A systematic approach to illuminate a new hot spot of avian influenza circulation in South Vietnam

Short running title

A new AI hotspot in South Vietnam

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Summary (285/300)

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 factor to determine avian influenza (AI) prevalence in the southern part. Therefore, a risk analysis of AIV spread was conducted by including all possible value chain factors. A cross-sectional study was performed in backyard farms, high-biosecurity farms (bio-farms), LBMs, and poultry delivery stations (PDSs) in the four districts of Vinh Long Province in December 2016 and August 2017. A total of 3 597 swab samples were collected from individual poultry at 101 backyard farms, 50 bio-farms, 58 sellers in LBMs, and 17 traders in PDSs and then investigated for AIV isolation. Concurrently, information related to participants and birds was collected and used to identify the fixed and random effects of factors in AIV infection. A total of 274 birds were positive for virus isolation, with a prevalence of 7.6% (95% confidence interval [CI]: 6.8–8.5) at the individual poultry level, and the adjusted prevalence based on the sampling weight was 7.9% (95% CI: 7.6–8.2). The significantly higher prevalence in PDSs (20.7%) and LBMs (14.2%) compared to backyard farms (3.0%) and bio-farms (0.6%) suggested that PDSs are another hot spot for AIV circulation. The high diversity in the seller and trader population characteristics was revealed using multiple-correspondence analysis to analyze the participants' demographic factors in LBM and PDS. The mixed-effect logistic regression model revealed that keeping duck at the sampling time and the owner's older age should be risk factors of AIV infection in PDS. Therefore, functional AI control efforts to monitor the PDS system should be emphasized to minimize AIV circulation risk in Vietnam.

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. Particularly, outbreaks of high pathogenicity avian influenza (HPAI) have occurred in poultry throughout Southeast Asia despite large-scale vaccination campaigns in Vietnam and Indonesia together with stamping-out interventions (Alexander, 2007; Brown, 2010). Although the number of HPAI outbreaks in Vietnam due to infection with H5N1 subtype viruses has decreased markedly since 2004 (FAO Vietnam, 2017), substantial losses in the domestic poultry sector continuously occur. A large number of studies have greatly contributed to the improvement of the understanding of AI virus (AIV) epidemiology by highlighting the importance of several drivers owning to its spread (Nomura et al., 2012; Okamatsu et al., 2013; Nguyen et al., 2014; Chu et al., 2016; Chu et al., 2017; Nguyen et al., 2020). In the efforts to control AIV risk, the government has developed both active and passive surveillance systems. One of the advantages of active surveillance can capture new virus introduction or strain evolution rapidly. In contrast, a passive AI surveillance system is appropriate to figure out the cause of the outbreaks through the passive reporting of disease events by farmers.

Effective surveillance system results imply that diversification in AIV subtypes has increased due to a wide range of its infection in poultry populations in East and Southeast Asia, including Vietnam (Li et al., 2013; de Vries et al., 2015). These viruses can cause major poultry production losses, such as an increase in mortality and a reduction of egg production (Kinde et al., 2003), and pose a concern for global health security to cause zoonotic infection. Therefore, monitoring the virus subtypes circulating in the field is essential (Pfeiffer et al., 2013).

In previous studies, the trade and movement of live birds are demonstrated as a major pathway for promoting a wider spreading of AIV (Kung et al., 2007; Kim et al., 2010; Gilbert et al., 2014). Along with that, live bird markets (LBMs) have been identified to 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 a zoonotic outbreak caused by the H7N9 AIV infection in China in 2013, the closure of LBMs was remarkably effective in reducing its human infection by up to 99% (Yu et al., 2014). Although rest days in LBMs effectively break the viral amplification cycle within LBMs, they do not prevent virus reintroduction from the outside (Kung et al., 2003). A previous study in Vietnam about the control measures in LBMs has indicated no differences in AIV prevalence between intervention and non-intervention LBM (Chu et al., 2017), meaning that the introduction of AIV into LBMs might occur continuously. This evidence indicated that the source of AIV circulation in the value chain of poultry products in Vietnam has not yet been fully identified and controlled.

During the active surveillance in Vietnam since 2015, it was indicated that a sector which should be named as poultry delivery station (PDS) would play a role in the connection among poultry farms, LBMs, and slaughterhouses. PDS usually works within a wide range of distance, which is up to 100 km, with mixing several species of poultry under poor biosecurity conditions, whereas LBMs tends to take smaller-scale operations with poultry, most of which are transported from nearby household and semicommercial enterprises (Phan et al., 2013).

In this study, the active surveillance of AIV and a questionnaire study in the four poultry production sectors: high-biosecurity farm (bio-farm), backyard farm, LBM, and PDS in Vinh Long Province, Vietnam, were conducted in 2016 and 2017. This study specifically aimed to (1) estimate the AIV prevalence in each of the four sectors and compare the characteristics of AIV-positive samples and (2) identify the factors that might more likely cause positive AIV infection in birds. Identifying the relatively important factors influencing the AIV spread at each sector should be definitively the first step to design more effective evidence-based measures to reduce the risk of AIV infection through the value chain of poultry products in Vietnam.

Materials and Methods

Study design and study area

A cross-sectional study was conducted in 101 backyard farms, 50 bio-farms, 58 sellers of LBMs, and 17 traders of PDSs in the four districts of Vinh Long Province, Vietnam (Figure 1) in December 2016 and August 2017. From the list provided by the local government, the number of participants in each district was determined from 20 to 25 for backyard farms and from 10 to 15 for bio-farms. A farm that had not applied any prevention measures following local authority guidelines, such as keeping poultry in a separate place, vaccination, and disinfection, was categorized as a backyard farm. A farm categorized as a bio-farm satisfied to apply at least more than one experience out of several control measures, including keeping poultry in a separate place, vaccination, and disinfection. Two LBMs per district were selected at each sampling round, and a total of 12 LBMs were selected in this study. Two PDSs per district were selected at each sampling round, and a total of 13 PDSs were selected. Each of the PDSs, LBMs, and farms was visited by the investigators (KTL, LTN, D-HC, and TNN) and the Sub-Department of Animal Health (SDAH) staff of Vinh Long Province. Each category's sampling weight in the poultry value chain was computed based on Lumley's (2010) through the survey package (Lumley, 2020) developed in R.

Laboratory procedures

All oropharyngeal swabs, cloacal swabs, and fecal samples were collected from chickens, ducks, or Muscovy ducks in each poultry farmer, seller, and trader at each sampling round. The sterile tube contained transport medium was used to kept oropharyngeal and cloacal swabs, as described previously (Le et al., 2020). Samples were then 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 presence of influenza type A virus in the samples were tested by using real-time reverse transcription-polymerase chain reaction (RT-PCR) targeting on M gene with the primer design and thermal cycle (Das et al., 2006) following by the manual of OIE (OIE, 2018). All samples were transferred to the Laboratory of Microbiology, Faculty of Veterinary Medicine, Hokkaido University, for virus isolation. The shipment of samples containing AIV was classified into Biological Substance, Category B, following the International Air Transport Association's instructions in the Dangerous Goods Regulation Manual (Pearson, 2007).

Virus isolation

The 10-day-old chicken embryonated eggs produced by conventional chickens tested free of AIV antibody were use to isolated the AIV. Each sample was resuspended with a transport medium and inoculated into the allantoic cavity. The incubation was carried out in 30 to 48 h at 35°C, allantoic fluid was collected to check the hemagglutination activity. The hemagglutination inhibition and neuraminidase inhibition tests with antisera to the reference influenza virus strains were used to determine the subtypes of the isolated influenza virus (Kida & Yanagawa, 1979).

The true prevalence was estimated based on the sensitivity and specificity of the real-time RT-PCR (in RAHO7) and virus isolation (in Hokkaido University) by applying the epiR package (Stevenson et al., 2021) on R.

Questionnaire and interview

By referring to the previous questionnaires administrated by the Vietnamese Department of Animal Health (DAH), Ha Noi, a questionnaire to collect details of knowledge, attitudes, and practices regarding AIV was developed in this study by the authors in partnership with the DAH staff. The questionnaire and response is stored in corresponding authors' data and available on request. Three different questionnaires were developed in Vietnamese, each for the farmer in backyard farms and bio-farms, the seller in LBM, and the trader in PDS, respectively. All three questionnaires consisted of the following sections: (1) the poultry worker's demographic, (2) details of the source and type of poultry on the interviewing day, (3) knowledge of poultry worker regarding AIV, (4) details of their attitudes about AI control measures, and (5) the typically implemented for AI biosecurity measures.

Questionnaire surveys were administered by trained interviewers of the SDAH Vinh Long Province and each district veterinary station. Under the supervision and technical assistance of DAH staff, and the support of the SDAH veterinarians at the study communes, 226 face-to-face interviews were conducted with poultry workers in the two sampling rounds in the four districts. Each sampling round was divided into two stages: In the first stage, the sample and questionnaire collection was performed in the bio-farm then backyard farm in the former stage. In the latter stage, the same procedure was applied for LBMs and PDS. The sampling schedule was announced to the stakeholders and the local veterinarian in advance, and all sample collection and questionnaire surveys in each stage were completed in 8 days. The field activities to manage the sampling process and collect the participants' information were conducted under the responsibility of the DAH and SDAH Vinh Long Province.

Data management

In the beginning, a unique key assigned for each poultry worker as an identifier during this study. Questionnaire responses at each sampling round and results of AIV isolation were recorded in two separate tables. The unique poultry worker identifier was used to link two tables within the relational database.

Multiple-correspondence analysis

The pairwise crosstabulation of the individual variable was used to construct an $I \times J$ indicator matrix, where I is the set of i individual records, and J is the set of j variable categories. Then MCA was performed on this indicator matrix with each cell (i, j) contains an individual record i and category j (Snijders & Bosker, 1999).

In the MCA, each individual variable represented by a mark on the MCA two-dimensional graph and the association of the categories are expressed as the distance of these marks. The relative location of a representative point depends on the distance (interaction) between the variables in the dataset. The square of the distance between the representative marks is introduced according to the following equation:

$$d_m^{\,2}(i,i')=\frac{1}{f_j}+\frac{1}{f_j^{'}} \qquad (1)$$

where ${d_m}^2(i,i')$ is the squared distance between representatives *i* and *i'* for variable *m*. f_j is the relative frequency of representative records that selected category *j*. $f_j^{'}$ is the relative frequency of representative records that selected category *j'*. Relative frequency of each category is introduced as the rate of the number of representatives in category per total number of individual records in the dataset. The sum of all square distances between each set of the two representative records is calculated using the following equation:

$$D^2(i,i')=\frac{1}{M}\sum_{m\in M} {d_m}^2(i,i') \qquad (2)$$

where $D^2(i,i')$ is the sum of squared distance between individuals *i* and *i'*. *M* is a set of all variables.

Since both clouds (the set's position of variables) of representative variables and their variable categories are at the same dimension, the relative location between representative points (individual variables) and variable categories was determined by a point and a weight. The squared distance between two different categories *j* and *j'* is introduced according to the following equation. The *njj* will be zero if two categories are at the same variable.

$$(N_j N_{j'})^2=\frac{n_j+n_{j'}-2n_{jj'}}{\frac{n_j n_{jj'}}{n}} \qquad (3)$$

where $(N_j N_{j'})^2$ is the squared distance between categories *j* and *j'*. n_j is the number of representatives that selected category *j*. $n_{j'}$ is the number of representatives that selected category *j'*. $n_{jj'}$ is the number of representatives that selected both categories *j* and *j'*. MCA was performed using R version 4.0.0 (R Development Core Team 2016) with the FactoMineR package (Husson et al., 2008).

Mixed-effect logistic regression

The prevalence of AIV at the individual bird level was defined as the proportion of the total number of individual birds with AIV positive samples per the total number of birds sampled. Unconditional associations between responses on the questionnaire (the explanatory variables) and the laboratory results (the presence or absence of AIV in an individual bird) were expressed as the odds ratio. Analysis of variance (ANOVA) was performed to determine the effect of independent variables to the dependent variable in a regression study aiming to figure out the potential factor that might relate to AIV infection. Any explanatory variables with P < 0.2 (two-sided) of unconditional association were applied in the multivariable modeling.

The probability that a bird is positive for AIV infection was parameterized as a function of the *m* explanatory variables in a fixed-effects multiple logistic regression model. This model takes the following form under the assumption of $p_i = P(Y_i = 1)$ and that Y_i are mutually independent:

$$\log\left(\frac{p_i}{1-p_i}\right)=\beta_0+\beta_1 x_{1i}+\ldots+\beta_m x_{mi}+\epsilon_i \quad (4)$$

In Eq. (4), β_0 represents the intercept term and $\beta_1, \ldots, \beta_m$ represent the regression coefficients of each m explanatory variable in the model. Unnecessary explanatory variables were selected and taken out from the regression model by the backward elimination method until all of the explanatory variables satisfied significance at $\alpha < 0.05$. Explanatory variables excluded at univariable analysis were included into the final model to check whether they cause more than 20% of change in any of estimated regression coefficients. Furthermore, none of the biologically plausible two-way interactions were significant at $\alpha = 0.05$.

To account for the lack of independence arising from the hierarchical structure of the data, including individual birds clustered within poultry worker clustered within sampling rounds and LBMs or PDS, the model in Eq. (4) was extended to a mixed-effects model.

$$\log\left(\frac{p_{ijk}}{1-p_{ijk}}\right)=\beta_0+\beta_1 x_{1ijk}+\ldots+\beta_m x_{mijk}+M_k+S_{jk}+\epsilon_{ijk} \qquad (5)$$

In Eq. (5), p_{ijk} represents the probability of being AIV positive in the *i*th bird from the *j*th poultry worker in the *k*th LBM/PDS. The M_k is a zero mean random effect term parameter with variance σ_M^2 indicating the affect of the *k*th LBM/PDS on the probability of being AIV positive.The S_{jk} is a zero mean random effect term with variance σ_S^2 indicating the affect of the *j*th seller/trader in the *k*th LBM/PDS. The S_{jk} and M_k were included in the model to describe unexplained extrabinomial variation existing at the poultry worker level and LBM/PDS level on AIV positive.

The assumptions of normality and homogeneity of variance were investigatedby constructing the histograms of the residuals in the multilevel model, and plots betwee the residuals and predicted values, respectively. In the multilevel model, extrabinomial variation was not included to individual bird variance, and estimates of variance at each of three levels (LBS/PDS, worker, and bird) were assumed that the variance at the lowest level on the logit scale was π2/3, where π = 3.1416 (Snijders & Bosker, 1999).

Descriptive analysis, measures of unconditional association, and fixed-effects logistic regression models were accomplished in R. The MlwiN (Rasbash et al., 2015) was applied to develop the mixed-effects model by using the R2MLwiN package (Zhang et al., 2016) in R.

Results

Descriptive statistics and unconditional associations

The sample size and the relative AIV prevalence among sectors are shown in Table 1. During the study period, the average number of birds sampled per trader in PDS was 40 (minimum of 19, maximum of 52), per seller in LBM was 11 (minimum of 10, maximum of 52), per backyard farm was 10 (minimum of 5, maximum of 20), per bio-farm was 26 (minimum of 10, maximum of 50). The final dataset comprised details from 3 597 birds from 17 traders of 13 PDSs, 58 sellers of 12 LBMs, 101 backyard farms, and 50 bio-farms in eight communes in the four districts of Vinh Long Province. A total of 274 out of 3 597 birds (7.6%; 95% confidence interval [95% CI]: 6.8–8.5) were positive for AIV isolation. Isolation rates for AIV varied between sectors (Figure 2), with PDS accounting for 20.7% (95% CI: 17.7–24.0), followed by LBM with 14.2% (95% CI: 11.7–17.1), backyard farm with 3.0% (95% CI: 2.1–4.3), and bio-farm with 0.6% (95% CI: 0.2–1.2). The sensitivity and specificity of the diagnostic tests used in this study were defined as 1, meaning that the prevalence obtained in each category of the poultry value chain in this study approached true prevalence. The total numbers in each category of the poultry value chain provided by the SDAH Vinh Long Province were 228 for bio-farm; 1,288 for backyard farm; 123 for LBM; and 98 for PDS (Table 2). The sampling weight of each category of the poultry value chain was obtained as 5; 13; 10; 8; respectively. True AI prevalence was estimated through the sampling weight, and the true prevalence in each category of the poultry value chain was 7.9% (95% CI: 7.6–8.2).

The number of chicken and duck samples were 1 801 (50.1%) and 1 575 (43.8%), respectively. Because the number of Muscovy duck, goose, and environment samples was only 221 (6.1%), only the numbers of chicken and duck samples were compared. A significant difference was confirmed between AIV prevalence in chicken (5.6%; 95% CI: 4.5–6.7) and duck (10.0%; 95% CI: 8.5–11.6). This result reflected the field situation that the natural living environment of duck was more facilitated for the survival of AIV compared to the environment of chicken feeding.

The average number of samples in each district was 899 (minimum of 867, maximum of 911), and 10.9% (95% CI: 8.9–13.1) of AIV prevalence was confirmed in Tam Binh District, which was significantly higher than one in the others (6.1%–6.7%; 95% CI: 4.6–8.5). A significant difference in AI prevalence was observed between samples collected in 2016 (5.9%; 95% CI: 4.9–7.1) and those in 2017 (9.4%; 95% CI: 8.1–10.8), in which many AI outbreaks were officially reported. The cycle of AIV in nature was demonstrated as the major factor affecting AI prevalence and AI outbreak.

Multiple-correspondence analysis

The relationships between different independent categorical variables could be described in MCA without the identification of a dependent variable or any assumptions on the data' distribution (Greenacre, 1984; Tenenhaus & Young, 1985). The relationships among the different categories of the variables were reflected by the distance between the representative marks in a two-dimensional space. The category variables located near the axes' origin meaning that their characteristics corresponding to the most frequent categories. In contrast, category variables located far from the origin of the axes have unique characteristics. A discrimination measure for each variable and in each dimension is computed that can be regarded as a squared component loading and is also the variance of the quantified variable in that dimension. As a result of the MCA factor map, the profiles of each section of the questionnaires are visualized. In the demographic section, the level of correlation among the factors was lowest in PDS (Figure 3a) and LBM (Figure 3b), followed by backyard farms (Figure 3c). A high correlation was confirmed in a bio-farm (Figure 3d). This result indicated that a variety of participants' characteristics was highest in PDS and LBM in the four sectors. This phenomenon might attribute to the diversity of the worker population in LBM and PDS. In the knowledge section, a similar pattern was observed in all sectors. The correlation level of the factors was lowest in PDS (Figure 4a) and LBM (Figure 4b), whereas the opposite results were confirmed in backyard farms (Figure 4c) and bio-farms (Figure 4d). In the attitude section, LBM (Figure 5b) and bio-farm (Figure 5d) showed a higher correlation among factors compared to PDS (Figure 5a) and backyard farm (Figure 5c). Actually, LBM had been the target for applying AI control measures, including an education campaign for a long time, and most bio-farm owners were well-trained and gained good knowledge about AI. Therefore, the participants of LBM and bio-farm could respond to the present questionnaire correctly and consistently. The most important sections for reducing AIV risk are the practice section because the failure of achieving good practice would result in the ineffectiveness in AI control regardless of good knowledge or excellent attitude. A lower correlation was reported in the practice section of PDS (Figure 6a) and LBM (Figure 6b). The diversity of the participant's characteristics in PDS and LBM might lead to biases in their knowledge, attitude, and practice.

Multivariable logistic regression analyses

The screening step shows that there were 11, 24, 10, and 4 potential factors retained for the mixed-effect analysis in PDS (Table 3), LBM (Table 4), backyard farm (Supplementary Table S1), and bio-farm (Supplementary Table S2), respectively. The interactions among these potential factors were assessed using estimated regression coefficients via generalized linear models. In PDS, two of the explanatory variables, keeping duck in PDS and the PDS owner's age range from 51 to 60 years, showed the association with the risk of AIV positive ($P < 0.001$), which was statistically significant in the final mixed-effects model. Specifically, if the PDS owner kept the duck in their PDS at the sampling time, their poultry flock has a higher chance of being infected with AIV at 10.46 (95% CI: 6.76–16.19) times compared to those who kept the chicken. The dependence of AIV infection risk to the PDS owner's age was observed in this study. The PDS owners' age ranging from 51 to 60 years obtained a higher risk of AIV infection into their poultry at 41.4 (95% CI: 10.07–170.13) times compared to a younger age. In LBM, one explanatory variable, gain AI knowledge via the neighbor in LBM, showed a negative association with AIV infection risk ($P = 0.028$). Precisely, if the seller gains knowledge from their neighbor in LBM during their business time, their poultry flock has a lower chance of infected by AIV at 0.55 (95% CI: 0.35–0.86) times compared to the others. In backyard farms and bio-farms, none of the explanatory variables were associated with the risk of being AIV positive ($P < 0.05$).

Discussion

This is the first cross-sectional study to assess AI prevalence in the four different sectors in the poultry product value chain, including PDS, LBM, backyard farm, and bio-farm. The control measures for AI have been applied on LBMs and farms for a long time in Vietnam since the first report of AI outbreak in 2003, but the results of this study indicated that PDSs should be included as a new stakeholder for AI control measures. In that way, AI control measures should be updated over the years based on the field situation to reach the highest effectiveness. Although the study area was selected in the south of Vietnam, the network system of poultry products illustrated in this study is a traditionally common structure throughout Vietnam. This study would first reveal that PDS, indicated as a wholesale market in another epidemiological study, was a hot spot for AIV circulation by being functional in the poultry product value chain for a long time (Soares Magalhães et al., 2010). In this study, the role of PDSs for AIV spread in the community was fully accessed by multiple approaches (i.e., virological and epidemiological methods).

Like this study, very few studies in Vietnam carried out a systematic and concurrently approach to almost all of the most important sectors along with the poultry movement by combining virological and epidemiological methods. Therefore, this result might provide a more accurate overview of AIV circulation in southern Vietnam. A previous study in southern Vietnam under a similar setting to this study demonstrated lower AI prevalence (5.3%) in farms and LBMs (Okamatsu et al., 2013) compared to this study (7.9%), which may mean that the AI prevalence was increasing in the last several years in southern Vietnam. Furthermore, the AI prevalence at LBM in this study was 14.2%, which was significantly higher compared to 6.9% in the central area (Chu et al., 2017) and 5.8% in the northern area (Thuy et al., 2016), which might imply that LBM in southern Vietnam seems to play a more critical role in the increase of AIV circulation compared to the other areas. Although AIV positivity varied by sector, a relatively high prevalence was found in PDSs followed by LBMs in both rounds (Figure 2). The higher AI prevalence could be explained by the low biosecurity in the trading behavior (Nguyen et al., 2017) and farming system, especially in Mekong Delta, where the free-grazing duck is still a common farming system (Meyer et al., 2017a). A previous study pointed out that PDS or a part of it with different names, such as wholesale market and duck yards, were commonly lacking regular disinfection, kept poultry from different sources in the same cage, and rejected the assessment of local veterinary (Meyer et al., 2017b). These issues would lead to high AIV prevalence in PDS. The result indicated that if a critical imbalance of sampling strategy existed in surveillance, the bias in the conclusion of AIV circulation might come easily. Although the adjustment of the overall prevalence in this study did not change the whole picture of AIV circulation in southern Vietnam, statistical analysis for the adjustment of sampling strategy in future surveillance is essential to obtain higher accurate findings.

MCA results visualized the characteristics of the whole population that participated in this study, although it might not directly indicate the risk of AIV in each sector. The low correlation shown in PDS and LBM implies that high variances were confirmed in knowledge, attitude, and practice among participants. This result means that a single participant with little knowledge and incorrect practice might introduce a high risk of AI infection into their poultry and indirectly expose other participants in the same area at high risk. Most traders of PDSs and sellers of LBMs usually run their business depending on the market demand (Meyer et al., 2017b), meaning that they tend to change their business if it does not give them any benefit. Therefore, the trader or seller population characteristics tend to change over time and lead to a lack of AI knowledge among newcomers. In contrast, farmers usually run their businesses based on their ability and resources, meaning that they tend to obtain more knowledge and follow better practices to earn more income (Chilonda & Van Huylenbroeck, 2001). In other words, through the education campaign ever conducted, farmers' accumulated good knowledge and manipulated them into their practice (Pham-Duc et al., 2019). This explanation was supported in this study by the result of MCA; a high correlation was observed in almost all sections of both bio-farms and backyard farms, implying that most farmers obtain similar knowledge, attitude, and practice. Together with the result of virus isolation, it can be concluded that farmers in this study had obtained excellent knowledge, attitude, and practice for AI. This result would be in accordance with the multivariable logistic regression results that any risk factors were identified in neither bio-farms nor backyard farms. It is recommended that AI control efforts at the individual level of traders in PDSs and sellers in LBMs should be emphasized rather than at the market or commune level.

Encouragingly, at the multilevel analysis, the factors related to AIV infection in PDS were identified; keeping or selling duck increases the proportion of AIV-positive birds. Because the duck is already known as a high-risk bird to incubate AIV without manifesting apparent clinical signs (Nomura et al., 2012; Okamatsu et al., 2013), virus incubation or transmission by holding ducks would affect all sections of the poultry value chain (Hulse-Post et al., 2005; Meyer et al., 2017b). Furthermore, similar findings were confirmed in previous works that analyzed the AI outbreak in Vietnam that duck and Muscovy duck might increase the risk of AI outbreak (Nguyen et al., 2019, 2020). Unlike LBM, where control measures are applied by the local veterinarian and supported by the authority, AI control measures in PDS were not implemented by the local veterinarian or authorities but done mainly by the traders of PDS themselves because PDSs are not recognized as official areas. It may support the current result that ducks were kept commonly in both PDS and LBM; however, a higher AI prevalence was observed only in PDS. In this study, the owner's age range from 51 to 60 years was also indicated as a positive factor related to AIV infection in PDS compared to the other age ranges. Normally, PDS owners have joined many training courses and accumulated experiences over time (personal communication, SDAH Vinh Long Province). At an older age, they could become trainers for their workers and transfer knowledge by keeping close contact, which is documented as an effective training method in a previous study (Manabe et al., 2012), meaning that the senior owner's knowledge and practice are inherited directly via practice to the junior workers. Unfortunately, older age seems to be slower to accept and update new ideas or knowledge (Thomas & Kunzmann, 2014), meaning that out-of-date information might exist in the PDS of an older-age owner. The above evidence indicated that AI control measures in PDS should be focused on education to perform better behaviors and obtain AI knowledge for both owners and workers, followed by a clear instruction in the policy documents for AI control in PDS as the best combination (Manabe et al., 2011).

In conclusion, PDS and LBM are identified to possess big issues for AI control in Vietnam. The specific resolutions for each section based on their characteristics are needed. This study proposed that the most suitable measure for the control of AIV circulation should be a combination of a systematic education campaign to enhance the knowledge, attitude, and practice of all participants under strong policy measures to strengthen and update the surveillance system, conduct field investigations regularly, and release the clear instructions of AI control measures in the law.

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Conflict of interest

The authors declare that no competing interests exist.

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Tables

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