery of education programs specifically designed for those in each enterprise will assist in this regard. The timing and frequency of delivery of education programs are likely to be important if the turnover of those working in LBMs and PDSs is high.

Keywords: avian influenza; Vietnam; poultry delivery station; knowledge attitude and practice survey

Introduction

Avian influenza (AI) virus circulation has been reported in many countries, including Vietnam (FAO, 2021). Particularly, since 1996 outbreaks of high pathogenicity avian influenza (HPAI) have occurred in poultry throughout Asia and Southeast Asia despite large-scale vaccination campaigns and stamping-out programs in a number of countries (Alexander, 2007; Brown, 2010). Although the number of HPAI outbreaks in Vietnam due to infection with H5N1 subtype viruses has markedly decreased since 2004 (FAO Vietnam, 2017) substantial losses in the domestic poultry sector continue to occur. A number of studies have improved our understanding of the epidemiology of avian influenza by identifying drivers of virus spread (Nomura et al., 2012; Okamatsu et al., 2013; Nguyen et al., 2014; Chu et al., 2016, 2017; Nguyen et al., 2020). As part of their efforts to reduce AIV infection risk, the Vietnamese government has developed both active and passive surveillance programs. One of the advantages of active surveillance programs is that they can detect the introduction of new virus strains into a population or detect the evolution of virus strains relatively quickly. In contrast, passive surveillance programs rely on prompt reporting by poultry farmers for timely disease event detection.

The results of data collected by active surveillance programs that have been operational in Asia, Europe, and North America since 2014 show that diversification of AIV subtypes has increased (Li et al., 2013; de Vries et al., 2015). Despite some AIVs being categorized as low pathogenicity (LPAIV), LPAIV can cause substantial poultry production losses such as high rates of mortality and reductions in egg production (Kinde et al., 2003) and pose a concern for global health security arising from the risk of zoonotic infection. Due to variations in the pathogenicity of AIVs dependent on subtype it is essential to monitor virus subtypes circulating in the field (Pfeiffer et al., 2013).

In a number of previous studies the movement of live birds arising from trade has shown to be an important determinant of AIV spread (Kung et al., 2007; Kim et al., 2010; Gilbert et al., 2014). In addition, live bird markets (LBMs) play an important role in AIV circulation (Bulaga et al., 2003; Choi et al., 2005; Chen et al., 2009; Indriani et al., 2010; Kang et al., 2015). During an outbreak of H7N9 AIV in China in 2013 which was the cause of up to 45 human deaths, the closure of LBMs was remarkably effective in reducing human infection rates by up to 99% (Yu et al., 2014). Although LBM closures break the viral amplification cycle, AIVs are often re-introduced once they are re-opened (Kung et al., 2003). A previous Vietnamese study investigating the effectiveness of virus control measures in LBMs showed no differences in AIV prevalence between LBMs with and without biosecurity interventions (Chu et al., 2017). One interpretation of these findings is that the introduction of AIV into LBMs occurs continuously. The absence of differences in AIV prevalence between intervention and non-intervention LBMs supports the hypothesis that the source of AIV in the value chain of poultry products in Vietnam has not yet been fully identified and controlled.

As a result of active surveillance programs for AI that have been operational in Vietnam since 2015, it was shown that poultry delivery stations (PDSs) play a role connecting poultry farms, LBMs and poultry slaughterhouses (Supplementary Figure 1). Backyard farms are characterized by their small-scale, the mixing of poultry species and relatively low levels of biosecurity whereas commercial farms routinely practice several AIV control measures such as separating poultry species, routinely disinfecting those entering and leaving premises and limiting contact between poultry and wildlife. LBMs tend to receive poultry from nearby backyard and semi-commercial poultry enterprises (Phan et al., 2013). In contrast, PDSs are private businesses which usually receive birds from much larger catchment areas (up to 100 km) and mix several species of poultry under relatively poor biosecurity conditions.

We conducted a cross-sectional study of avian influenza and biosecurity practices among four poultry enterprise groups (backyard farms, commercial farms, LBMs and PDSs) in Vinh Long Province, Vietnam in 2016 and 2017. Our specific aims were to: (1) estimate the individual bird-level prevalence of AIV in each of the four enterprise groups; and (2) identify characteristics of those responsible for the management of birds that were associated with AIV infection positivity. Identifying poultry flock manager characteristics that increase the risk of AIV positivity across different industry players is a necessary step towards the design of effective, evidence-based measures to reduce the risk of AIV infection through the supply chain of poultry products in Vietnam.

Materials and methods

Study design and study area

This was a cross-sectional study of owners of backyard poultry farms, managers of commercial poultry farms, poultry sellers at LBMs and PDS traders in four of the eight districts of Vinh Long Province, Vietnam (Figure 1). Data were collected over two sampling rounds: the first in December 2016 and the second in August 2017. From a sampling frame of enterprises provided by local Department of Animal Health (DAH) officials those eligible for the study were selected at random from each of the four poultry enterprise groups. The key decision maker of each selected enterprise was contacted by the authors and asked if they consented to take part in the study. A total of 228 decision-makers agreed to take part representing 101 backyard farms, 50 commercial poultry farms, 58 sellers at LBMs and 19 traders at PDSs. For the purpose of this study enterprises that had not applied any prevention measures following local authority guidelines such as keeping poultry in a separate place, vaccination, and disinfection were defined as backyard farms. Enterprises, where at least more than one of several control measures (such as keeping poultry in a separate place, the use of routine vaccination and disinfection) were applied, were defined as commercial poultry farms. Up to two LBMs from each of the four study districts of Vinh Long were selected at each of the two sampling rounds leading to a total of 12 individual LBMs included in the study. Similarly, up to two PDSs per study district were selected at each sampling round returning 13 individual PDSs included in the study. In each of the two sampling rounds, the average number of birds sampled was 10 for backyard farms (minimum of 5, maximum of 20), 26 for commercial poultry farms (minimum of 10, maximum of 50), 11 for LBM sellers (minimum of 10, maximum of 52) and 40 for PDS traders (minimum of 19, maximum of 52). At the time of bird sampling key decision makers from selected backyard farms, commercial poultry farms, LBM traders and PDSs were interviewed by the first author and staff from the Sub-Department of Animal Health (SDAH) staff of Vinh Long Province for the purpose of questionnaire administration.

Laboratory procedures

Oropharyngeal swabs, cloacal swabs and fecal samples were collected from chickens, ducks, and Muscovy ducks from each participant enterprise at each sampling round. The oropharyngeal and cloacal swabs from the same poultry were kept in one sterile tube containing transport medium, as described previously (Le et al., 2020). This medium comprised Eagle's minimum essential medium (Nissui, Japan) containing 10,000 U/mL penicillin G (Meiji Seika, Japan), 10 mg/mL streptomycin (Meiji Seika, Japan), 0.3 mg/mL gentamicin (Schering Plough, USA), 250 U/mL nystatin (Sigma, USA), and 0.5% bovine serum albumin fraction V (Roche, Switzerland). Samples were transported to the Regional Animal Health Office No. 7 (RAHO7), Can Tho, Vietnam. Under ISO 17025:2017 certification for the diagnostic procedure in RAHO7, the aliquot of ten samples collected from the same enterprise were pooled to test for the presence of influenza type A virus using real-time reverse transcription-polymerase chain reaction (RT-PCR) targeting the M gene with the primer design and thermal cycle (Das et al., 2006) following methods described by the World Organisation for Animal

Health (OIE, 2018). All samples were then transferred to the Laboratory of Microbiology in the Faculty of Veterinary Medicine, Hokkaido University, Japan for virus isolation.

Virus isolation

Ten-day-old chicken embryonated eggs produced by conventional chickens tested for freedom from AIV antibody by ELISA were used to isolate AIV. The M-gene-positive pooled samples were selected for virus isolation. Each of ten samples was resuspended with transport medium and inoculated into the allantoic cavity. Inoculated eggs were incubated for 30 to 48 hours at 35 °C and the allantoic fluid collected to check the hemagglutination activity. The hemagglutination inhibition and neuraminidase inhibition tests with antisera to the reference influenza virus strains were performed to determine isolated influenza virus subtypes (Kida & Yanagawa, 1979).

Questionnaire and interview

By referring to previous survey documents developed by the Vietnamese DAH, Ha Noi, a questionnaire to collect details of knowledge, attitudes, and practices regarding AIV was developed in partnership with provincial DAH staff. This questionnaire was then modified to suit the specific conditions for respondents from backyard farms and commercial poultry farms, LBM sellers and PDS traders. In detail, the questionnaires comprised of 87, 82 and 118 questions were established for farms, LBM and PDS, respectively. All three questionnaires asked key decision makers (referred to as 'respondents' in the remainder of this paper) to provide details on: (1) their demographic status; (2)

the source, type and numbers of poultry present on their enterprise on the day of interview; (3) their general knowledge regarding AIV; (4) their attitudes about AI control measures; and (5) AI biosecurity measures routinely used.

At the start of the first sampling round SDAH staff from Vinh Long (n = 8) who were recruited for data collection received instruction on questionnaire administration. Questionnaire surveys were administered by SDAH staff to each respondent. A total of 228 face-to-face interviews were carried out during the two sampling rounds in the four districts. In each sampling round, birds were sampled and questionnaires administered to key decision makers on each of the participant backyard farms and commercial farms in the early stage. Immediately after the early stage was finished, the same procedure was then applied in LBMs and PDSs at the later stage. The sampling schedule was announced to respondents and local veterinarians well in advance and, for both rounds, samples were collected and questionnaires administered over a period of 8 days.

Data management

Each of the respondents enrolled into the study were assigned a unique identification code. Questionnaire responses at each sampling round and the results of AIV isolation from sampled poultry were recorded in two tables in a relational database with the respondent identification code providing the link between each table.

The diagnostic sensitivity and specificity of the RT-PCR was assumed to be both 100% (Das et al., 2006). Furthermore, the diagnostic sensitivity and specificity of virus isolation was assumed to be both 100% (Suarez et al., 2007).

Multiple correspondence analysis

Multiple correspondence analysis (MCA) (Snijders & Bosker, 1999) was used to produce a graphic representation of the relationships between responses provided in each of the four sections of the questionnaire: demographic details, AIV knowledge, AIV attitude and AIV practice.

MCA is a generalization of principle component analysis suitable for categorical variables. In an MCA, the rows and columns of an $I \times J$ indicator matrix (where I is the set of i individual responses to a given question and J is the set of j categories of responses for each question) are assumed to be points in a high-dimensional Euclidean space. The method aims to redefine the dimensions of the space so that the principal dimensions ('components') capture the most variance. The results of an MCA are presented as a scatterplot for the first and second principle components – that is, the dimensions that capture most of the variability in the data. In an MCA scatterplot, questionnaire responses that are similar in distribution across respondents are positioned close on the plot. MCA scatterplots were produced using responses to each of the four sections of the questionnaire (demographic details, AIV knowledge, AIV attitude and AIV practice) and, for each plot, cluster analysis using hierarchical clustering on principal components (HCPC) was carried out using Ward's method. This allowed us to aggregate respondents into relatively homogeneous subgroups ('clusters') for each section of the questionnaire. These assigned clusters were then used as explanatory variables in a multivariable logistic regression model of bird-level AIV infection risk. Our MCA analyses were performed using the contributed FactoMineR package (Husson et al., 2008) in R version 4.0.5 (RCoreTeam, 2021).

Mixed-effects logistic regression

A mixed-effects logistic regression model was developed to quantify the association between respondent-level explanatory variables and the risk of a bird being AIV positive at the time of sampling. Unconditional associations between each of the explanatory variables and the outcome variable (AIV status) were expressed as the odds ratio. Explanatory variables associated with the outcome at P < 0.2 (two-sided) at the unconditional level were selected for multivariable modeling.

For our multivariable model, the probability that a bird was AIV positive p_i was parameterized as a function of the candidate cluster variables (as described above) in addition to a single categorical variable comprised of four levels defining respondent enterprise type (backyard farm, commercial poultry farm, LBM seller and PDS trader). If Y_i defines AIV positivity status for the ith bird this model takes the following form under the assumption of $p_i = P(Y_i = 1)$ and that Y_i are mutually independent:

$$log\left(\frac{p_i}{1 - p_i}\right) = \beta_0 + \beta_1 x_{1i} + \cdots + \beta_m x_{mi} + \epsilon_i \qquad \text{Equation 1}$$

In Eq. (1) β_0 represents the intercept term and $\beta_1, \dots, \beta_m$ the regression coefficients for each of the m explanatory variables in the model.

To account for the lack of independence arising from the hierarchical structure of the data, that is, individual birds clustered within respondents Eq. (1) was extended to a mixed-effects model as follows:

$$log\left(\frac{p_{ij}}{1-p_{ij}}\right) = \beta_0 + \beta_1 x_{1ij} + \cdots + \beta_m x_{mij} + P_j + \varepsilon_{ij} \qquad \text{Equation 2}$$

In Eq. (2), p_{ij} represents the probability of the ith bird from the jth respondent being AIV positive. Variable P_j is a zero mean random effect term with variance σ_P^2 indicating the effect of the jth respondent on AIV positivity. The term P_j was included in the model to account for unexplained extrabinomial variation arising from unmeasured respondent-level influences on AIV positivity.

A backward stepwise approach was used for explanatory variable selection. Each of the explanatory variables unconditionally associated with the outcome at P < 0.2 were included in the fixed-effects model (Equation 1). Explanatory variables were removed from the model, one at a time, starting with the least significant until all variables that remained were associated with the outcome at $\alpha < 0.05$. Explanatory variables that were excluded in univariable analyses were tested for inclusion in the final model and were retained if their inclusion changed any of the estimated regression coefficients by more than 20%. Biologically plausible two-way interactions between explanatory variables were assessed: none were found to be significant at $\alpha = 0.05$. The model was then extended to include the random effect term P_i (Equation 2). Explanatory variables were retained in the mixed-effects model, regardless of their statistical significance.

The assumptions of normality and homogeneity of variance were investigated by constructing histograms of residuals from the multilevel model and scatterplots of the residuals as a function of the predicted values, respectively. Estimates of the variance attributable to the three levels of the data (respondent, bird) were calculated assuming the level 1 (bird) variance on the logit scale was $\frac{\pi^2}{3}$ where π = 3.1416 (Snijders & Bosker, 1999).

A Receiver Operating Characteristic (ROC) curve was constructed on the basis of the bird-level AIV positivity status predicted by the model. The area under the ROC curve, which ranges from zero to one, provided a measure of the model's ability to discriminate between AIV-positive and AIV-negative birds. The greater the area under the ROC curve the better the model's discriminatory power.

Our unconditional measures of association analyses were carried out using the contributed epiR package (Stevenson et al., 2021) in R. The mixed-effects logistic regression model was developed using the contributed lme4 package (Bates et al., 2015) in R.

Results

Descriptive statistics and unconditional associations

Details of the number of birds sampled, the number of samples AIV positive and the prevalence of AIV positivity stratified by enterprise type, species, sampling round and district are shown in Table 1. A total of 3,597 birds were sampled: 1,056 from 101 backyard farms; 1,200 from 50 commercial poultry farms; 660 from 58 sellers at 12 LBMs and 681 from 19 traders at 13 PDSs. Two hundred and seventy-four of 3,597 birds (7.6%; 95% confidence interval [95% CI]: 6.8% to 8.5%) were AIV positive. In total, 13 H3N2, 21 H5N1, 127 H6N6, 105 H9N2, 2 H10N3, 5 H11N9, and 1 H12N5 AIVs were identified from collected samples (Supplementary Table S1). Isolation rates for AIV varied by poultry enterprise type (Figure 2) with the highest prevalence among birds sampled from PDSs (21%, 95% CI: 18% to 24%), followed by LBMs (14%, 95% CI: 12% to 17%), backyard farms (3.0%, 95% CI: 2.1% to 4.3%) and commercial poultry farms (0.6%, 95% CI: 0.2% to 1.2%).

The numbers of chickens and ducks sampled were 1,801 (50%) and 1,575 (44%), respectively. Because the total number of Muscovy ducks, geese and environment samples was only 221 (6.1%), only AIV positivity for chicken and duck samples were compared. The prevalence of AIV positivity for ducks (10%, 95% CI: 8.5% to 12%) was statistically significantly higher than the prevalence of AIV positivity for chickens (5.6%; 95% CI: 4.5% to 6.7%; χ^2 test statistic 23.29; *df* 1; $P < 0.01$). This result reflects the field situation in that the environment in which ducks are typically kept facilitates AIV survival, much more so than that of the environment in which chickens are kept. The prevalence of AIV positivity differed across the two sampling rounds with a lower prevalence in 2016 (5.9%; 95% CI: 4.9% to 7.1%) compared with 2017 (9.4%; 95% CI: 8.1% to 11%).

Multiple correspondence analysis

The results of our MCA analyses are based on questionnaire responses from key decision makers of 100 backyard farms and 40 commercial poultry farms, 58 sellers from LBMs and 19 PDS traders. MCA scatterplots developed from responses to each of the four sections of the questionnaire (demographic details, AIV knowledge, AIV attitude and AIV practice) are shown in Figures 3a to 6a. Accompanying each scatterplot is an error bar plot showing the prevalence of AIV positivity as a function of the identified cluster group, stratified by enterprise type (Figures 3b to 6b). In an MCA scatterplot, the relationships among categories of questionnaire responses are reflected by the distance between pairs of marks with questionnaire responses further from the origin more discriminating in the data. Superimposed on each MCA scatterplot (Figures 3a to 6a) are ellipses delineating the clusters identified using the hierarchical clustering on principal components method. Details of the questionnaire responses for each identified cluster are provided in Tables 2 to 5. In effect, the above tables are interpreted as the 'profiles' for questionnaire responses of respondents through demographic details, AIV knowledge, AIV attitude, and AIV practice section.

In the demographic section of the questionnaire, three clusters were identified (Table 2). The first (n = 158) was comprised predominantly of female respondents from backyard farms working with poultry for up to 10 years. The second (n = 46 respondents) were mostly males from LBMs working with poultry for a shorter period of time, up to five years. The third, smaller cluster (n = 13 respondents) was similar to the second with the exception that a greater proportion working with poultry for more than 10 years. In Table 2 and Figure 3, the first, second and third clusters are labelled 'Female backyard', 'Male LBM ≤10 yrs' and 'Male LBMs >10 yrs', respectively.

For AIV knowledge three clusters were identified (Table 3). The first cluster (n = 29 respondents) was comprised predominantly of those that had heard about AI and knew that infected birds were a source of infection, primarily domestic poultry and interactions with those from backyard farms, commercial farms, LBMs and PDSs. Most in this cluster obtained their information about AI from the television and local veterinarians; 59% had seen AI before, and most had received training on AI control and prevention. Respondents in the second cluster (n = 67) were evenly divided in terms of having heard about AI. Questions regarding the way how AIV can be spread (by domestic poultry, wild birds, domestic animals) were similarly evenly split. Most in this cluster obtained information about AI from the radio and local veterinarians. Interestingly, 94% of those in this cluster had attended training on AI control and prevention. The third cluster (n = 121 respondents) was comprised predominantly of those that had heard about AI but were not so sure which was the cause of AI.. While those in this cluster were generally not of the belief that AI could be spread by domestic poultry, wild birds, domestic animals (apart from poultry) and interactions with other poultry farmers, poultry traders and LBMs at a reasonably high proportion were of the belief that AIV could be spread by interactions with those from backyard poultry farms. Most in this cluster obtained information about AI from the television and less than 50% receiving information from their local veterinarian. Most in this cluster (70%) had not seen AI and had not received formal training on AI control and prevention (76%). In Table 3 and Figure 4 the first, second and third clusters are labelled 'Good knowledge', 'Mixed knowledge' and 'Low knowledge', respectively.

For AIV attitudes, three clusters were identified (Table 4). For the first, all respondents (n = 55) were willing to report an AI outbreak if detected, mostly to local veterinarians (87%) but not to local Department of Animal Health officials. For the second cluster (n = 41) there was relatively even split between willingness to report an AI outbreak if detected (44% yes; 56% no). If an outbreak was to be reported, it would be to a local Department of Animal Health official. For the third cluster (comprised of n = 121 respondents) all declared that they would not be willing to report an AI outbreak if detected. If an outbreak was to be reported, 55% of them stated that they would report to local veterinarians and 100% stated that they would not report the outbreak to a local Department of Animal Health official. In Table 4 and Figure 5 clusters 1, 2 and 3 are labelled 'Report AI yes', 'Report AI mixed' and 'Report AI no', respectively.

Finally, for AIV practice two clusters were identified (Table 5). Respondents that comprised the first cluster (n = 135) mostly kept chickens (90%) and around 40% of them used personal protective equipment when handling live or dead birds. This group disposed of dead birds using usual methods for garbage disposal and were less likely to manage sick birds by selling them. Respondents that comprised the second cluster (n = 82) kept a mix of poultry species (chickens, ducks and Muscovy ducks), did not generally use personal protective equipment when handling live or dead birds, disposed of dead birds using usual methods for garbage disposal and sold sick birds. In Table 5 and Figure 6, clusters 1 and 2 are labelled 'High biosecurity' and 'Low biosecurity', respectively.

Multivariable logistic regression analyses

Estimated regression coefficients for enterprise type, knowledge cluster and attitude cluster and estimates of the variability of the farm and bird-level random effect terms from the mixed-effects logistic regression model are shown in Table 6. Not surprisingly, with the marked difference in AIV prevalence by enterprise type, the odds of a bird being AIV positive if it was from an LBM or PDS was 45 (95% CI: 3.4 to 590) and 25 (95% CI: 1.4 to 460), respectively, times higher to the odds of a bird from a commercial poultry farm being AIV positive. Although cluster 1 ('Good knowledge') in the AI knowledge section and cluster 1 ('Report AI yes') in the AI attitude section showed the difference in the odds of birds being AIV positive, the significant difference was not recorded.

After adjusting for the fixed effects included in the model, the proportions of unexplained variance at the enterprise and bird level was $10.37 \div (10.37 + \frac{\pi^2}{3})$ = 0.76 and $\frac{\pi^2}{3} \div (10.37 + \frac{\pi^2}{3})$ = 0.24, respectively. The area under the ROC curve for the fixed-effects model was 0.81, indicating a satisfactory to good ability to discriminate between AIV-positive and AIV-negative birds. The area under the ROC curve for the mixed-effects model was 0.98.

Discussion

This cross-sectional study quantified the prevalence of AIV positivity among poultry from backyard farms, commercial poultry farms, live bird markets (LBMs) and poultry delivery stations (PDSs) in Vinh Long province over two sampling rounds in 2016 and 2017. In Vietnam control measures for AI have been applied to backyard poultry farms, commercial poultry farms and LBMs since the first outbreaks of AI were reported in 2003. Our finding that one in five poultry sampled from PDSs were AIV positive (21%; 95% CI: 18% to 24%, Table 1) demonstrate a relatively high prevalence of AIV in poultry in this sector and indicate that PDSs should receive emphasis for interventions in AI control programs. Unlike LBMs, where control measures for AI are supervised by local veterinarians and supported by local government authorities, AI control measures in PDSs are primarily implemented by PDS traders themselves mainly because PDSs are not recognized as official areas. The inevitable variability in the application and effectiveness of sanitary measures that occurs as a result makes the relatively high prevalence of AIV positivity a not unexpected finding. Our results support the proposal that PDSs receive AI control measure oversight similar to that applied to LBMs (Manabe et al., 2011). These findings are consistent with the cross-sectional study of Soares Magalhães et al. (2010) who identified wholesale markets as hot spots for AIV circulation in greater Ha Noi in 2006 and 2007 and the study of (Meyer et al., 2017) who found that PDSs and PDS-like enterprises (such as wholesale markets and duck yards) often lacked regular disinfection procedures, routinely kept poultry from different sources in the same cage and received a low level of oversight from local veterinary authorities.

A previous study carried out in the south of Vietnam under similar conditions identified a slightly lower prevalence of AI (5.3%) among farms and LBMs (Okamatsu et al., 2013) compared to the 7.6% identified in this study. Furthermore, the prevalence of AIV positivity among LBMs in this study (14%) was higher than the AIV positivity prevalence of 6.9% among LBMs in the center of Vietnam identified by (Chu et al., 2017) and 5.8% in the north of Vietnam identified by (Thuy et al., 2016). Assuming these differences in prevalence are real and not due to, for example, seasonal and yearly fluctuations in the incidence of AI, our results imply that LBMs in southern Vietnam play a more dominant role in maintaining AIV circulation in the poultry population compared to other areas of the country. Similar to PDSs the higher prevalence of AIV positivity among poultry sampled from LBMs is likely to be due to the routine mixing of large numbers of birds from different sources (Nguyen et al., 2017) and generally lower levels of biosecurity compared with both backyard and commercial poultry farms.

The questionnaire designed for this study was comprehensive and sought to solicit respondent demographic information and details of their knowledge, attitude and practice with respect to AI. The questionnaire comprised a total of 46 questions which presented difficulties when developing a parsimonious regression model to identify risk factors for AIV positivity. To address this issue MCA analyses were carried out using responses from each of the four sections of the questionnaire (demography, AI knowledge, AI attitude and AI practice). Clusters of responses for each section were identified and used as explanatory variables for our multivariable logistic regression model. In effect these clusters can be interpreted as respondent ‘profiles’ for demographics, AIV knowledge, AIV attitude and AIV practice. This allowed us to develop a model indicative of broad trends in the questionnaire data as opposed to developing a model starting with 46 candidate explanatory variables and attempting to identify responses to single, highly specific questions that were predictive of AIV positivity. It is our belief that this 'profile-based' approach provided results allowing us to identify broad trends in the data sufficient to guide policy development.

For the fixed-effects logistic regression model the explanatory variable representing the three cluster categories of AI knowledge (good knowledge, mixed knowledge and low knowledge) and the explanatory variable representing the three cluster categories of AI attitude (report AI yes, report AI mixed and report AI no) were significantly associated with bird level AIV positivity status. After accounting for unmeasured, individual enterprise level effects through inclusion of enterprise identifier as a random effect term the sign and magnitude of the point estimates of the regression coefficients were similar to that of the fixed effects regression model but both explanatory variables were no longer significantly associated with AIV positivity status. Respondents with a level of knowledge about AI classified as 'mixed' (i.e., where some facts regarding AI transmission and spread were correctly recalled and others were not) and respondents where their level of knowledge about AI was classified as 'low' had a 5.0 (95% CI: 0.2 to 130) and 3.5 (95% CI: 0.2 to 62) fold increase in the odds of their birds being AIV positive compared with respondents classified as having a good knowledge of AIV transmission and spread. Similar trends were noted for AI attitude. Respondents that provided inconsistent responses in terms of their likelihood to report an outbreak of AI to authorities ('Report AI mixed') and those that were unlikely to report an outbreak of AI to authorities ('Report AI no') had a 1.5 (95% CI: 0.10 to 26) and 1.1 (95% CI: 0.20 to 6.7) fold increase in the odds of their birds being AIV positive compared with respondents classified as being likely to report an outbreak of AI to authorities ('Report AI yes'). The substantial increase in the uncertainty around each of these measures of association after inclusion of the enterprise level random effect term reflect what is believed to be substantial individual enterprise-level influence on these associations.

Traders in PDSs and sellers at LBMs usually run their business dependent on market demand (Meyer et al., 2017) which means that they tend to leave the industry if a sufficient financial return is not achieved. For this reason, there is a relatively high population turnover of PDS traders and LBM sellers with those that are new to the industry often lacking knowledge about AI and its control. The knowledge and practice of participants from LBMs and PDSs are likely to be important in a given area because these industry players directly influence AIV circulation risk in a given market catchment area. In contrast, backyard and commercial poultry farmers run their businesses based on their ability and resources, meaning that they strive to obtain more knowledge and adopt better practices to generate more income (Chilonda & Van Huylenbroeck, 2001). This explanation is indirectly supported by the findings from this study: AIV positivity among birds from backyard farms and commercial farms was relatively low. We attempted to assess the interaction between enterprise type and AI knowledge, attitude and practice cluster assignment on AIV positivity risk to investigate this hypothesis further. Zero counts of AIV positive birds in some strata combinations made this analysis not possible.

In conclusion, consistent with previous studies we identified a higher prevalence of AIV positivity among poultry sampled from LBMs and PDSs compared with poultry sampled from backyard and commercial poultry farms which means that LBMs and PDSs should receive specific emphasis in AI control programs. Our findings provide evidence to support the hypothesis that incomplete respondent knowledge of AI and how it is spread was associated with an increased risk of AIV positivity. Delivery of education programs specifically designed for each industry sector (backyard farms, commercial farms, LBMs and PDSs) are likely to assist in this regard. The timing and frequency of delivery of education programs is likely to be important if the turnover of those working in LBMs and PDSs is high. Furthermore, the previous studies in Mekong Delta suggested that the farming practice of the farmers and trading system in this region was similar among the provinces. Implying that the result in this study might be applied for AI control in the other provinces of the Mekong Delta.

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Conflict of interest

The authors declare that no competing interests exist.

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Figure legends

Figure 1. (a) Map of Vietnam showing the location of Vinh Long province; (b) map showing the district boundaries in Vinh Long and the location of the four districts in which sampling was carried out (gray).

Figure 2. Error bar plot showing AIV prevalence (and its 95% confidence interval for backyard farms, commercial farms, live bird markets (LBM) and poultry delivery stations (PDS) by sampling round (2016 and 2017).

Figure 3. (a) Multiple correspondence analysis biplot showing questionnaire responses related to respondent demographics; (b) error bar plot showing AIV prevalence (and its 95% confidence interval) for the three clusters shown in (a) by enterprise type.

Figure 4. (a) Multiple correspondence analysis biplot showing questionnaire responses related to respondent AI knowledge; (b) error bar plot showing AIV prevalence (and its 95% confidence interval) for the three clusters shown in (a) by enterprise type.

Figure 5. (a) Multiple correspondence analysis biplot showing questionnaire responses related to respondent AI attitude; (b) error bar plot showing AIV prevalence (and its 95% confidence interval) for the three clusters shown in (a) by enterprise type.

Figure 6. (a) Multiple correspondence analysis biplot showing questionnaire responses related to respondent AI practice; (b) error bar plot showing AIV prevalence (and its 95% confidence interval) for the three clusters shown in (a) by enterprise type.

List of supplementary figure

Supplementary Figure S1. Flowchart to indicate the role of poultry delivery station in the poultry value chain.

Fig. 1.
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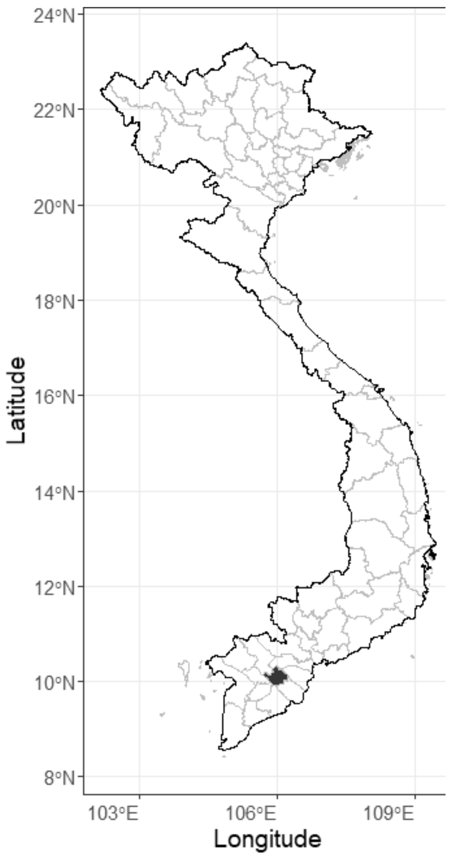
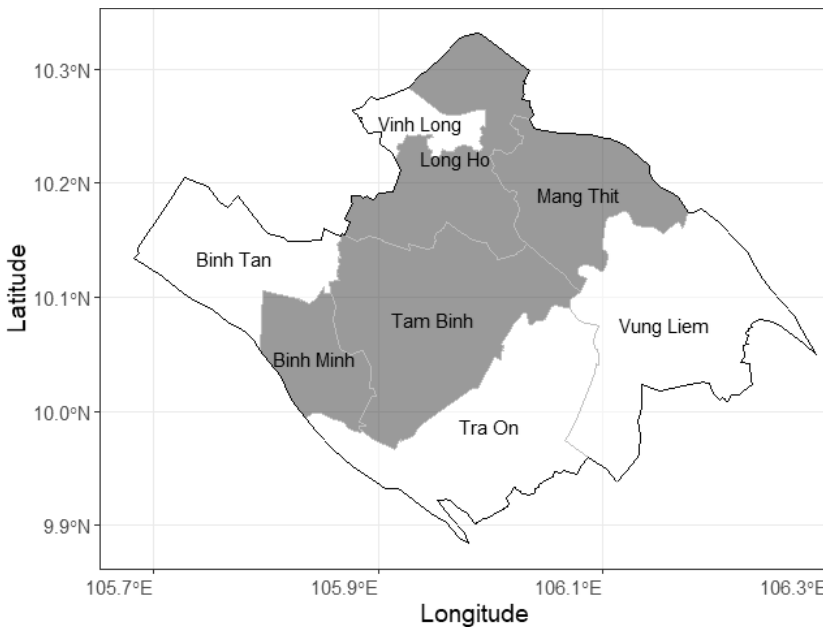


(a)



(b)

Fig. 2.
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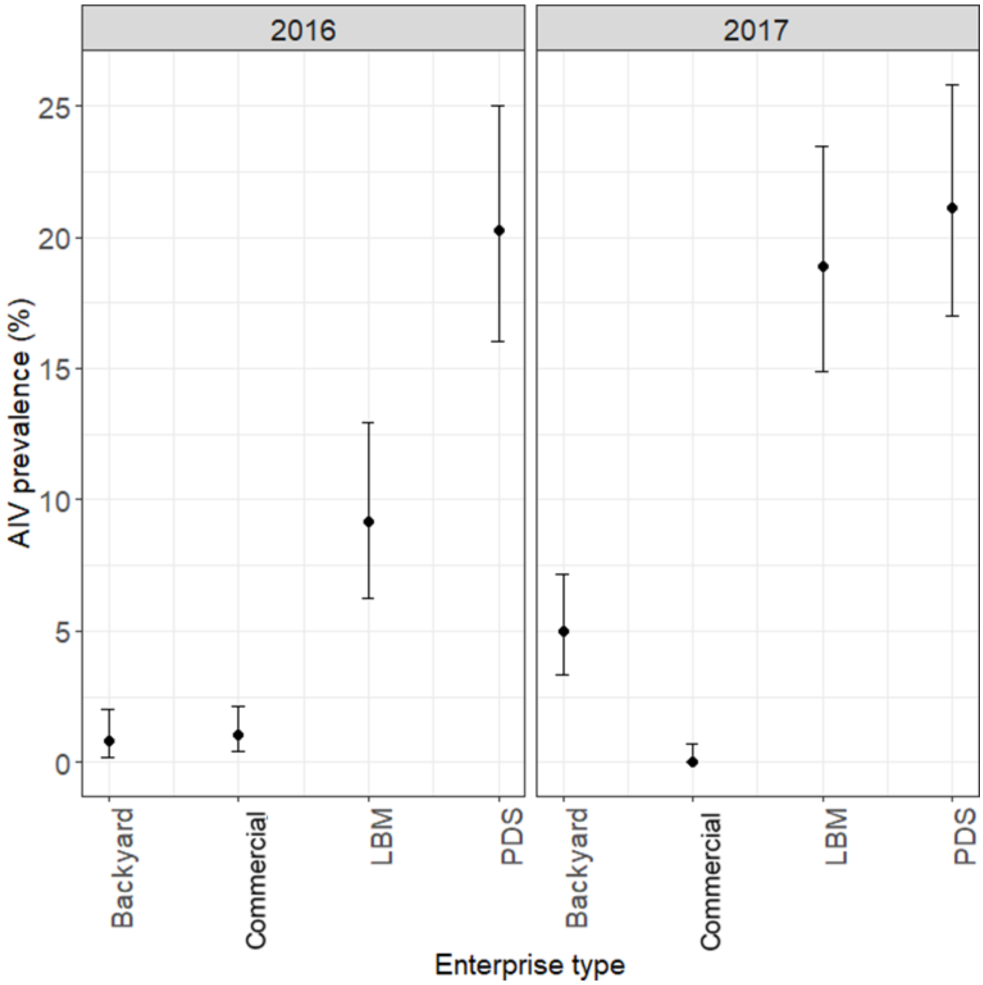
2016
2017
AIV prevalence (%)
0
5
10
15
20
25
Backyard
Commercial
LBM
PDS
Backyard
Commercial
LBM
PDS
Enterprise type

Fig. 3.
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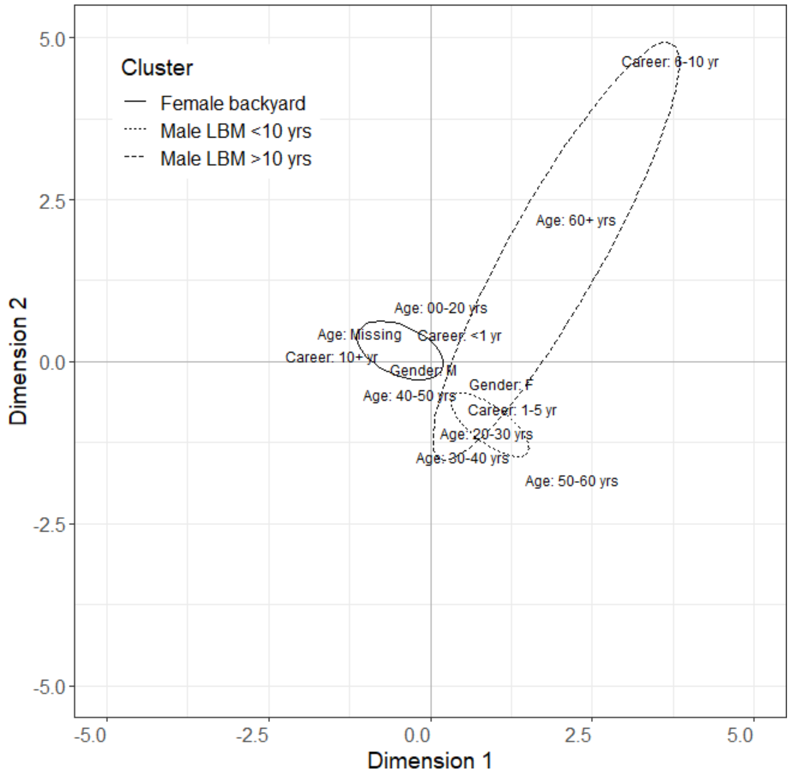
(a)



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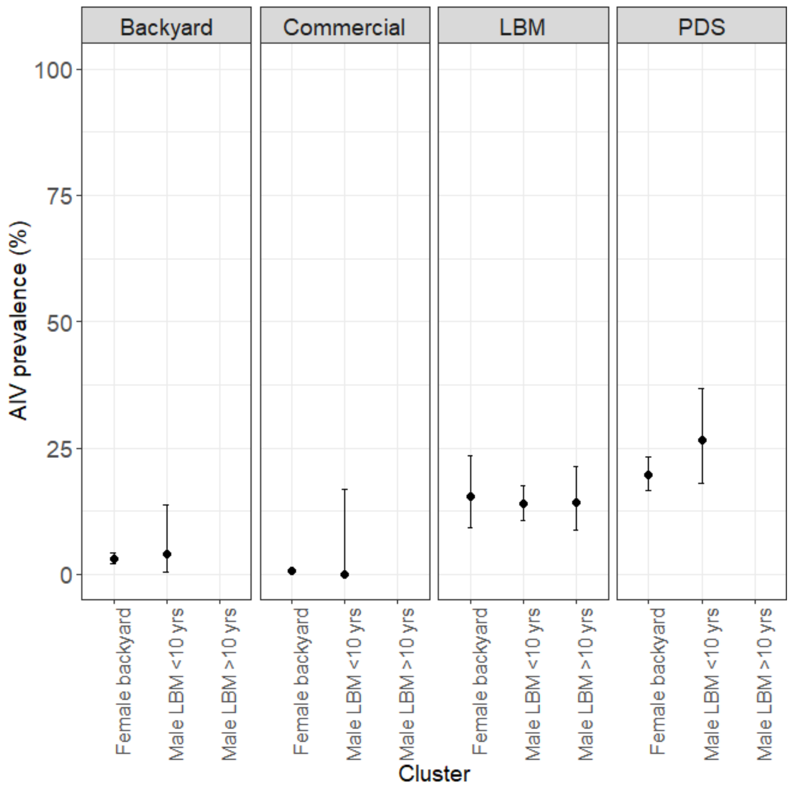
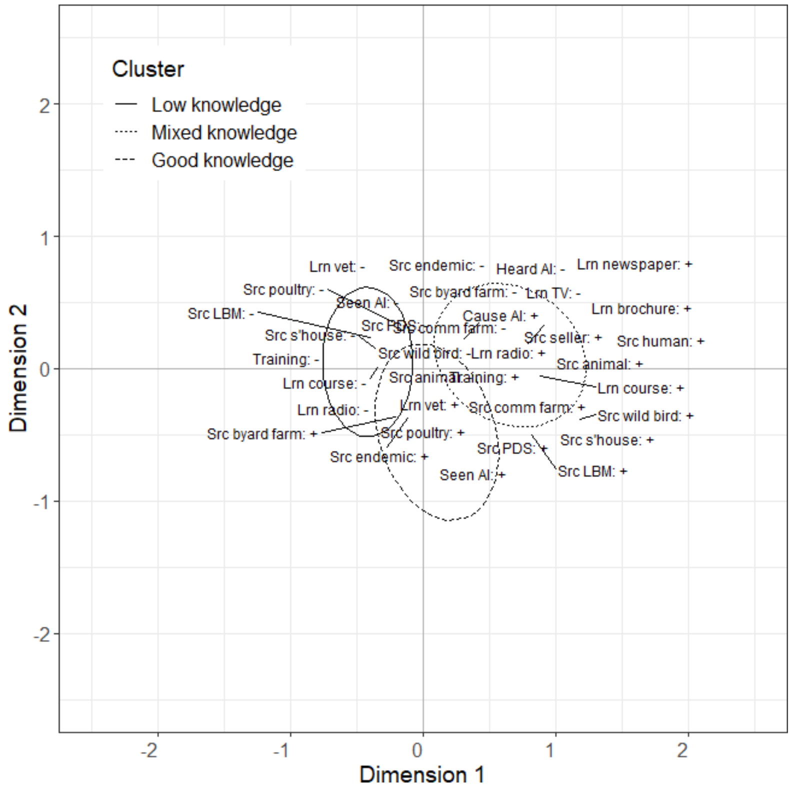


Fig. 4.
Le et al.

(a)



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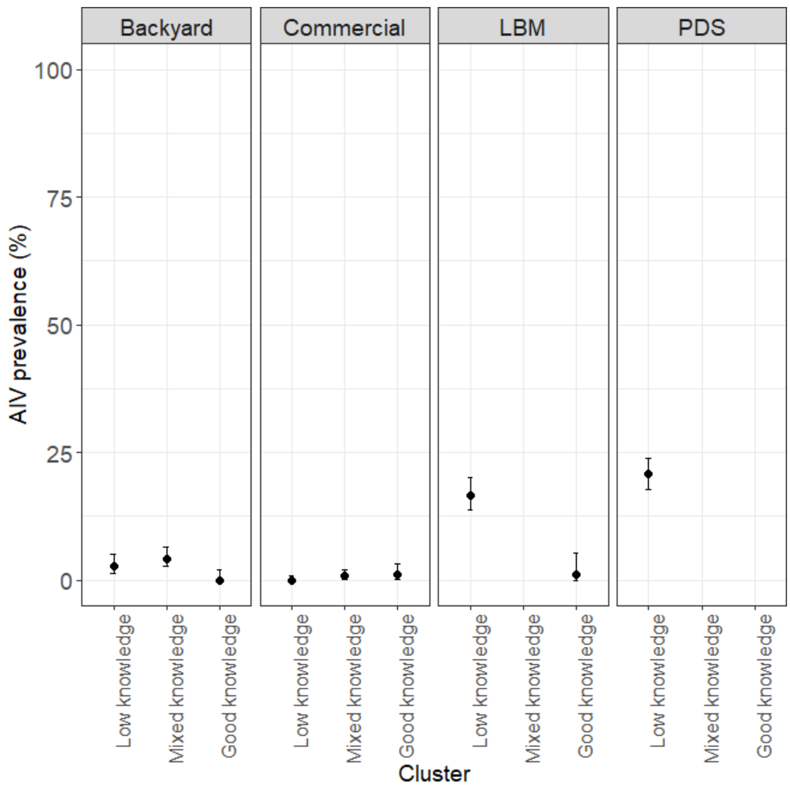
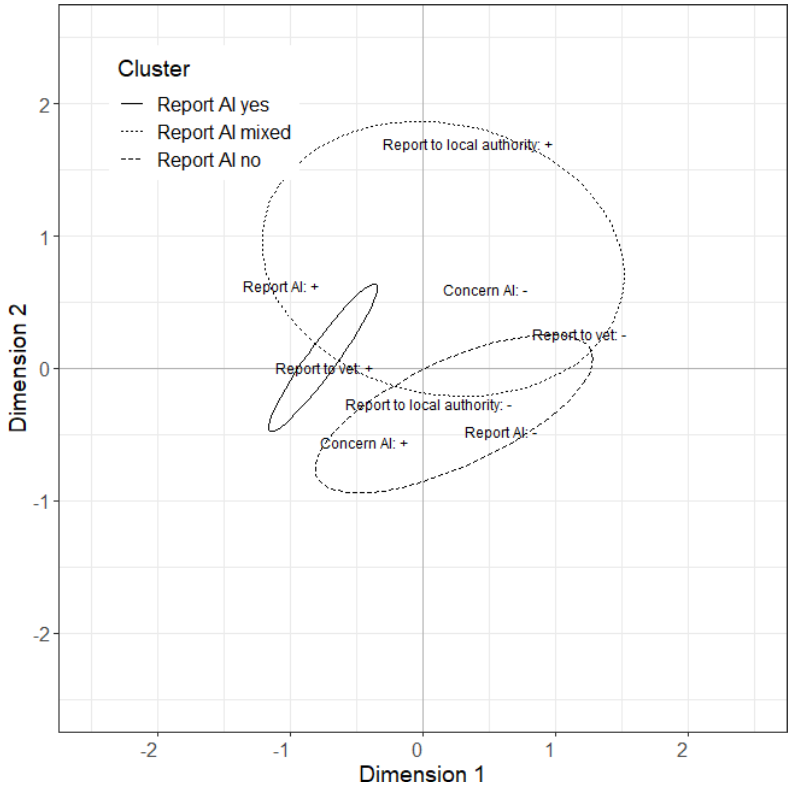


Fig. 5.
Le et al.

(a)



(b)

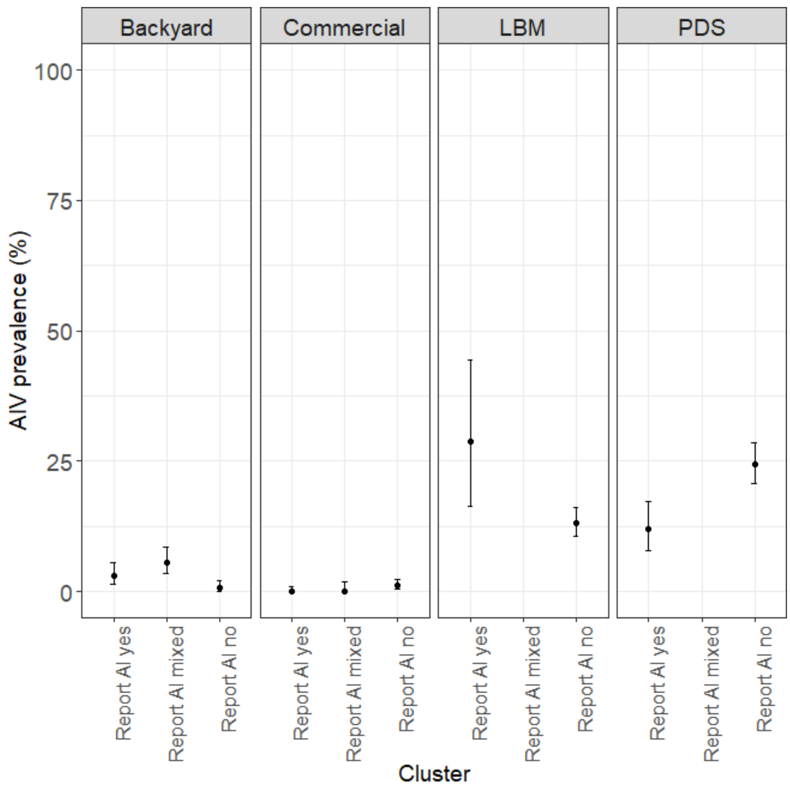


Fig. 6.
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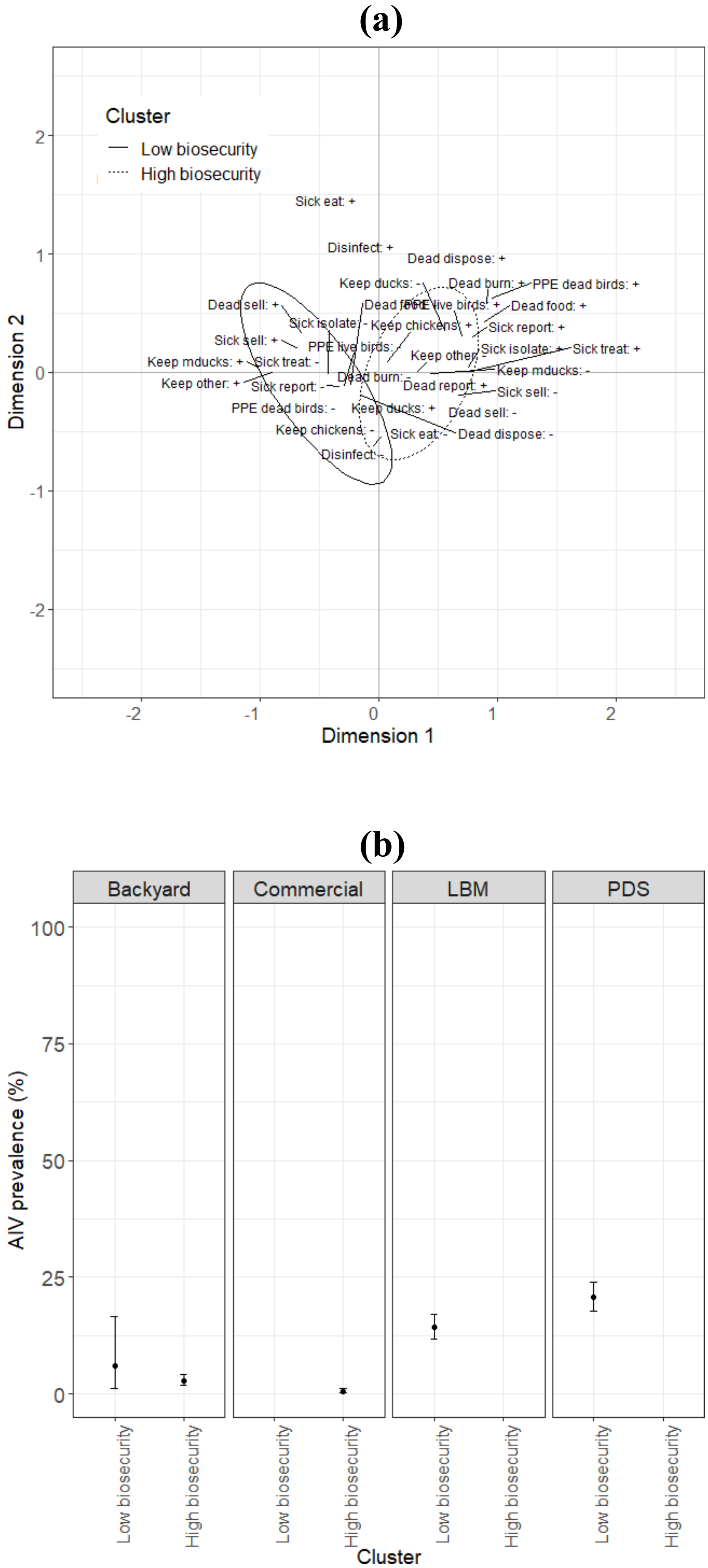

(a)
Cluster
Low biosecurity
High biosecurity
Dimension 1
Dimension 2
Sick eat: +
Disinfect: +
Dead dispose: +
Keep ducks: -
Dead burn: +
PPE dead birds: +
Dead sell: +
PPE live birds: +
Dead food: +
Sick isolate: -
Keep chickens: +
Sick report: +
Sick sell: +
PPE live birds: -
Keep other: -
Sick isolate: +
Sick treat: +
Keep mducks: +
Sick treat: -
Keep mducks: -
Keep other: +
Sick report: -
Dead burn: -
Dead report: +
Sick sell: -
PPE dead birds: -
Keep ducks: +
Dead sell: -
Keep chickens: -
Sick eat: -
Dead dispose: -
Disinfect: -
(b)
Backyard
Commercial
LBM
PDS
AIV prevalence (%)
Low biosecurity
High biosecurity
Cluster

Table 1. Numbers of birds sampled, numbers of samples AIV positive and AIV positivity prevalence, expressed as the number of AIV-positive birds per 100 birds at risk by enterprise type, species, sampling round and district.

Variable	No. of samples	AIV positive	Prevalence (95% CI) [a]	P-value
Enterprise type:				
Commercial	1,200	7	0.6 (0.2 to 1.2)	Reference
Backyard farm	1,056	32	3 (2.1 to 4.3)	<0.01
LBM	660	94	14 (12 to 17)	<0.01
PDS	681	141	21 (18 to 24)	<0.01
Species:				
Chicken	1,801	100	5.6 (4.5 to 6.7)	Reference
Duck	1,575	157	10 (8.5 to 12)	<0.01
Muscovy duck	189	16	8.5 (4.9 to 13)	0.11
Environment	18	0	0 (0 to 18)	0.97
Goose	14	1	7.1 (0.2 to 34)	0.27
Sampling round:				
1 (2016)	1,814	107	5.9 (4.9 to 7.1)	Reference
2 (2017)	1,783	167	9.4 (8.1 to 11)	<0.01
District:				
Binh Minh	910	61	6.7 (5.2 to 8.5)	Reference
Long Ho	909	61	6.7 (5.2 to 8.5)	0.84
Mang Thit	867	53	6.1 (4.6 to 7.9)	0.61
Tam Binh	911	99	10.9 (8.9 to 13)	<0.01

CI: Confidence interval.
[a] Number of AIV positive birds per 100 birds at risk.

Table 2. Numbers of respondents in each identified of the three respondent demographic cluster groups (n = 217) and percentages of responses for each question type.

Variable	**'Female backyard'**	**'Male LBM ≤10 yrs'**	**'Male LBM >10 yrs'**
	n = 158	**n = 46**	**n = 13**
Enterprise type:			
Backyard farm	60.8	8.7	0
Commercial	24.7	2.2	0
LBM	4.4	82.6	100
PDS	10.1	6.5	0
Gender:			
Female	70.9	21.7	30.8
Male	29.1	78.3	69.2
Length of career:			
Less than 1 year	40.5	4.3	15.4
1 to 5 years	7	95.7	46.2
6 to 10 years	52.5	0	0
More than 10 years	0	0	38.5

Table 3. Numbers of respondents in each identified of the three respondent AI knowledge cluster groups (n = 217) and percentages of responses for each question type.

Variable	**'Good knowledge'**	**'Mixed knowledge'**	**'Low knowledge'**
	n **= 29**	n **= 67**	n **= 121**
Heard of AI:			
Yes	89.7	52.2	92.6
No	10.3	47.8	7.4
Know the cause of AI:			
Yes	55.2	55.2	31.4
No	44.8	44.8	68.6
Know that the source of AIV is an infected bird:			
Yes	75.9	52.2	70.2
No	24.1	47.8	29.8
Know that AIV can be spread by domestic poultry:			
Yes	75.9	47.8	23.1
No	24.1	52.2	76.9
Know that AIV can be spread by wild birds:			
Yes	27.6	41.8	1.7
No	72.4	58.2	98.3
Believe that AIV can be spread by domestic animals (excluding poultry):			
Yes	20.7	53.7	3.3
No	79.3	46.3	96.7
Believe that AIV can be spread by interactions with other poultry farmers:			
Yes	3.4	55.2	0.8
No	96.6	44.8	99.2
Believe that AIV can be spread by interactions with poultry traders:			
Yes	10.3	64.2	0.8
No	89.7	35.8	99.2
Believe that AIV can be spread by interactions with those from backyard farms:			
Yes	69.0	40.3	62.8
No	31.0	59.7	37.2
Believe that AIV can be spread by interactions with those from commercial farms:			
Yes	65.5	53.7	18.2
No	34.5	46.3	81.8
Believe that LBMs are a source of AI:			
Yes	96.6	55.2	4.1
No	3.4	44.8	95.9
Believe that PDSs are a source of AI:			
Yes	96.6	49.3	7.4
No	3.4	50.7	92.6
Believe that slaughterhouses are a source of AI:			
Yes	65.5	52.2	1.7
No	34.5	47.8	98.3
Obtain information about AI from the television:			
Yes	86.2	43.3	94.2
No	13.8	56.7	5.8
Obtain information about AI from printed material:			
Yes	3.4	55.2	0.0
No	96.6	44.8	100.0
Obtain information about AI by attending training courses:			
Yes	13.8	61.2	13.2
No	86.2	38.8	86.8
Obtain information about AI from the radio:			
Yes	20.7	85.1	14.9
No	79.3	14.9	85.1

Obtain information about AI from the newspaper:			
Yes	0.0	53.7	2.5
No	100.0	46.3	97.5
Obtain information about AI from their local veterinarian:			
Yes	86.2	83.6	48.8
No	13.8	16.4	51.2
Have seen AI and are familiar with the clinical signs of AI:			
Yes	58.6	40.3	29.8
No	41.4	59.7	70.2
Have attended training on AI control and prevention:			
Yes	62.1	94.0	24.0
No	37.9	6.0	76.0

Table 4. Numbers of respondents in each identified of the three respondent AI attitude cluster groups (n = 217) and percentages of responses for each question type.

Variable	**'Report AI yes'**	**'Report AI mixed'**	**'Report AI no'**
	n **= 55**	n **= 41**	n **= 121**
Concerned about AI:			
Yes	58.2	48.8	50.4
No	41.8	51.2	49.6
Willing to report an AI outbreak:			
Yes	100	43.9	0
No	0	56.1	100
Would report an AI outbreak to local veterinarians:			
Yes	87.3	51.2	55.4
No	12.7	48.8	44.6
Would report an AI outbreak to local Department of Animal Health officials:			
Yes	0	100	0
No	100	0	100

Table 5. Numbers of respondents in each identified of the three respondent AI practice cluster groups (n = 217) and percentages of responses for each question type.

Variable	**'High biosecurity'** n **= 135**	**'Low biosecurity'** n **= 82**
Keep chickens:		
Yes	90.4	69.5
No	9.6	30.5
Keep ducks:		
Yes	54.8	84.1
No	45.2	15.9
Keep Muscovy ducks:		
Yes	13.3	56.1
No	86.7	43.9
Keep other domestic species:		
Yes	14.1	46.3
No	85.9	53.7
Use personal protective equipment when handling live birds:		
Yes	40.7	12.2
No	59.3	87.8
Use personal protective equipment when handling dead birds:		
Yes	40.0	0.0
No	60.0	100.0
Routinely disinfect their vehicle after transporting poultry:		
Yes	37.0	34.1
No	63.0	65.9
Dispose of dead birds using usual methods for garbage disposal:		
Yes	100.0	76.8
No	0.0	23.2
Dispose of dead birds by cremation:		
Yes	31.9	0.0
No	68.1	100.0
Dispose of dead birds by selling:		
Yes	22.2	82.9
No	77.8	17.1
Dispose of dead birds by feeding them to livestock:		
Yes	34.1	0.0
No	65.9	100.0
Dispose of dead birds by composting:		
Yes	28.1	1.2
No	71.9	98.8
Isolate sick birds:		
Yes	58.5	0.0
No	41.5	100.0
Sell sick birds:		
Yes	22.2	93.9
No	77.8	6.1
Treat sick birds:		
Yes	71.1	0.0
No	28.9	100.0
Feed sick birds to livestock:		
Yes	17.8	31.7
No	82.2	68.3

Table 6. Regression coefficients and their standard errors from a mixed-effects logistic regression model quantifying the association between enterprise type, cluster membership and AIV positivity.

Explanatory variable	Samples	AIV positive	Coefficient (SE)	*z*	*P* -value	OR (95% CI)
Intercept	3,597	274	-7.8884 (1.6737)			
Enterprise type:						
Commercial	1,200	7	Reference	-	-	1.0
Backyard farm	1,056	32	0.9482 (1.2031)	0.788	0.43	2.6 (0.2 to 27) [a]
LBM	660	94	3.8104 (1.3164)	2.895	<0.01	45 (3.4 to 590)
PDS	681	141	3.2215 (1.4823)	2.173	0.03	25 (1.4 to 460)
Knowledge:						
Good knowledge	547	4	Reference	-	-	1.0
Mixed knowledge	1014	25	1.6018 (1.6809)	0.953	0.34	5.0 (0.2 to 130)
Low knowledge	2036	245	1.2422 (1.4750)	0.842	0.40	3.5 (0.2 to 62)
Attitude:						
Report AI yes	1,000	48	Reference	-	-	1.0
Report AI mixed	527	19	0.4036 (1.4656)	0.275	0.78	1.5 (0.10 to 26)
Report AI no	2,070	207	0.0831 (0.9282)	0.090	0.93	1.1 (0.20 to 6.7)
Random effects:	Variance	SE				
Enterprise	10.37	3.221				

SE: standard error; OR: odds ratio; CI: confidence interval.

[a] Interpretation: After adjusting for the effect of respondent knowledge category, attitude category and unmeasured enterprise-level effects the odds of a bird being AIV positive if it was from a backyard farm was 2.6 (95% CI: 0.2 to 27) times the odds of a bird from a commercial poultry farm being AIV positive.