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1 **A cross-sectional study to quantify the prevalence of avian influenza viruses in poultry at**
2 **intervention and non-intervention live bird markets in central Vietnam, 2014**

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41 **Summary**

42 In Vietnam, live bird markets are found in most populated centers, providing the means by which
43 fresh poultry can be purchased by consumers for immediate consumption. Live bird markets are
44 aggregation points for large numbers of poultry and therefore it is common for a range of avian
45 influenza viruses to be mixed within live bird markets as a result of different poultry types and species
46 being brought together from different geographical locations. We conducted a cross-sectional study
47 in seven live bird markets in four districts of Thua Thien Hue province in August and December,
48 2014. The aims of this study were to: (1) document the prevalence of avian influenza in live bird
49 markets (as measured by virus isolation); and (2) quantify individual bird-, seller-, and market-level
50 characteristics that rendered poultry more likely to be positive for avian influenza virus at the time of
51 sale. A questionnaire soliciting details of knowledge, attitude and avian influenza practices was
52 administered to poultry sellers in study markets. At the same time, swabs and fecal samples were
53 collected from individual poultry and submitted for isolation of avian influenza virus. The final
54 dataset comprised samples from 1,629 birds from 83 sellers in the seven live bird markets. A total of
55 113 birds were positive for virus isolation; a prevalence of 6.9 (95% CI 5.8 to 8.3) avian influenza
56 virus positive birds per 100 birds submitted for sale. After adjusting for clustering at the market- and
57 individual seller-level, none of the explanatory variables solicited in the questionnaire were
58 significantly associated with avian influenza virus isolation positivity. The proportions of variance at
59 the individual market-, seller-, and individual bird-level were 6%, 48% and 46%, respectively. We

60 conclude that the emphasis of avian influenza control efforts in Vietnam should be at the individual
61 seller- as opposed to the market-level.

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63 **Key words:** Avian influenza; live bird markets; multi-level modeling; seller-level; Vietnam

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80 **Introduction**

81 Live bird markets (LBMs) are known to be reservoirs and transmission hubs for avian influenza
82 viruses (AIV) (Biswas et al., 2015). In Southeast Asian countries, LBMs are ubiquitous and integral
83 components of the semi-intensive poultry industries that are common in this part of the world (Wan
84 et al., 2011; Indriani et al., 2010). In Vietnam, LBMs are found in most populated centers, providing
85 the means by which the majority of the population access fresh poultry for immediate consumption
86 (Fournié et al., 2012). LBMs tend to be small scale operations where poultry are mixed together with
87 other animals under conditions of relatively poor infrastructure, mostly trading poultry derived from
88 household and semi-commercial enterprises situated closely to the area in which markets are located
89 (Phan et al., 2012). In LBMs, it is common for a range of subtypes of AIV to be mixed as a result of
90 different poultry types and species being brought together from different geographical locations (Li
91 et al., 2015). In Southeast Asia, highly pathogenic avian influenza (HPAI) viruses are known to
92 circulate in LBMs (Biswas et al., 2015; Nasreen et al., 2015; Nguyen et al., 2013; Phan et al., 2012;
93 Indriani et al., 2010) and it has been hypothesized that LBMs may facilitate the emergence and spread
94 of new viral reassortants due to close contact amongst the infected birds (Zhou et al., 2015).
95 Furthermore, it has also been shown that, in China, human infections with AIV, in particular, of the
96 subtypes H5N1 and H7N9 are associated with recent exposure to poultry in LBMs (Li et al., 2015;
97 Wan et al., 2011).

98

99 An effective strategy for reducing the likelihood of AIV transmission to the general public is to
100 close LBMs indefinitely (Yu et al., 2015; He et al., 2014; Wu et al., 2014). This approach was used
101 in the outbreak of HPAI that occurred in the Hong Kong Special Administrative Region of the
102 People's Republic of China in 1997 (Chan, 2002). Although this strategy is effective for reducing the
103 risk of AIV infection, it is an unpopular approach with poultry consumers (Scoones, 2010) and
104 difficult in terms of promoting effective long term control of AI because poultry sellers that are
105 displaced from LBMs that have been closed tend to rapidly establish 'black market' poultry trading
106 locations (Vietnam Department of Animal Health, personal communication, 2014). For these reasons,
107 a less draconian approach has been to adopt interventions aimed to improve LBM biosecurity and
108 hygiene. In this way, the risk of AIV infection within LBMs can be minimized and, at the same time,
109 poultry trade can be permitted to continue. In a previous study, the characteristics of LBMs with
110 improved infrastructure ('intervention LBMs', $n = 3$) were compared with those operating in a routine
111 manner ($n = 6$) under the Vietnam Avian and Human Influenza Control and Preparedness Project
112 (VAHIP) in Thua Thien Hue province, in the central region of Vietnam (Chu et al., 2016; VAHIP-
113 World Bank Group, 2015). The study showed that HPAI H5N6 viruses were isolated from apparently
114 healthy ducks, Muscovy ducks and environmental samples in one of the intervention LBMs. Although
115 the number of LBMs that took part in the study was small, it appears that physical improvements in
116 the market biosecurity and hygiene had little apparent effects on the prevalence of AIV amongst
117 poultry present for sale at those markets.

118

119 In this study, the data of Chu et al. (2016) were used to identify characteristics associated with
120 the presence of AIV in poultry submitted for sale at seven LBMs in Thua Thien Hue province,
121 Vietnam. Our specific aims were to: (1) document the prevalence of AIV in seven LBMs that took
122 part in this study and compare the prevalence of AIV positive samples amongst intervention and non-
123 intervention markets; and (2) quantify bird-, poultry seller- and market-level characteristics that
124 rendered individual birds more likely to be AIV isolation positive at the time of sale. Identifying the
125 relative importance of factors influencing the poultry submitted for sale at LBMs is a critical first step
126 towards the design of evidence-based better measures to reduce the number of AIV positive birds
127 (and therefore the risk of virus infection) within LBMs in Vietnam.

128

129 **Materials and methods**

130 **Study design and study area**

131 A cross-sectional study was carried out in seven LBMs in four districts of Thua Thien Hue
132 province (Figure 1 and Table 1), Vietnam in August and December, 2014. At three of the seven LBMs,
133 biosecurity had been improved as part of the VAHIP program in Thua Thien Hue province. In
134 intervention LBMs, there was a good standard of infrastructure with poultry from different sources
135 physically separated by the seller; besides, disinfection procedures were performed twice, in the
136 morning and evening, on a given sale day. The other four (non-intervention) LBMs were wet markets
137 at which no particular intensive biosecurity interventions were carried out and at which poultry and

138 other animals were mixed together under relatively low biosecurity conditions; details are described
139 in Chu et al. (2016).

140 Each of the LBMs were visited by the investigators (D-H C, TNN and LVN) and Sub-
141 Department of Animal Health (SDAH) staff of Thua Thien Hue province on two occasions: August
142 and December 2014. At the time of each market visit, a list of all poultry sellers present was obtained
143 from the market manager. Each poultry seller was contacted with the investigators and samples were
144 taken from individual birds for AIV isolation. At the conclusion of sampling, a questionnaire
145 (described below) was administered to the poultry sellers.

146

147 **Laboratory procedures**

148 Oropharyngeal, cloacal swabs and fecal samples were collected from chickens, ducks, Muscovy
149 ducks for each poultry seller on each of the two sampling rounds. For each bird, the oropharyngeal
150 and cloacal swabs were collected in a sterile tube with transport medium, as described by Chu et al.
151 (2016). Samples were then transported to the National Center for Veterinary Diagnostics (NCVD),
152 Hanoi, Vietnam. At the NCVD, the samples were tested for the presence of influenza type A viruses
153 (M gene detection) using real time RT-PCR. All samples were then prepared for transfer to the
154 Laboratory of Microbiology, Department of Disease Control, Graduate School of Veterinary
155 Medicine, Hokkaido University, Sapporo, Japan. The shipment of samples containing AIV was
156 classified into Biological Substance, Category B, following the instructions of the International Air
157 Transport Association (IATA) in Dangerous Goods Regulation Manual (Pearson, 2007). At the

158 Laboratory of Microbiology, all samples were submitted for virus isolation. After which,
159 representative isolates, such as H5, H6, and H9 AIV, were phylogenetically and antigenically
160 analyzed to characterize the genetic and antigenic variation of AIVs currently circulating in LBMs in
161 Vietnam, further detail is provided by Chu et al. (2016).

162

163 **Questionnaire and interview**

164 A questionnaire developed to solicit details of knowledge, attitudes and practices concerning
165 AIV was developed by the authors in conjunction with staffs from the Vietnamese Department of
166 Animal Health (DAH), Hanoi. A copy of the questionnaire is available from the corresponding
167 authors on request. The questionnaire was developed in Vietnamese and was comprised of 45 open
168 and two closed questions organized into the following sections: (1) demographic details of the seller;
169 (2) a description of the source and type of poultry on sale on the given market day; and (3) details of
170 AI biosecurity measures typically used by the seller.

171

172 A total of 83 face-to-face interviews with poultry sellers were carried out over the two sampling
173 rounds in the seven markets (45 in intervention and 38 in non-intervention LBMs). Questionnaire
174 survey were administered by trained interviewers from the SDAH of Thua Thien Hue and the District
175 Veterinary Stations of each of the districts in which the study markets were located. Interviews were
176 carried out with assistance from SDAH veterinarians located in communes adjacent to each market.
177 DAH staff provided technical supervision and assistance during each market visit. On average, the

length of time taken to visit all of the sellers within a market and to complete sampling and administration of the questionnaires was two days (minimum 1 day; maximum 7 days).

180

181 **Data management**

Questionnaire responses for each sampling round were entered into a relational database with a numeric poultry seller identifier (assigned at the time of interview in the first round) used as a unique key. The results of AIV isolation were entered into this database as a separate table. The two tables were linked within the database using the unique poultry seller identifier.

186

187 **Statistical analyses**

The prevalence of AIV at the individual bird level was calculated as the total number of individual bird samples that were AIV positive as the numerator and the total number of birds sampled as the denominator.

191

Unconditional associations between questionnaire responses (the explanatory variables) and the outcome of interest (the presence or absence of AIV in an individual bird) were computed using the odds ratio. Explanatory variables with unconditional associations at the $P < 0.2$ level (2-sided) were selected for multivariable modelling.

196

197 A fixed-effects logistic regression model was developed where the probability of a bird being
 198 AIV positivity was parameterized as a function of the m explanatory variables with unconditional
 199 associations significant at $P < 0.2$, as described above. Given $p_i = P(Y_i = 1)$ and assuming that Y_i are
 200 mutually independent, this model takes the form:

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

201 In Equation 1, β_0 represents the intercept term and $\beta_1, \dots, \beta_m$ represent the regression coefficients for
 202 each of the m explanatory variables included in the model. Explanatory variables that were not
 203 statistically significant were removed from the model one at a time, beginning with the least
 204 significant, until the estimated regression coefficients for all explanatory variables retained were
 205 significant at an alpha level of less than 0.05. Explanatory variables that were excluded at the initial
 206 screening stage were tested for inclusion in the final model and were retained in the model if they
 207 changed any of the estimated regression coefficients by more than 20%. Biologically plausible two-
 208 way interactions were tested and none were significant at an alpha level of 0.05.

209

210 To account for lack of independence arising from the hierarchical structure of the data, that is,
 211 individual birds clustered within seller and sellers clustered within sampling rounds and markets we
 212 extended the model shown in Equation 1 to a mixed-effects model:

$$\log\left(\frac{p_{ijk}}{1 - p_{ijk}}\right) = \beta_0 + \beta_1 x_{1ijk} + \dots + \beta_m x_{mijk} + M_k + S_{jk} + \epsilon_{ijk} \quad \text{Equation 2}$$

213 In Equation 2 p_{ijk} represents the probability of being influenza A virus isolation positive for the
 214 i th bird from the j th seller in the k th market. Parameter M_k is a zero mean random effect term with

215 variance σ_M^2 representing the influence of the k th market on the probability of being AIV positive.
216 Similarly, parameter S_{jk} is a zero mean random effect term with variance σ_S^2 representing the
217 influence of the j th seller in the k th market on the probability of being AIV positive. Our reason for
218 including S_{jk} and M_k in the model was to account for unexplained extra-binomial variation operating
219 at the seller- and market-level on AIV risk.

220

221 Frequency histograms of the residuals from the multilevel model and plots of the residuals versus
222 predicted values were constructed to check that the assumptions of normality and homogeneity of
223 variance had been met. In the multilevel model, the level 1 (individual bird) variance was constrained
224 to 1 (that is, no extra-binomial variation was permitted). Because this variance was expressed on the
225 binomial rather than the logit scale, the estimates of the proportion of variation of each level of the
226 hierarchy (market, seller, and bird) were computed assuming the level 1 variance on the logit scale
227 was $\pi^2/3$, where $\pi = 3.1416$. This calculation is based on interpreting the presence or absence of
228 virus isolation as the result of an underlying latent process with a continuous, logistic distribution
229 (Snijders and Bosker et al., 1999).

230

231 Descriptive analyses, the unconditional measures of association and the fixed-effects logistic
232 regression models were carried out using R version 3.2.3 (R Development Core Team 2016). The
233 mixed-effects model was developed with MLwiN (Rasbash et al., 2012) using the R2MLwiN package
234 (Zhang et al., 2016) in R.

235

236 **Results**

237 **Descriptive statistics and unconditional associations**

238 Table 1 describes the structure of the data. The final dataset comprised details from 1,629 birds
239 from 83 sellers in seven LBMs in seven communes in four districts of Thua Thien Hue province. The
240 average number of birds sampled per seller over the study period was 20 (minimum 2; maximum
241 142). The average number of sellers per market was 21 (minimum 6; maximum 32).

242

243 Table 2 presents the 16 questionnaire derived explanatory variables that were associated with
244 virus isolation positivity at $P < 0.2$. Most of the birds sampled were sold by women (1,558 of 1,629;
245 96%) and the odds of birds sold by women sellers being AIV positive was 0.57 (95% CI 0.27 to 1.22)
246 times that of birds sold by male sellers. A relatively small proportion of birds were sold by sellers
247 with high school education (71 of 1,629) and the odds of birds sold by those with high school
248 education being AIV positive was 2.68 (95% CI 1.17 to 5.90) times that of birds sold by those with
249 no formal education. Most of the birds submitted for sale were sourced from the same commune as
250 the commune in which the market was located (1,128 of 1,629; 69%) and the odds of birds sourced
251 from the same commune being AIV positive was 0.36 (95% CI 0.25 to 0.53) times that of birds
252 sourced from different communes. Most (1,050 of 1,629; 64%) birds were handled by their sellers
253 without gloves and a similar proportion (1,037 of 1,629; 64%) were handled without the seller
254 washing their hands afterwards. While the number of birds sold by sellers who had attended an AI

255 training course was relatively high (968 of 1,629; 60%) only 11% (180 of 1,629) were sold by sellers
256 who were confident of the clinical signs of AI and a high proportion (1,331 of 1,629; 82%) of birds
257 were sold by those who believed that control of AI would have a positive effect on their business. A
258 total of 1,144 of the 1,629 birds (70%) were sold at the three intervention markets which had a higher
259 volume of sale than the non-intervention markets.

260

261 Figure 2 demonstrates the variation of AIV isolation positivity prevalence amongst intervention
262 and non-intervention LBMs. After the 2nd round of sampling, there was no reduction of AIV
263 prevalence, in either the intervention or non-intervention LBMs.

264

265 **Multivariable logistic regression analyses**

266 Estimated regression coefficients for the effect of the district in which the market was located
267 and estimates of the variability of the market- and seller-level random effect terms from the mixed
268 effects model are provided in Table 3. In the mixed-effects model, district was retained as an
269 explanatory variable because *a priori* it was considered to comprise part of the hierarchical structure
270 of the data. None of the explanatory variables that were associated with the risk of being AIV positive
271 at the $P < 0.2$ level were statistically significant in the final mixed-effects model.

272

273 After adjusting for the effect of the district in which a given market was located, the proportions
274 of variance at the individual market-, seller-, and individual bird-level were $(0.4041 \div (0.4041 +$

275 $3.3652 + \pi^2/3 = 0.06$, $(3.3652 \div (0.4041 + 3.3652 + \pi^2/3) = 0.48$ and $(\pi^2/3 \div$
276 $(0.4041 + 3.3652 + \pi^2/3) = 0.46$, respectively. There were relatively large numbers of sellers
277 and individual birds where AIV likelihood was positively associated with unmeasured seller-level as
278 well as bird-level effects. The identifiers of the 83 sellers were sorted in order of their estimated
279 random effect terms and an error bar plot produced showing the point estimate of the seller-level
280 random effect (and its 95% confidence interval) as a function of seller rank (Figure 3).

281

282 **Discussion**

283 This was a cross-sectional study to quantify the prevalence of AIV in poultry submitted for sale
284 at seven LBMs in Thua Thien Hue province, central Vietnam. Across the two sampling rounds, a
285 total of 113 out of the 1,629 sampled birds were positive for the AIV, with a prevalence of 6.9 (95%
286 CI 5.8 to 8.3) AIV positive birds per 100 birds submitted for sale. AIV positivity varied by market
287 and sampling round (Figure 2) with the intervention markets having a relatively low prevalence of
288 AIV in the first round and marked variation in positivity prevalence in the second. For the non-
289 intervention markets, the prevalence of AIV positivity was variable across both sampling rounds. Our
290 ability to draw definitive conclusions from these data is limited given the relatively small numbers of
291 markets in the intervention and non-intervention groups in each sampling round. At the very least, it
292 is evident that AIV positivity amongst poultry submitted for sale at LBMs varies over time and the
293 prevalence of AIV positivity in birds sampled from LBMs on one occasion will not necessarily be
294 similar to the prevalence of positivity on a second occasion. If sampling of live birds for AIV isolation

295 is to be carried out in future studies, and ignoring the effect of clustering of AIV positivity at the
296 seller-level we estimate that at least 580 birds need to be sampled and tested at the 95% level of
297 confidence under the estimation of 6.9% of the expected prevalence introduced from the present study
298 and desired absolute precision of 2.1% which is equal to 30% of the expected prevalence (Thrusfield,
299 2007).

300

301 While some questionnaire responses were significantly associated with AIV positivity at the
302 unconditional level, adjustment for confounding using the mixed-effects logistic regression model
303 rendered none of the questionnaire variables significantly associated with AIV positivity. There are
304 two explanations for these findings. Firstly, it is possible that a considerable amount of confounding
305 was present in the data which meant that after adjustment the association between each of the fixed-
306 effect explanatory variables and the study outcome was no longer statistically significant. A second
307 explanation is that the number of birds sampled in our study provided insufficient power to detect
308 associations between certain explanatory variables and the outcome at the alpha level of 0.05 (Altman
309 and Bland, 1995), as indicated, more birds were sold in intervention than non-intervention LBMs
310 (Table 2). Although this was more than likely to be the case for some explanatory variables where
311 the prevalence of exposure for AIV positive and negative birds was similar (e.g. gender, where the
312 proportion of AIV positive birds sold by females was 0.93 and the proportion of AIV negative birds
313 sold by females was 0.96) it was not so for others, for example whether or not sellers sourced their
314 birds from the same commune as the LBM (Table 2).

315

316 In the multivariable model the inclusion of market-, seller- and individual bird random effect
317 terms was useful in terms of providing an indication of the proportions of variance in AIV positivity
318 that was explained by unmeasured effects operating at each of the three levels. This extension to the
319 model was informative because it provided the opportunity to distinguish the influence of the
320 individual bird, the seller and the market in which birds were sold on the risk of being AIV positive.
321 Our mixed-effects logistic regression model shows that only 6% of the variation in AIV positivity
322 risk was at the market level whereas 48% and 46% of the variation in AIV positivity risk was at the
323 seller and individual bird level, respectively (Table 3). These findings indicate that characteristics of
324 the seller (apart from those measured in the questionnaire) and the birds themselves should be much
325 more likely to contribute the AIV positivity prevalence. Furthermore, of the 45 interviewed sellers
326 selling their birds in intervention LBMs in which the odds of the sellers in the intervention group
327 being AIV positive bird was 3.59 (95% CI 1.39 to 9.96) times that of those in the non-intervention
328 group of 38 sellers. Our inference from these findings is that the emphasis of AI control efforts needs
329 to be at the individual seller-level rather than the market-level. Furthermore, to be effective,
330 interventions need to recognize that sellers at LBMs are a diverse group demographically (Table 3)
331 and, ideally, intervention measures should target specific demographic groupings. Encouragingly, at
332 the bivariate level (at least), those sellers that attended a training course had a reduced risk of having
333 AIV positive birds.

334

335 If it is assumed that AIV enter a market via poultry submitted for sale by individual sellers, it is
336 perhaps not surprising that only 6% of the variation in AIV positivity risk was due to factors operating
337 at the market-level. This finding is biologically plausible, since birds enter a market on a given sale
338 day from a number of geographic locations and it is reasonable to expect that the risk of virus entry
339 into a market depends largely on the location from which birds are sourced. LBMs are licensed or
340 registered under local law to operate from a fixed address and must have a certificate for tracking the
341 source of birds introduced into the market on a given day. Because LBMs are the congregation point
342 for relatively large numbers of (presumably) AI naïve birds they represent ideal surveillance points
343 for estimation of AI prevalence (Trock et al., 2008). Poultry remains in the LBMs environment for a
344 relatively short period of time (typically one to two days) so the risk of within-market spread of AIV
345 is likely to be small. The length of time birds is kept in an LBM, the effectiveness of disinfection and
346 biosecurity procedures may therefore contribute to the prevalence of AIV positivity, although based
347 on our findings the contribution of market effects on AIV positivity prevalence was relatively small.
348 We expected that within market transmission of AIV to be less in the intervention LBMs. However,
349 field observations showed that there were periodic lapses in cleaning procedures including incomplete
350 coverage of disinfectant and use of disinfectants diluted at incorrect concentrations.

351

352 A limitation of this study is that our observations were based on a cross-sectional survey in which
353 LBMs were sampled on only two occasions and the interval between the two sampling rounds was
354 relatively short (approximately 3 months). Reports from market managers and sellers about their

355 biosecurity practices in the LBMs were not verified. Although around 60% of birds were handled by
356 sellers who did not use gloves it is likely that this proportion has been underestimated because of
357 obsequiousness on behalf of questionnaire respondents (that is, sellers altering their responses to a
358 given question to conform with the perceived expectations of the person administering the
359 questionnaire).

360

361 **Conclusion**

362 The prevalence of AIV positivity in poultry submitted for sale at the LBMs included in this study
363 was 6.9 (95% CI 5.8 to 8.3) AIV positive birds per 100 birds submitted for sale. After adjusting for
364 clustering at the market- and individual seller-level none of the explanatory variables solicited in the
365 questionnaire were significantly associated with AIV positivity. A relatively small component of the
366 variation in AIV positivity risk was at the individual market-level. We conclude that the emphasis of
367 AI control efforts should be at the seller-level rather than market-level.

368

369 **Conflict of interest**

370 None.

371

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382

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480

481 **Fig. 3.** Caterpillar plot showing the point estimate of the individual seller-level random effect terms
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Table 1

Structure of the data from 1,629 individual bird samples from 83 sellers in seven live bird markets.

Level	Number	Number per unit at next-higher level	
		Mean	Range
District ^a (highest level)	4	—	—
Live bird market	7	2	1 – 3
Seller	83	21	6 – 32
Birds sampled	1,629	20	2 – 142

^a Each district had mean number, 2 (range 1–3) live bird markets.

Table 2

Unconditional associations between the outcome variable (virus isolation positive) and the sixteen explanatory variables.

Variable	VI positive	Birds	OR (95%CI)	P-value
Gender:				
Female	105	1,558	1.00	Reference
Male	8	71	1.76 (0.76 – 3.56)	0.15
Education:				
None	18	281	1.00	Reference
Elementary	35	497	1.11 (0.62 – 2.03)	0.73
Middle school	49	780	0.98 (0.57 – 1.75)	0.94
High school	11	71	2.68 (1.17 – 5.90)	0.01
Number of years trading:				
1–5 years	7	294	1.00	Reference
6–10 years	79	922	3.84 (1.88 – 9.25)	< 0.01
Over 10 years	27	413	2.87 (1.30 – 7.23)	0.01
Do you source birds from the same commune as the market?				
No	60	501	1.00	Reference
Yes	53	1,128	0.36 (0.25 – 0.53)	< 0.01
What is the cause of avian influenza (AI)?				
Unknown	34	627	1.00	Reference
Bacteria	1	10	1.94 (0.10 – 10.8)	0.53
Virus	78	992	1.49 (0.99 – 2.28)	0.06
Do you separate ducks and chickens at the market?				
No	99	1,490	1.00	Reference
Yes	14	139	1.57 (0.84 – 2.75)	0.13
Do you wash your hands with soap after handling poultry?				
No	58	1,037	1.00	Reference
Yes	55	592	1.73 (1.18 – 2.54)	< 0.01
Do you wear gloves when handling poultry?				
No	81	1,050	1.00	Reference
Yes	32	579	0.70 (0.45 – 1.06)	0.10
Are you confident of the clinical signs of AI?				
No	49	828	1.00	Reference
Not sure	58	621	1.64 (1.10 – 2.44)	0.01
Yes	6	180	0.55 (0.21 – 1.20)	0.17
Do you believe personal protective equipment will protect you from AI?				
No	28	599	1.00	Reference
Not sure	56	695	1.79 (1.13 – 2.89)	0.01
Yes	29	335	1.93 (1.13 – 3.32)	0.02
What benefit will AI control have for your business?				
Very little	9	166	1.00	Reference
Not sure	1	132	0.13 (0.01 – 0.72)	0.06
A lot	103	1,331	1.46 (0.77 – 3.16)	0.29
Why do you not use PPE?				
No answer	57	818	1.00	Reference
Cost money	5	143	0.48 (0.17 – 1.12)	0.13
Inconvenience	51	668	1.10 (0.74 – 1.63)	0.62
Are your poultry kept at the market overnight?				
No	45	769	1.00	Reference
Yes	68	860	1.38 (0.94 – 2.05)	0.10
Have you attended a course on AI?				
No	57	661	1.00	Reference
Yes	56	968	0.65 (0.44 – 0.95)	0.03
Market type:				
Non-intervention	40	485	1.00	Reference
Intervention	73	1,144	0.76 (0.51 – 1.14)	0.18
Sampling round:				
The 1 st (August 2014)	64	1,078	1.00	Reference
The 2 nd (December 2014)	49	551	1.55 (1.05 – 2.27)	0.03

Table 3

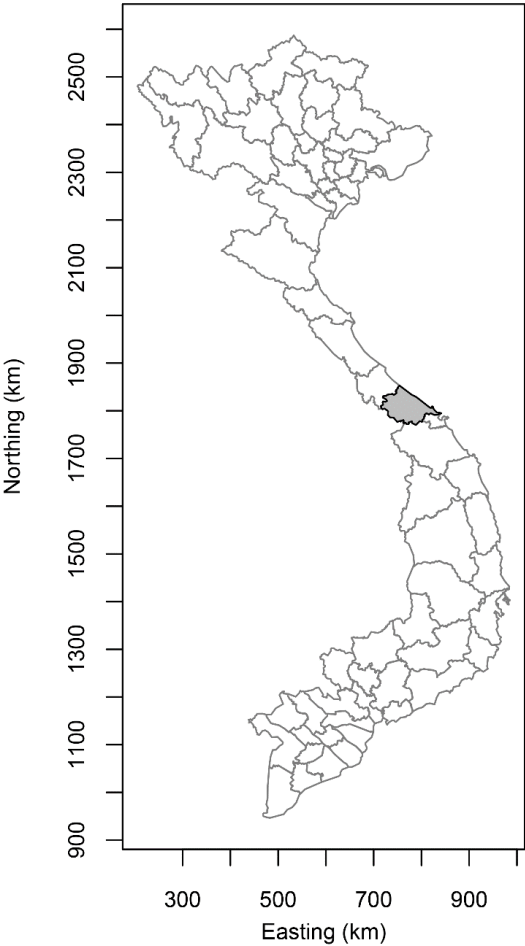
Estimated regression coefficients from a mixed-effects logistic regression model.

Explanatory variable	VI positive ^a	Total ^b	Coefficient (SE)	P-value	OR (95% CI)
<i>Fixed effects</i>					
Intercept	113	1,629	-2.5280 (0.4291)	< 0.01	—
District:					
Huong Thuy	48	606	Reference		1.00
Phong Dien	17	436	-0.6857 (0.7063)	0.33	0.50 (0.13 – 2.01) ^c
Phu Vang	48	587	0.0193 (0.6470)	0.97	1.02 (0.29 – 3.62)
<i>Random effects</i> ^d					
			Variance	SE	
Market	113	1,629	0.4041	0.3812	
Seller	113	1,629	3.3652	0.6935	

^a Number of bird samples were positive with avian influenza virus isolation.^b Total of bird samples.^c Interpretation: The proportion of AI virus isolation positive poultry from sellers from Phong Dien was 0.50 (95% CI 0.13 – 2.10) times that of poultry whose sellers were from Huong Thuy.^d Variance and standard error of the variance of the random effect terms.

Fig. 1. Chu *et al.*

a)



b)

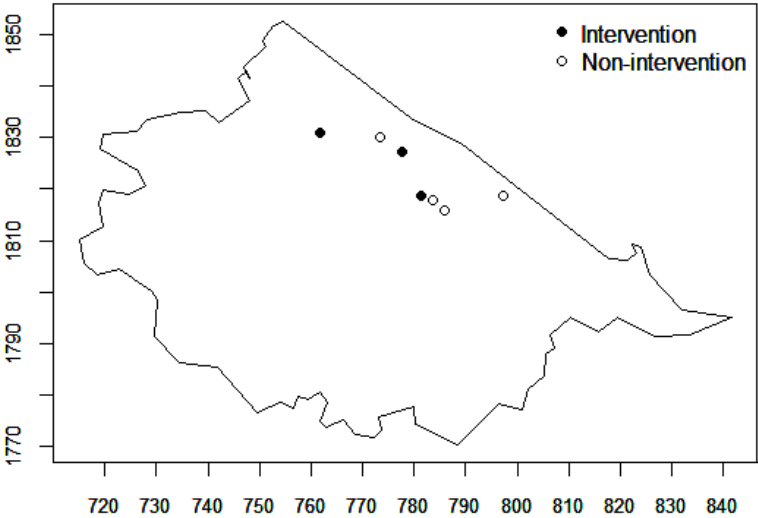


Fig. 2. Chu *et al.*

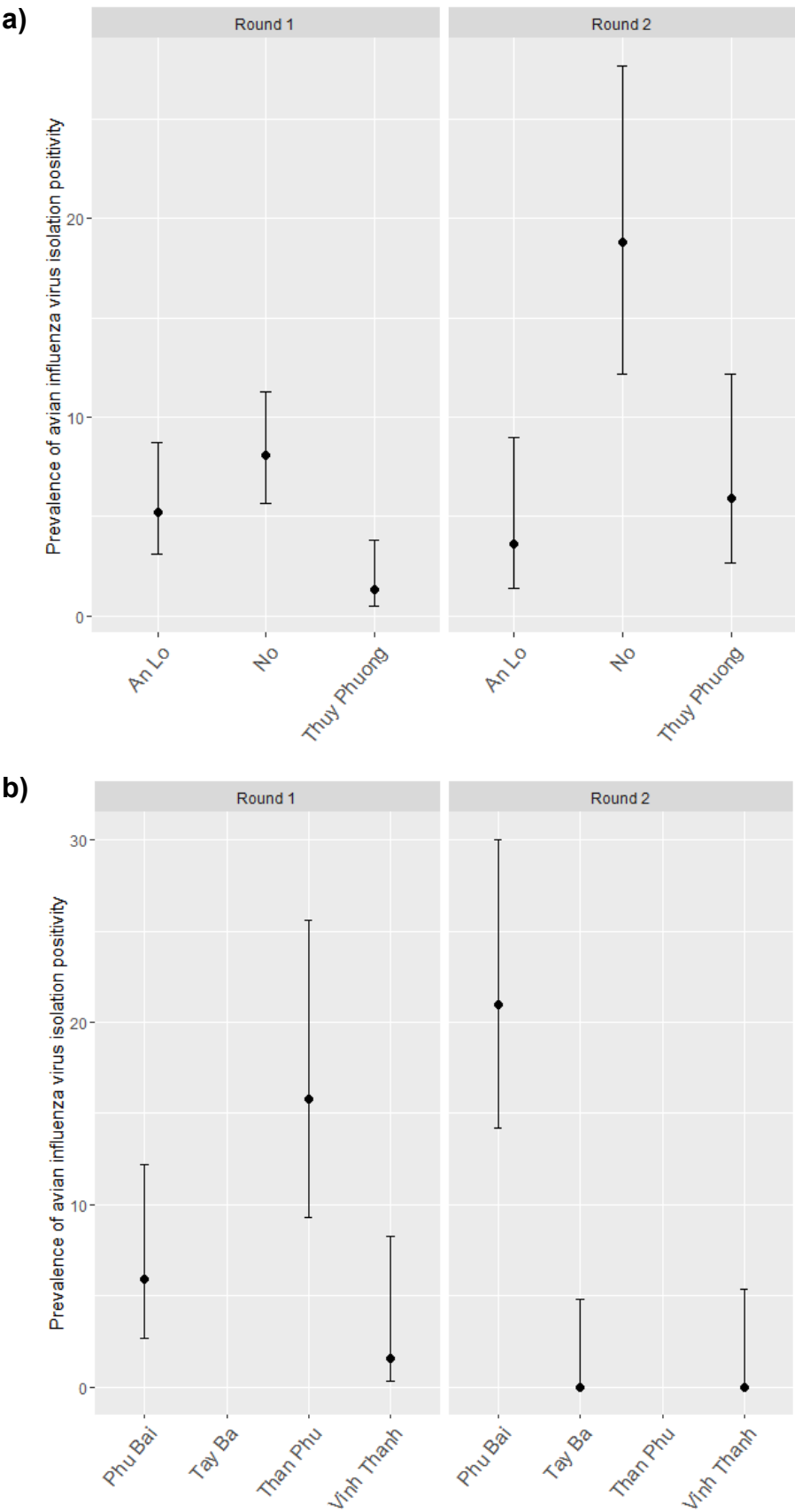


Fig. 3. Chu *et al.*

