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| 1 | A systematic approach to illuminate a new hot spot of avian influenza virus circulation in South |
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| 5 | A new AI hotspot in South Vietnam |
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39 Summary

40 In South Vietnam live bird markets (LBMs) are key in the value chain of poultry products and spread 41 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. 42 43 A cross-sectional study of poultry flock managers and poultry on backyard farms, commercial (high 44 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 45 46 collected from birds from 101 backyard farms, 50 commercial farms, 58 sellers in LBMs and 19 47 traders in PDSs. Swab samples were submitted for AIV isolation. At the same time a questionnaire 48 was administered to flock managers asking them to provide details of their knowledge, attitude and 49 practices related to avian influenza. Multiple correspondence analysis and a mixed-effects 50 multivariable logistic regression model were developed to identify enterprise and flock manager 51 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 52 8.5%). The odds of a bird being AIV positive if it was from an LBM or PDS were 45 (95% CI: 3.4 53 54 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 55 56 (uncertain or inconsistent) level and a low level of knowledge about AI were 5.0 (95% CI: 0.20 to 57 130) and 3.5 (95% CI: 0.2 to 62), respectively, times higher to the odd of birds being positive for 58 respondents with a good knowledge of AI. LBMs and PDSs should receive specific emphasis in AI

| 59 | control programs in Vietnam. Our findings provide evidence to support the hypothesis that |
|----|--|
| 60 | incomplete respondent knowledge of AI and AIV spread mechanism were associated with an |
| 61 | increased risk of AIV positivity. Delivery of education programs specifically designed for those in |
| 62 | each enterprise will assist in this regard. The timing and frequency of delivery of education programs |
| 63 | are likely to be important if the turnover of those working in LBMs and PDSs is high. |
| 64 | |
| | |

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

67 Introduction

68 Avian influenza (AI) virus circulation has been reported in many countries, including 69 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 70 71 campaigns and stamping-out programs in a number of countries (Alexander, 2007; Brown, 2010). 72 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 73 74 sector continue to occur. A number of studies have improved our understanding of the epidemiology 75 of avian influenza by identifying drivers of virus spread (Nomura et al., 2012; Okamatsu et al., 2013; 76 Nguyen et al., 2014; Chu et al., 2016, 2017; Nguyen et al., 2020). As part of their efforts to reduce 77 AIV infection risk, the Vietnamese government has developed both active and passive surveillance 78 programs. One of the advantages of active surveillance programs is that they can detect the 79 introduction of new virus strains into a population or detect the evolution of virus strains relatively 80 quickly. In contrast, passive surveillance programs rely on prompt reporting by poultry farmers for 81 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).

| 90 | In a number of previous studies the movement of live birds arising from trade has shown to |
|-----|--|
| 91 | be an important determinant of AIV spread (Kung et al., 2007; Kim et al., 2010; Gilbert et al., 2014). |
| 92 | In addition, live bird markets (LBMs) play an important role in AIV circulation (Bulaga et al., 2003; |
| 93 | Choi et al., 2005; Chen et al., 2009; Indriani et al., 2010; Kang et al., 2015). During an outbreak of |
| 94 | H7N9 AIV in China in 2013 which was the cause of up to 45 human deaths, the closure of LBMs |
| 95 | was remarkably effective in reducing human infection rates by up to 99% (Yu et al., 2014). Although |
| 96 | LBM closures break the viral amplification cycle, AIVs are often re-introduced once they are re- |
| 97 | opened (Kung et al., 2003). A previous Vietnamese study investigating the effectiveness of virus |
| 98 | control measures in LBMs showed no differences in AIV prevalence between LBMs with and |
| 99 | without biosecurity interventions (Chu et al., 2017). One interpretation of these findings is that the |
| 100 | introduction of AIV into LBMs occurs continuously. The absence of differences in AIV prevalence |
| 101 | between intervention and non-intervention LBMs supports the hypothesis that the source of AIV in |
| 102 | the value chain of poultry products in Vietnam has not yet been fully identified and controlled. |
| 103 | As a result of active surveillance programs for AI that have been operational in Vietnam |
| 104 | since 2015, it was shown that poultry delivery stations (PDSs) play a role connecting poultry farms, |
| 105 | LBMs and poultry slaughterhouses (Supplementary Figure 1). Backyard farms are characterized by |

- 106 their small-scale, the mixing of poultry species and relatively low levels of biosecurity whereas

107 commercial farms routinely practice several AIV control measures such as separating poultry species, routinely disinfecting those entering and leaving premises and limiting contact between 108 109 poultry and wildlife. LBMs tend to receive poultry from nearby backyard and semi-commercial 110 poultry enterprises (Phan et al., 2013). In contrast, PDSs are private businesses which usually 111 receive birds from much larger catchment areas (up to 100 km) and mix several species of poultry 112 under relatively poor biosecurity conditions. 113 We conducted a cross-sectional study of avian influenza and biosecurity practices among 114 four poultry enterprise groups (backyard farms, commercial farms, LBMs and PDSs) in Vinh Long 115 Province, Vietnam in 2016 and 2017. Our specific aims were to: (1) estimate the individual bird-116 level prevalence of AIV in each of the four enterprise groups; and (2) identify characteristics of 117 those responsible for the management of birds that were associated with AIV infection positivity. 118 Identifying poultry flock manager characteristics that increase the risk of AIV positivity across 119 different industry players is a necessary step towards the design of effective, evidence-based 120 measures to reduce the risk of AIV infection through the supply chain of poultry products in 121 Vietnam.

122 Materials and methods

123 Study design and study area

124 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 125 126 Vinh Long Province, Vietnam (Figure 1). Data were collected over two sampling rounds: the first in 127 December 2016 and the second in August 2017. From a sampling frame of enterprises provided by 128 local Department of Animal Health (DAH) officials those eligible for the study were selected at 129 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 130 131 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 132 133 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 134 least more than one of several control measures (such as keeping poultry in a separate place, the use 135 of routine vaccination and disinfection) were applied, were defined as commercial poultry farms. Up 136 137 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 138 139 PDSs per study district were selected at each sampling round returning 13 individual PDSs included 140 in the study. In each of the two sampling rounds, the average number of birds sampled was 10 for 141 backyard farms (minimum of 5, maximum of 20), 26 for commercial poultry farms (minimum of 10,

| 142 | maximum of 50), 11 for LBM sellers (minimum of 10, maximum of 52) and 40 for PDS traders |
|-----|--|
| 143 | (minimum of 19, maximum of 52). At the time of bird sampling key decision makers from selected |
| 144 | backyard farms, commercial poultry farms, LBM traders and PDSs were interviewed by the first |
| 145 | author and staff from the Sub-Department of Animal Health (SDAH) staff of Vinh Long Province for |
| 146 | the purpose of questionnaire administration. |
| 147 | |
| 148 | Laboratory procedures |
| 149 | Oropharyngeal swabs, cloacal swabs and fecal samples were collected from chickens, ducks, |
| 150 | and Muscovy ducks from each participant enterprise at each sampling round. The oropharyngeal and |
| 151 | cloacal swabs from the same poultry were kept in one sterile tube containing transport medium, as |
| 152 | described previously (Le et al., 2020). This medium comprised Eagle's minimum essential medium |
| 153 | (Nissui, Japan) containing 10,000 U/mL penicillin G (Meiji Seika, Japan), 10 mg/mL streptomycin |
| 154 | (Meiji Seika, Japan), 0.3 mg/mL gentamicin (Schering Plough, USA), 250 U/mL nystatin (Sigma, |
| 155 | USA), and 0.5% bovine serum albumin fraction V (Roche, Switzerland). Samples were transported |
| 156 | to the Regional Animal Health Office No. 7 (RAHO7), Can Tho, Vietnam. Under ISO 17025:2017 |
| 157 | certification for the diagnostic procedure in RAHO7, the aliquot of ten samples collected from the |
| 158 | same enterprise were pooled to test for the presence of influenza type A virus using real-time reverse |
| 159 | transcription-polymerase chain reaction (RT-PCR) targeting the M gene with the primer design and |
| 160 | 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.

163

164 Virus isolation

| 165 | Ten-day-old chicken embryonated eggs produced by conventional chickens tested for |
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| 166 | freedom from AIV antibody by ELISA were used to isolate AIV. The M-gene-positive pooled |
| 167 | samples were selected for virus isolation. Each of ten samples was resuspended with transport |
| 168 | medium and inoculated into the allantoic cavity. Inoculated eggs were incubated for 30 to 48 hours |
| 169 | at 35 °C and the allantoic fluid collected to check the hemagglutination activity. The hemagglutination |
| 170 | inhibition and neuraminidase inhibition tests with antisera to the reference influenza virus strains |
| 171 | were performed to determine isolated influenza virus subtypes (Kida & Yanagawa, 1979). |

172

173 *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)

| 181 | the source, type and numbers of poultry present on their enterprise on the day of interview; (3) their |
|-----|--|
| 182 | general knowledge regarding AIV; (4) their attitudes about AI control measures; and (5) AI |
| 183 | biosecurity measures routinely used. |
| 184 | At the start of the first sampling round SDAH staff from Vinh Long $(n = 8)$ who were recruited |
| 185 | for data collection received instruction on questionnaire administration. Questionnaire surveys were |
| 186 | administered by SDAH staff to each respondent. A total of 228 face-to-face interviews were carried |
| 187 | out during the two sampling rounds in the four districts. In each sampling round, birds were sampled |
| 188 | and questionnaires administered to key decision makers on each of the participant backyard farms |
| 189 | and commercial farms in the early stage. Immediately after the early stage was finished, the same |
| 190 | procedure was then applied in LBMs and PDSs at the later stage. The sampling schedule was |
| 191 | announced to respondents and local veterinarians well in advance and, for both rounds, samples were |
| 192 | collected and questionnaires administered over a period of 8 days. |
| 193 | |
| 194 | Data management |
| 195 | Each of the respondents enrolled into the study were assigned a unique identification code. |
| 196 | Questionnaire responses at each sampling round and the results of AIV isolation from sampled |
| 197 | poultry were recorded in two tables in a relational database with the respondent identification code |
| 198 | providing the link between each table. |

199 The diagnostic sensitivity and specificity of the RT-PCR was assumed to be both 100% (Das 200 et al., 2006). Furthermore, the diagnostic sensitivity and specificity of virus isolation was assumed to 201 be both 100% (Suarez et al., 2007). Multiple correspondence analysis 202 203 Multiple correspondence analysis (MCA) (Snijders & Bosker, 1999) was used to produce a 204 graphic representation of the relationships between responses provided in each of the four sections of 205 the questionnaire: demographic details, AIV knowledge, AIV attitude and AIV practice. 206 MCA is a generalization of principle component analysis suitable for categorical variables. In an MCA, the rows and columns of an $I \times I$ indicator matrix (where I is the set of i individual 207 responses to a given question and *J* is the set of *j* categories of responses for each question) are 208 209 assumed to be points in a high-dimensional Euclidean space. The method aims to redefine the 210 dimensions of the space so that the principal dimensions ('components') capture the most variance. 211 The results of an MCA are presented as a scatterplot for the first and second principle components -212 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 213 plot. MCA scatterplots were produced using responses to each of the four sections of the 214 215 questionnaire (demographic details, AIV knowledge, AIV attitude and AIV practice) and, for each 216 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 217 subgroups ('clusters') for each section of the questionnaire. These assigned clusters were then used 218

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).

222 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 *i*th bird this model takes the following form under the assumption of $p_i = P(Y_i = 1)$ and that Y_i are mutually independent:

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

In Eq. (1) β_0 represents the intercept term and β_1, \dots, β_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:

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

239 In Eq. (2), p_{ij} represents the probability of the *i*th bird from the *j*th respondent being AIV positive. Variable P_i is a zero mean random effect term with variance σ_P^2 indicating the effect of the 240 241 *j*th respondent on AIV positivity. The term P_i was included in the model to account for unexplained extrabinomial variation arising from unmeasured respondent-level influences on AIV positivity. 242 A backward stepwise approach was used for explanatory variable selection. Each of the 243 244 explanatory variables unconditionally associated with the outcome at P < 0.2 were included in the 245 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 246 247 at $\alpha < 0.05$. Explanatory variables that were excluded in univariable analyses were tested for 248 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 249 explanatory variables were assessed: none were found to be significant at $\alpha = 0.05$. The model was 250 then extended to include the random effect term P_i (Equation 2). Explanatory variables were retained 251 252 in the mixed-effects model, regardless of their statistical significance.

253 The assumptions of normality and homogeneity of variance were investigated by constructing 254 histograms of residuals from the multilevel model and scatterplots of the residuals as a function of 255 the predicted values, respectively. Estimates of the variance attributable to the three levels of the data 256 (respondent, bird) were calculated assuming the level 1 (bird) variance on the logit scale was $\frac{\pi^2}{3}$ where 257 $\pi = 3.1416$ (Snijders & Bosker, 1999).

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| 259 | A Receiver Operating Characteristic (ROC) curve was constructed on the basis of the bird- |
|-----|---|
| 260 | level AIV positivity status predicted by the model. The area under the ROC curve, which ranges from |
| 261 | zero to one, provided a measure of the model's ability to discriminate between AIV-positive and |
| 262 | AIV-negative birds. The greater the area under the ROC curve the better the model's discriminatory |
| 263 | power. |
| 264 | 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

266 developed using the contributed lme4 package (Bates et al., 2015) in R.

267 Results

268 Descriptive statistics and unconditional associations

269 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 270 shown in Table 1. A total of 3,597 birds were sampled: 1,056 from 101 backyard farms; 1,200 from 271 272 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 273 274 8.5%) were AIV positive. In total, 13 H3N2, 21 H5N1, 127 H6N6, 105 H9N2, 2 H10N3, 5 H11N9, 275 and 1 H12N5 AIVs were identified from collected samples (Supplementary Table S1). Isolation rates 276 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 277 farms (3.0%, 95% CI: 2.1% to 4.3%) and commercial poultry farms (0.6%, 95% CI: 0.2% to 1.2%). 278 279 The numbers of chickens and ducks sampled were 1,801 (50%) and 1,575 (44%), respectively. 280 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 281 282 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 283 284 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 285

- 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%).
- 288

289 Multiple correspondence analysis

290 The results of our MCA analyses are based on questionnaire responses from key decision 291 makers of 100 backyard farms and 40 commercial poultry farms, 58 sellers from LBMs and 19 PDS 292 traders. MCA scatterplots developed from responses to each of the four sections of the questionnaire 293 (demographic details, AIV knowledge, AIV attitude and AIV practice) are shown in Figures 3a to 6a. 294 Accompanying each scatterplot is an error bar plot showing the prevalence of AIV positivity as a 295 function of the identified cluster group, stratified by enterprise type (Figures 3b to 6b). In an MCA 296 scatterplot, the relationships among categories of questionnaire responses are reflected by the distance 297 between pairs of marks with questionnaire responses further from the origin more discriminating in 298 the data. Superimposed on each MCA scatterplot (Figures 3a to 6a) are ellipses delineating the 299 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 300 301 tables are interpreted as the 'profiles' for questionnaire responses of respondents through 302 demographic details, AIV knowledge, AIV attitude, and AIV practice section.

303

304 In the demographic section of the questionnaire, three clusters were identified (Table 2). The 305 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 = 13respondents) 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.

311 For AIV knowledge three clusters were identified (Table 3). The first cluster (n = 29312 respondents) was comprised predominantly of those that had heard about AI and knew that infected 313 birds were a source of infection, primarily domestic poultry and interactions with those from backyard 314 farms, commercial farms, LBMs and PDSs. Most in this cluster obtained their information about AI 315 from the television and local veterinarians; 59% had seen AI before, and most had received training 316 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 317 318 poultry, wild birds, domestic animals) were similarly evenly split. Most in this cluster obtained 319 information about AI from the radio and local veterinarians. Interestingly, 94% of those in this cluster 320 had attended training on AI control and prevention. The third cluster (n = 121 respondents) was 321 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 322 323 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 324 325 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.

330 For AIV attitudes, three clusters were identified (Table 4). For the first, all respondents (n =331 55) were willing to report an AI outbreak if detected, mostly to local veterinarians (87%) but not to 332 local Department of Animal Health officials. For the second cluster (n = 41) there was relatively even 333 split between willingness to report an AI outbreak if detected (44% yes; 56% no). If an outbreak was 334 to be reported, it would be to a local Department of Animal Health official. For the third cluster 335 (comprised of n = 121 respondents) all declared that they would not be willing to report an AI 336 outbreak if detected. If an outbreak was to be reported, 55% of them stated that they would report to 337 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', 338 'Report AI mixed' and 'Report AI no', respectively. 339

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.
- 348
- 349 Multivariable logistic regression analyses

350 Estimated regression coefficients for enterprise type, knowledge cluster and attitude cluster 351 and estimates of the variability of the farm and bird-level random effect terms from the mixed-effects 352 logistic regression model are shown in Table 6. Not surprisingly, with the marked difference in AIV 353 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 354 355 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 356 difference in the odds of birds being AIV positive, the significant difference was not recorded. 357

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.

363 Discussion

364 This cross-sectional study quantified the prevalence of AIV positivity among poultry from 365 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 366 367 measures for AI have been applied to backyard poultry farms, commercial poultry farms and LBMs 368 since the first outbreaks of AI were reported in 2003. Our finding that one in five poultry sampled 369 from PDSs were AIV positive (21%; 95% CI: 18% to 24%, Table 1) demonstrate a relatively high 370 prevalence of AIV in poultry in this sector and indicate that PDSs should receive emphasis for 371 interventions in AI control programs. Unlike LBMs, where control measures for AI are supervised 372 by local veterinarians and supported by local government authorities, AI control measures in PDSs 373 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 374 375 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 376 377 applied to LBMs (Manabe et al., 2011). These findings are consistent with the cross-sectional study 378 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 379 380 PDS-like enterprises (such as wholesale markets and duck yards) often lacked regular disinfection 381 procedures, routinely kept poultry from different sources in the same cage and received a low level 382 of oversight from local veterinary authorities.

| 383 | A previous study carried out in the south of Vietnam under similar conditions identified a |
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| 384 | slightly lower prevalence of AI (5.3%) among farms and LBMs (Okamatsu et al., 2013) compared to |
| 385 | the 7.6% identified in this study. Furthermore, the prevalence of AIV positivity among LBMs in this |
| 386 | study (14%) was higher than the AIV positivity prevalence of 6.9% among LBMs in the center of |
| 387 | Vietnam identified by (Chu et al., 2017) and 5.8% in the north of Vietnam identified by (Thuy et al., |
| 388 | 2016). Assuming these differences in prevalence are real and not due to, for example, seasonal and |
| 389 | yearly fluctuations in the incidence of AI, our results imply that LBMs in southern Vietnam play a |
| 390 | more dominant role in maintaining AIV circulation in the poultry population compared to other areas |
| 391 | of the country. Similar to PDSs the higher prevalence of AIV positivity among poultry sampled from |
| 392 | LBMs is likely to be due to the routine mixing of large numbers of birds from different sources |
| 393 | (Nguyen et al., 2017) and generally lower levels of biosecurity compared with both backyard and |
| 394 | commercial poultry farms. |
| 395 | The questionnaire designed for this study was comprehensive and sought to solicit respondent |
| 396 | demographic information and details of their knowledge, attitude and practice with respect to AI. The |
| 397 | questionnaire comprised a total of 46 questions which presented difficulties when developing a |
| 398 | parsimonious regression model to identify risk factors for AIV positivity. To address this issue MCA |
| 399 | analyses were carried out using responses from each of the four sections of the questionnaire |
| 400 | (demography, AI knowledge, AI attitude and AI practice). Clusters of responses for each section were |
| 401 | identified and used as explanatory variables for our multivariable logistic regression model. In effect |
| | |

402 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.

408 For the fixed-effects logistic regression model the explanatory variable representing the three 409 cluster categories of AI knowledge (good knowledge, mixed knowledge and low knowledge) and the 410 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 411 412 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 413 414 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 415 knowledge about AI classified as 'mixed' (i.e., where some facts regarding AI transmission and 416 spread were correctly recalled and others were not) and respondents where their level of knowledge 417 418 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 419 420 a good knowledge of AIV transmission and spread. Similar trends were noted for AI attitude. 421 Respondents that provided inconsistent responses in terms of their likelihood to report an outbreak of 422 AI to authorities ('Report AI mixed') and those that were unlikely to report an outbreak of AI to

| 423 | 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 |
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| 424 | in the odds of their birds being AIV positive compared with respondents classified as being likely to |
| 425 | report an outbreak of AI to authorities ('Report AI yes'). The substantial increase in the uncertainty |
| 426 | around each of these measures of association after inclusion of the enterprise level random effect term |
| 427 | reflect what is believed to be substantial individual enterprise-level influence on these associations. |
| 428 | Traders in PDSs and sellers at LBMs usually run their business dependent on market demand |
| 429 | (Meyer et al., 2017) which means that they tend to leave the industry if a sufficient financial return is |
| 430 | not achieved. For this reason, there is a relatively high population turnover of PDS traders and LBM |
| 431 | sellers with those that are new to the industry often lacking knowledge about AI and its control. The |
| 432 | knowledge and practice of participants from LBMs and PDSs are likely to be important in a given |
| 433 | area because these industry players directly influence AIV circulation risk in a given market |
| 434 | catchment area. In contrast, backyard and commercial poultry farmers run their businesses based on |
| 435 | their ability and resources, meaning that they strive to obtain more knowledge and adopt better |
| 436 | practices to generate more income (Chilonda & Van Huylenbroeck, 2001). This explanation is |
| 437 | indirectly supported by the findings from this study: AIV positivity among birds from backyard farms |
| 438 | and commercial farms was relatively low. We attempted to assess the interaction between enterprise |
| 439 | type and AI knowledge, attitude and practice cluster assignment on AIV positivity risk to investigate |
| 440 | this hypothesis further. Zero counts of AIV positive birds in some strata combinations made this |
| 441 | analysis not possible. |

| 442 | In conclusion, consistent with previous studies we identified a higher prevalence of AIV |
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| 443 | positivity among poultry sampled from LBMs and PDSs compared with poultry sampled from |
| 444 | backyard and commercial poultry farms which means that LBMs and PDSs should receive specific |
| 445 | emphasis in AI control programs. Our findings provide evidence to support the hypothesis that |
| 446 | incomplete respondent knowledge of AI and how it is spread was associated with an increased risk |
| 447 | of AIV positivity. Delivery of education programs specifically designed for each industry sector |
| 448 | (backyard farms, commercial farms, LBMs and PDSs) are likely to assist in this regard. The timing |
| 449 | and frequency of delivery of education programs is likely to be important if the turnover of those |
| 450 | working in LBMs and PDSs is high. Furthermore, the previous studies in Mekong Delta suggested |
| 451 | that the farming practice of the farmers and trading system in this region was similar among the |
| 452 | provinces. Implying that the result in this study might be applied for AI control in the other provinces |
| 453 | of the Mekong Delta. |
| | |

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Conflict of interest

471 The authors declare that no competing interests exist.

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| 621 | Figure | legends |
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| 623 | Figure 1. (a) Map of Vietnam showing the location of Vinh Long province; (b) map showing the |
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| 624 | district boundaries in Vinh Long and the location of the four districts in which sampling was carried |
| 625 | out (gray). |
| 626 | |
| 627 | Figure 2. Error bar plot showing AIV prevalence (and its 95% confidence interval for backyard farms, |
| 628 | commercial farms, live bird markets (LBM) and poultry delivery stations (PDS) by sampling round |
| 629 | (2016 and 2017). |
| 630 | |
| 631 | Figure 3. (a) Multiple correspondence analysis biplot showing questionnaire responses related to |
| 632 | respondent demographics; (b) error bar plot showing AIV prevalence (and its 95% confidence |
| 633 | interval) for the three clusters shown in (a) by enterprise type. |
| 634 | |
| 635 | Figure 4. (a) Multiple correspondence analysis biplot showing questionnaire responses related to |
| 636 | respondent AI knowledge; (b) error bar plot showing AIV prevalence (and its 95% confidence |
| 637 | interval) for the three clusters shown in (a) by enterprise type. |
| 638 | |

639 Figure 5. (a) Multiple correspondence analysis biplot showing questionnaire responses related to

- 640 respondent AI attitude; (b) error bar plot showing AIV prevalence (and its 95% confidence interval)
- 641 for the three clusters shown in (a) by enterprise type.

- 643 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)
- 645 for the three clusters shown in (a) by enterprise type.

646 List of supplementary figure

647

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

649 value chain.

Fig. 1. Le et al.



Fig. 2. Le et al.



Fig. 3. Le et al.



Fig. 4. Le et al.







Fig. 5. Le et al.







Fig. 6. Le et al.



(b)



| 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 |

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.

CI: Confidence interval.

^a Number of AIV positive birds per 100 birds at risk.

| Variable | 'Female backyard' | 'Male LBM ≤10 yrs' | 'Male LBM >10 yrs' |
|--------------------|-------------------|--------------------|--------------------|
| | n = 158 | <i>n</i> = 46 | <i>n</i> = 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 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 | 'Good knowledg | ge' 'Mixed knowledge' | 'Low knowledge' |
|---------------------------------------|-------------------------------------|-----------------------|-----------------|
| | <i>n</i> = 29 | <i>n</i> = 67 | <i>n</i> = 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 it | nfected bird: | | |
| Yes | 75.9 | 52.2 | 70.2 |
| No | 24.1 | 47.8 | 29.8 |
| Know that AIV can be spread by do | mestic poultry: | | |
| Yes | 75.9 | 47.8 | 23.1 |
| No | 24.1 | 52.2 | 76.9 |
| Know that AIV can be spread by wi | ld birds: | | |
| Yes | 27.6 | 41.8 | 1.7 |
| No | 72.4 | 58.2 | 98.3 |
| Believe that AIV can be spread by d | lomestic animals (excluding poultr | ry): | |
| Yes | 20.7 | 53.7 | 3.3 |
| No | 79.3 | 46.3 | 96.7 |
| Believe that AIV can be spread by i | nteractions with other poultry farm | ners: | |
| Yes | 3.4 | 55.2 | 0.8 |
| No | 96.6 | 44.8 | 99.2 |
| Believe that AIV can be spread by i | nteractions with poultry traders: | | |
| Yes | 10.3 | 64.2 | 0.8 |
| No | 89.7 | 35.8 | 99.2 |
| Believe that AIV can be spread by it | nteractions with those from backya | ard farms: | |
| Yes | 69.0 | 40.3 | 62.8 |
| No | 31.0 | 59.7 | 37.2 |
| Believe that AIV can be spread by i | nteractions with those from comm | ercial farms: | |
| Yes | 65.5 | 53.7 | 18.2 |
| No | 34.5 | 46.3 | 81.8 |
| Believe that LBMs are a source of A | AI: | | |
| Yes | 96.6 | 55.2 | 4.1 |
| No | 3.4 | 44.8 | 95.9 |
| Believe that PDSs are a source of A | I: | | |
| Yes | 96.6 | 49.3 | 7.4 |
| No | 3.4 | 50.7 | 92.6 |
| Believe that slaughterhouses are a se | ource of AI: | | |
| Yes | 65.5 | 52.2 | 1.7 |
| No | 34.5 | 47.8 | 98.3 |
| Obtain information about AI from the | ne television: | | |
| Yes | 86.2 | 43.3 | 94.2 |
| No | 13.8 | 56.7 | 5.8 |
| Obtain information about AI from p | rinted material: | | |
| Yes | 3.4 | 55.2 | 0.0 |
| No | 96.6 | 44.8 | 100.0 |
| Obtain information about AI by atte | nding training courses: | | |
| Yes | 13.8 | 61.2 | 13.2 |
| No | 86.2 | 38.8 | 86.8 |
| Obtain information about AI from the | ne radio: | | |
| Yes | 20.7 | 85.1 | 14.9 |
| No | 79.3 | 14.9 | 85.1 |

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.

| 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 | | | |

| Variable | 'Report AI yes' | 'Report AI mixed' | 'Report AI no' | | |
|---|-----------------|-------------------|----------------|--|--|
| | <i>n</i> = 55 | <i>n</i> = 41 | <i>n</i> = 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 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 | 'High biosecurity' | |
|---|--------------------|---------------|
| | <i>n</i> = 135 | <i>n</i> = 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: | 50.5 | |
| Yes | 28.1 | 1.2 |
| No | 71.9 | 98.8 |
| Isolate sick birds: | 1 2 . / | 2010 |
| Ves | 58 5 | 0.0 |
| No | 41.5 | 100.0 |
| Sell sick hirds | J.17 | 100.0 |
| | <i>22.2</i> | 03.0 |
| No | 22.2 77 8 | 73.7 6 1 |
| nu Traat siak hirds: | //.0 | 0.1 |
| Vee | 71 1 | 0.0 |
| I CS | /1.1 | 0.0 |
| NO | 28.9 | 100.0 |
| recu sick dirds to livestock: | 17.0 | 21.7 |
| Y es | 1/.8 | 31./ (0.2 |
| 1N0 | 82.2 | 08.5 |

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.

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 | $2.6 (0.2 \text{ to } 27)^{a}$ |
| 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.