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

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48 **Summary**

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

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

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Introduction

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

103 that, in China, human infections with AIV, in particular, of the subtypes H5N1 and H7N9 are
104 associated with recent exposure to poultry in LBMs (Li et al., 2015; Wan et al., 2011).

105

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

125

126 In this study, the data of Chu et al. (2016) were used to identify characteristics associated with
127 the presence of AIV in poultry submitted for sale at seven LBMs in Thua Thien Hue province,
128 Vietnam. Our specific aims were to: (1) document the prevalence of AIV in seven LBMs that took
129 part in this study and compare the prevalence of AIV positive samples amongst intervention and

130 non-intervention markets; and (2) quantify bird-, poultry seller- and market-level characteristics that
131 rendered individual birds more likely to be AIV isolation positive at the time of sale. Identifying the
132 relative importance of factors influencing the poultry submitted for sale at LBMs is a critical first
133 step towards the design of evidence-based better measures to reduce the number of AIV positive
134 birds (and therefore the risk of virus infection) within LBMs in Vietnam.

135

136 **Materials and methods**

137 **Study design and study area**

138 A cross-sectional study was carried out in seven LBMs in four districts of Thua Thien Hue
139 province (Figure 1 and Table 1), Vietnam in August and December, 2014. At three of the seven
140 LBMs, biosecurity had been improved as part of the VAHIP program in Thua Thien Hue province.
141 In intervention LBMs, there was a good standard of infrastructure with poultry from different
142 sources physically separated by the seller; besides, disinfection procedures were performed twice, in
143 the morning and evening, on a given sale day. The other four (non-intervention) LBMs were wet
144 markets at which no particular intensive biosecurity interventions were carried out and at which
145 poultry and other animals were mixed together under relatively low biosecurity conditions; details
146 are described in Chu et al. (2016).

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

153

154 **Laboratory procedures**

155 Oropharyngeal, cloacal swabs and fecal samples were collected from chickens, ducks,
156 Muscovy ducks for each poultry seller on each of the two sampling rounds. For each bird, the

157 oropharyngeal and cloacal swabs were collected in a sterile tube with transport medium, as
158 described by Chu et al. (2016). Samples were then transported to the National Center for Veterinary
159 Diagnostics (NCVD), Hanoi, Vietnam. At the NCVD, the samples were tested for the presence of
160 influenza type A viruses (M gene detection) using real time RT-PCR. All samples were then
161 prepared for transfer to the Laboratory of Microbiology, Department of Disease Control, Graduate
162 School of Veterinary Medicine, Hokkaido University, Sapporo, Japan. The shipment of samples
163 containing AIV was classified into Biological Substance, Category B, following the instructions of
164 the International Air Transport Association (IATA) in Dangerous Goods Regulation Manual
165 (Pearson, 2007). At the Laboratory of Microbiology, all samples were submitted for virus isolation.
166 After which, representative isolates, such as H5, H6, and H9 AIV, were phylogenetically and
167 antigenically analyzed to characterize the genetic and antigenic variation of AIVs currently
168 circulating in LBMs in Vietnam, further detail is provided by Chu et al. (2016).

169

170 Questionnaire and interview

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

178

179 A total of 83 face-to-face interviews with poultry sellers were carried out over the two
180 sampling rounds in the seven markets (45 in intervention and 38 in non-intervention LBMs).
181 Questionnaire survey were administered by trained interviewers from the SDAH of Thua Thien Hue
182 and the District Veterinary Stations of each of the districts in which the study markets were located.
183 Interviews were carried out with assistance from SDAH veterinarians located in communes adjacent

184 to each market. DAH staff provided technical supervision and assistance during each market visit.
185 One average, the length of time taken to visit all of the sellers within a market and to complete
186 sampling and administration of the questionnaires was two days (minimum 1 day; maximum 7
187 days).

189 **Data management**

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

195 **Statistical analyses**

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

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

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

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

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

217

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

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

221 In Equation 2 p_{ijk} represents the probability of being influenza A virus isolation positive for
 222 the i th bird from the j th seller in the k th market. Parameter M_k is a zero mean random effect term
 223 with variance σ_M^2 representing the influence of the k th market on the probability of being AIV
 224 positive. Similarly, parameter S_{jk} is a zero mean random effect term with variance σ_S^2 representing
 225 the influence of the j th seller in the k th market on the probability of being AIV positive. Our reason
 226 for including S_{jk} and M_k in the model was to account for unexplained extra-binomial variation
 227 operating at the seller- and market-level on AIV risk.

228

229 Frequency histograms of the residuals from the multilevel model and plots of the residuals
 230 versus predicted values were constructed to check that the assumptions of normality and
 231 homogeneity of variance had been met. In the multilevel model, the level 1 (individual bird)
 232 variance was constrained to 1 (that is, no extra-binomial variation was permitted). Because this
 233 variance was expressed on the binomial rather than the logit scale, the estimates of the proportion of

variation of each level of the hierarchy (market, seller, and bird) were computed assuming the level 1 variance on the logit scale was $\pi^2/3$, where $\pi = 3.1416$. This calculation is based on interpreting the presence or absence of virus isolation as the result of an underlying latent process with a continuous, logistic distribution (Snijders and Bosker et al., 1999).

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

Results

Descriptive statistics and unconditional associations

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

Table 2 presents the 16 questionnaire derived explanatory variables that were associated with virus isolation positivity at $P < 0.2$. Most of the birds sampled were sold by women (1,558 of 1,629; 96%) and the odds of birds sold by women sellers being AIV positive was 0.57 (95% CI 0.27 to 1.22) times that of birds sold by male sellers. A relatively small proportion of birds were sold by sellers with high school education (71 of 1,629) and the odds of birds sold by those with high school education being AIV positive was 2.68 (95% CI 1.17 to 5.90) times that of birds sold by those with no formal education. Most of the birds submitted for sale were sourced from the same commune as the commune in which the market was located (1,128 of 1,629; 69%) and the odds of birds sourced from the same commune being AIV positive was 0.36 (95% CI 0.25 to 0.53) times that of birds sourced from different communes. Most (1,050 of 1,629; 64%) birds were handled by

261 their sellers without gloves and a similar proportion (1,037 of 1,629; 64%) were handled without
262 the seller washing their hands afterwards. While the number of birds sold by sellers who had
263 attended an AI training course was relatively high (968 of 1,629; 60%) only 11% (180 of 1,629)
264 were sold by sellers who were confident of the clinical signs of AI and a high proportion (1,331 of
265 1,629; 82%) of birds were sold by those who believed that control of AI would have a positive
266 effect on their business. A total of 1,144 of the 1,629 birds (70%) were sold at the three intervention
267 markets which had a higher volume of sale than the non-intervention markets.

268
269 Figure 2 demonstrates the variation of AIV isolation positivity prevalence amongst
270 intervention and non-intervention LBMs. After the 2nd round of sampling, there was no reduction of
271 AIV prevalence, in either the intervention or non-intervention LBMs.

272 273 **Multivariable logistic regression analyses**

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

280
281 After adjusting for the effect of the district in which a given market was located, the
282 proportions of variance at the individual market-, seller-, and individual bird-level were $(0.4041 \div$
283 $(0.4041 + 3.3652 + \pi^2/3) = 0.06$, $(3.3652 \div (0.4041 + 3.3652 + \pi^2/3) = 0.48$ and $(\pi^2/$
284 $3 \div (0.4041 + 3.3652 + \pi^2/3) = 0.46$, respectively. There were relatively large numbers of
285 sellers and individual birds where AIV likelihood was positively associated with unmeasured seller-
286 level as well as bird-level effects. The identifiers of the 83 sellers were sorted in order of their

287 estimated random effect terms and an error bar plot produced showing the point estimate of the
288 seller-level random effect (and its 95% confidence interval) as a function of seller rank (Figure 3).

289

290 Discussion

291 This was a cross-sectional study to quantify the prevalence of AIV in poultry submitted for sale
292 at seven LBMs in Thua Thien Hue province, central Vietnam. Across the two sampling rounds, a
293 total of 113 out of the 1,629 sampled birds were positive for the AIV, with a prevalence of 6.9 (95%
294 CI 5.8 to 8.3) AIV positive birds per 100 birds submitted for sale. AIV positivity varied by market
295 and sampling round (Figure 2) with the intervention markets having a relatively low prevalence of
296 AIV in the first round and marked variation in positivity prevalence in the second. For the non-
297 intervention markets, the prevalence of AIV positivity was variable across both sampling rounds.
298 Our ability to draw definitive conclusions from these data is limited given the relatively small
299 numbers of markets in the intervention and non-intervention groups in each sampling round. At the
300 very least, it is evident that AIV positivity amongst poultry submitted for sale at LBMs varies over
301 time and the prevalence of AIV positivity in birds sampled from LBMs on one occasion will not
302 necessarily be similar to the prevalence of positivity on a second occasion. If sampling of live birds
303 for AIV isolation is to be carried out in future studies, and ignoring the effect of clustering of AIV
304 positivity at the seller-level we estimate that at least 580 birds need to be sampled and tested at the
305 95% level of confidence under the estimation of 6.9% of the expected prevalence introduced from
306 the present study and desired absolute precision of 2.1% which is equal to 30% of the expected
307 prevalence (Thrusfield, 2007).

308

309 While some questionnaire responses were significantly associated with AIV positivity at the
310 unconditional level, adjustment for confounding using the mixed-effects logistic regression model
311 rendered none of the questionnaire variables significantly associated with AIV positivity. There are
312 two explanations for these findings. Firstly, it is possible that a considerable amount of confounding
313 was present in the data which meant that after adjustment the association between each of the fixed-

314 effect explanatory variables and the study outcome was no longer statistically significant. A second
315 explanation is that the number of birds sampled in our study provided insufficient power to detect
316 associations between certain explanatory variables and the outcome at the alpha level of 0.05
317 (Altman and Bland, 1995), as indicated, more birds were sold in intervention than non-intervention
318 LBMs (Table 2). Although this was more than likely to be the case for some explanatory variables
319 where the prevalence of exposure for AIV positive and negative birds was similar (e.g. gender,
320 where the proportion of AIV positive birds sold by females was 0.93 and the proportion of AIV
321 negative birds sold by females was 0.96) it was not so for others, for example whether or not sellers
322 sourced their birds from the same commune as the LBM (Table 2).

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

340 the bivariate level (at least), those sellers that attended a training course had a reduced risk of
341 having AIV positive birds.

342

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

360

361 A limitation of this study is that our observations were based on a cross-sectional survey in
362 which LBMs were sampled on only two occasions and the interval between the two sampling
363 rounds was relatively short (approximately 3 months). Reports from market managers and sellers
364 about their biosecurity practices in the LBMs were not verified. Although around 60% of birds were
365 handled by sellers who did not use gloves it is likely that this proportion has been underestimated
366 because of obsequiousness on behalf of questionnaire respondents (that is, sellers altering their

367 responses to a given question to conform with the perceived expectations of the person
368 administering the questionnaire).

369

370 **Conclusion**

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

377

378 **Conflict of interest**

379 None.

380

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391

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492 (and their 95% confidence intervals) for the 83 sellers included in this study.

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

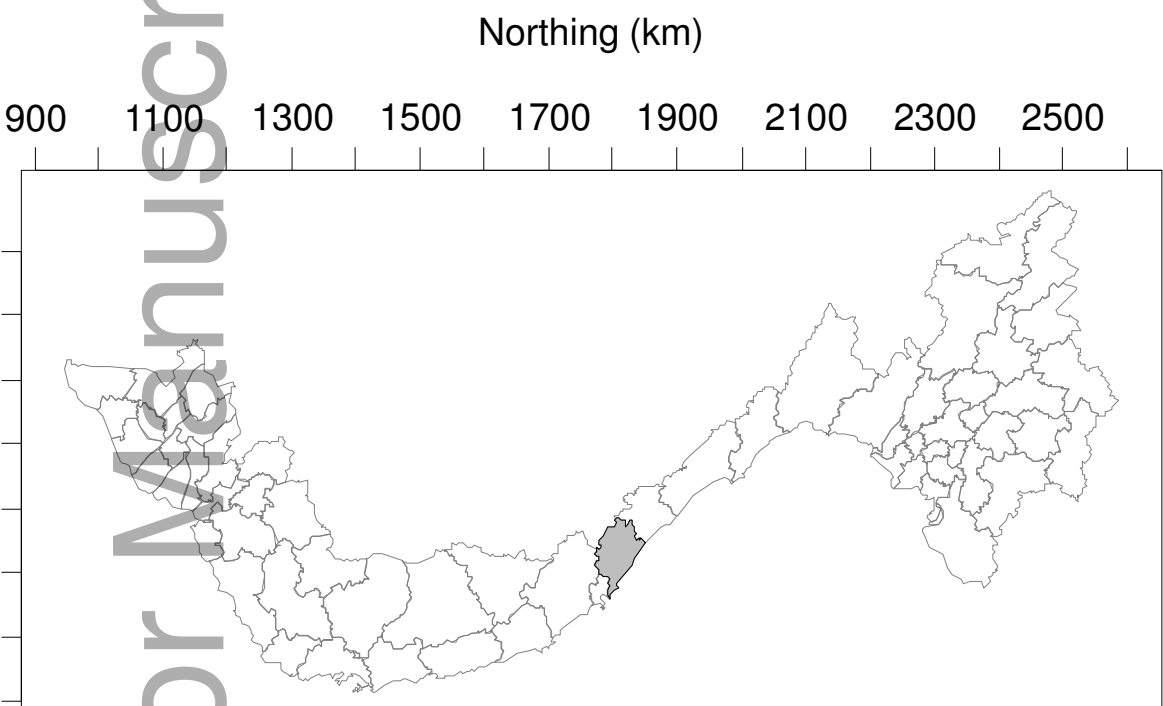
Explanatory variable	VI positive ^a	Total ^b	Coefficient (SE)	P-value	OR (95% CI)
Fixed effects					
Intercept	113	1,629	-2.5280 (0.4291)	< 0.01	—
District:					
Huong Thuy	48	606	Reference		1.00
Phong Dien	17	436	-0.6857 (0.7063)	0.33	0.50 (0.13 – 2.01) ^c
Phu Vang	48	587	0.0193 (0.6470)	0.97	1.02 (0.29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

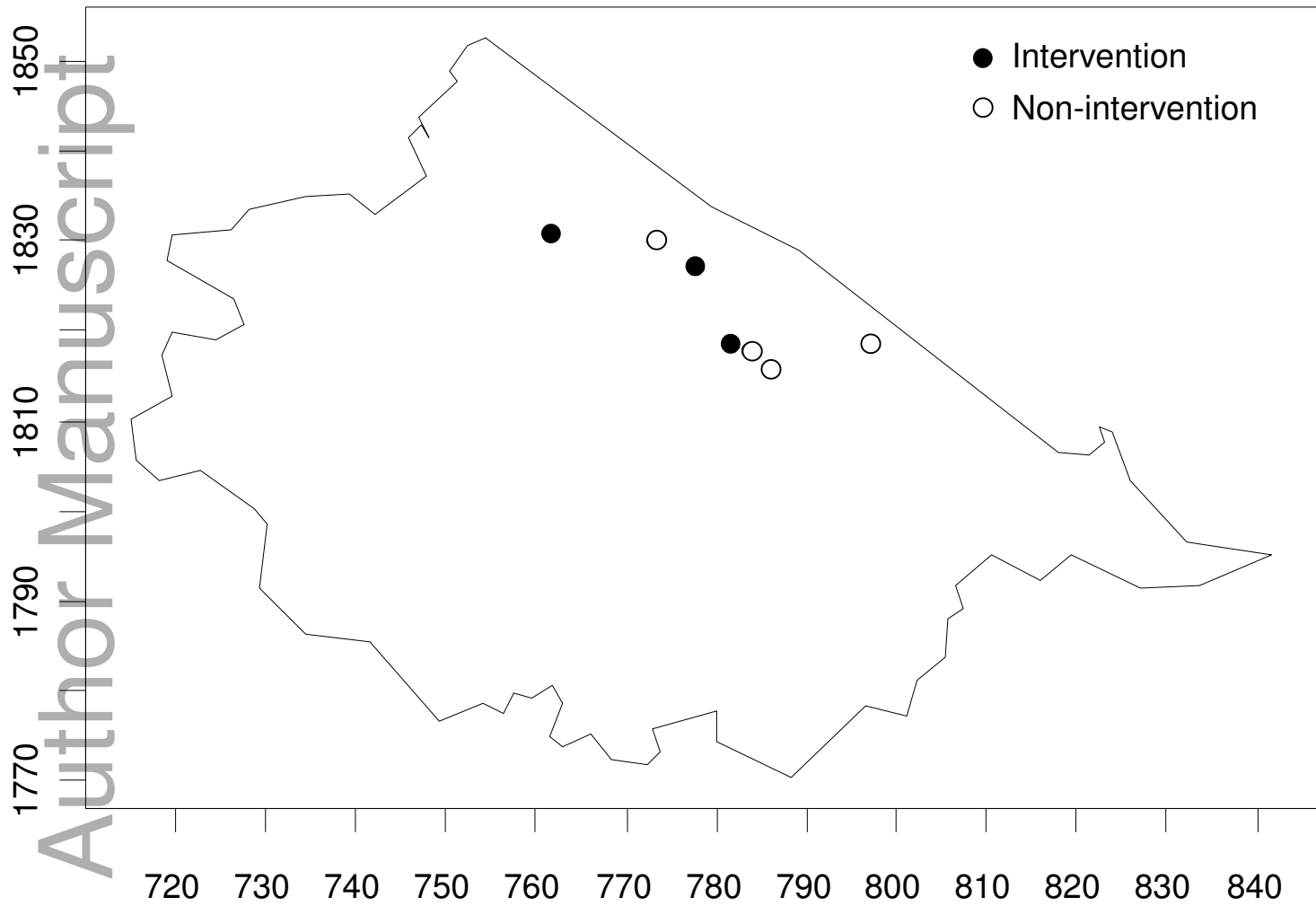
^a Number of bird samples were positive with avian influenza virus isolation.

^b Total of bird samples.

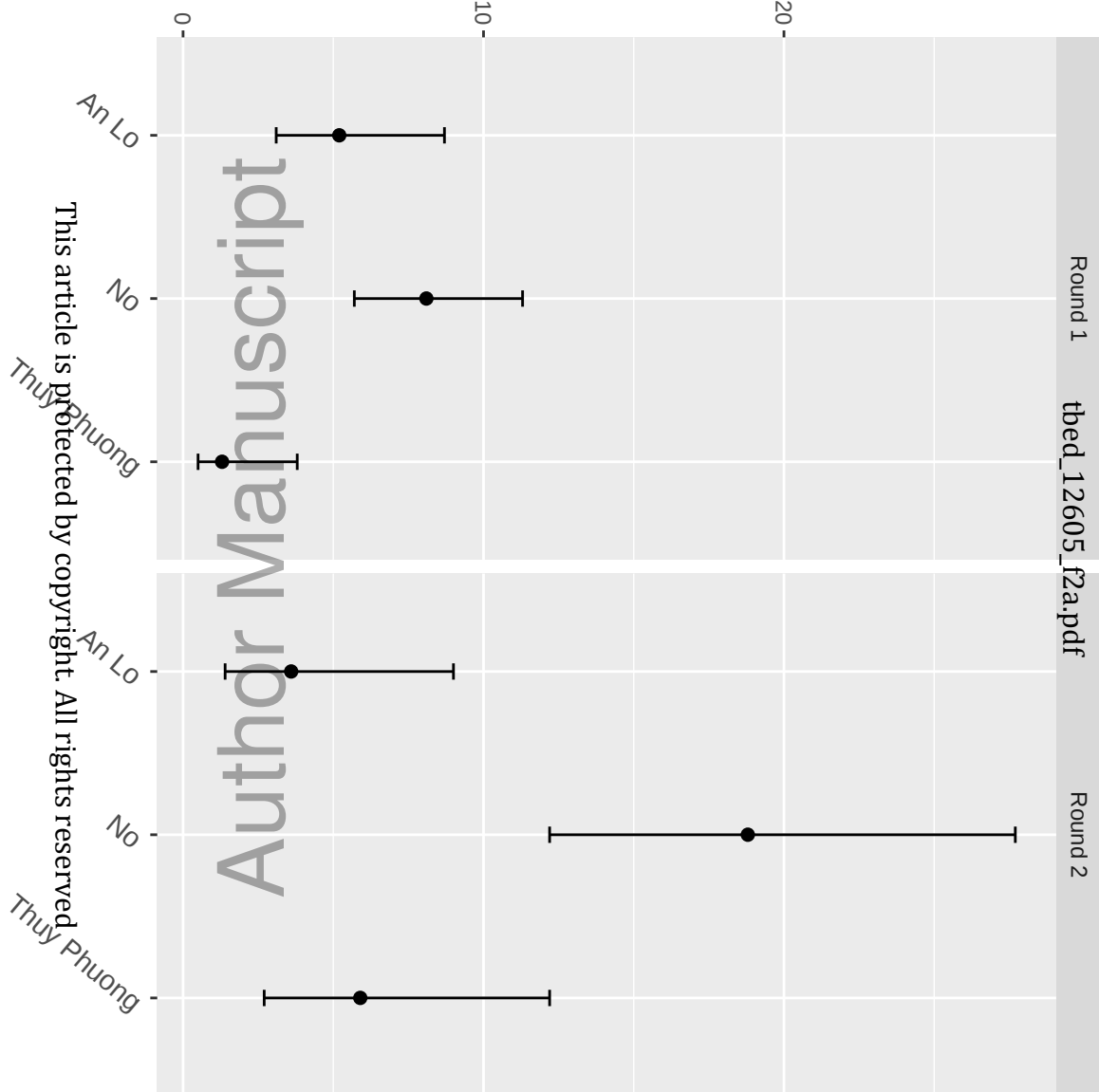
^c Interpretation: The proportion of AI virus isolation positive poultry from sellers from Phong Dien was 0.50 (95% CI 0.13 – 2.10) times that of poultry whose sellers were from Huong Thuy.

^d Variance and standard error of the variance of the random effect terms.





Prevalence of avian influenza virus isolation positivity



Prevalence of avian influenza virus isolation positivity

