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

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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

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

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

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

Materials and methods

Study design and study area

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

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

Laboratory procedures

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

Questionnaire and interview

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

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

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.

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.

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.

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

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

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

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

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

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

Frequency histograms of the residuals from the multilevel model and plots of the residuals versus predicted values were constructed to check that the assumptions of normality and homogeneity of variance had been met. In the multilevel model, the level 1 (individual bird) variance was constrained to 1 (that is, no extra-binomial variation was permitted). Because this 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 π = 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 their sellers without gloves and a similar proportion (1,037 of 1,629; 64%) were handled without the seller washing their hands afterwards. While the number of birds sold by sellers who had attended an AI training course was relatively high (968 of 1,629; 60%) only 11% (180 of 1,629) were sold by sellers who were confident of the clinical signs of AI and a high proportion (1,331 of 1,629; 82%) of birds were sold by those who believed that control of AI would have a positive effect on their business. A total of 1,144 of the 1,629 birds (70%) were sold at the three intervention markets which had a higher volume of sale than the non-intervention markets.

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

Multivariable logistic regression analyses

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

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

Discussion

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

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

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

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

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

Conclusion

The prevalence of AIV positivity in poultry submitted for sale at the LBMs included in this study was 6.9 (95% CI 5.8 to 8.3) AIV 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 AIV positivity. A relatively small component of the variation in AIV positivity risk was at the individual market-level. We conclude that the emphasis of AI control efforts should be at the seller-level rather than market-level.

Conflict of interest

None.

Acknowledgments

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References

Altman, D. G., and J. M. Bland, 1995: Absence of evidence is not evidence of absence. BMJ.

doi:10.1136/bmj.311.7003.485

Biswas, P. K., Giasuddin, M., Nath, B. K., Islam, M. Z., Debnath, N. C., and M. Yamage, 2015: Biosecurity and circulation of influenza A(H5N1) virus in live-bird markets in Bangladesh, 2012. Transbound. Emerg. Dis. 2012, 1–9. doi:10.1111/tbed.12454

Chan, P. K. S., 2002: Outbreak of avian influenza A(H5N1) virus infection in Hong Kong in 1997: Clin. Infect. Dis. 34, S58–S64. doi:10.1086/338820

Chu, D. -H., Okamatsu, M., Matsuno, K., Hiono, T., Ogasawara, K., Nguyen, L. T., Nguyen, L. Van, Nguyen, T. N., Nguyen, T. T., Pham, D. Van, Nguyen, H. H., Nguyen, T. D., To, T. L., Kida, H., and Y. Sakoda, 2016: Genetic and antigenic characterization of H5, H6 and H9 avian influenza viruses circulating in live bird markets with intervention in the center part of Vietnam. Vet. Microbiol. 192, 194-203. doi:10.1016/j.vetmic.2016.07.016

Fournié, G., Guitian, J., Desvaux, S., Mangtani, P., Ly, S., Cong, V. C., San, S., Dung, D. H., Holl, D., Pfeiffer, D. U., Vong, S., and A. C. Ghani, 2012: Identifying live bird markets with the potential to act as reservoirs of avian influenza A(H5N1) virus: A survey in Northern Viet Nam and Cambodia. PLoS One 7, 1–9. doi:10.1371/journal.pone.0037986

He, Y., Liu, P., Tang, S., Chen, Y., Pei, E., Zhao, B., Ren, H., Li, J., Zhu, Y., Zhao, H., Pan, Q., Gu, B., Yuan, Z., and F. Wu, 2014: Live poultry market closure and control of avian influenza A(H7N9), Shanghai, China. Emerg. Infect. Dis. 20, 1565–1566. doi:10.3201/eid2009.131243

Indriani, R., Samaan, G., Gultom, A., Loth, L., Indryani, S., Adjid, R., Dharmayanti, N. L. P. I., Weaver, J., Mumford, E., Lokuge, K., Kelly, and P. M. Darminto, 2010: Environmental sampling for avian influenza virus A(H5N1) in live-bird markets, Indonesia. Emerg. Infect. Dis. 16, 1889–1895. doi:10.3201/eid1612.100402

Li, J., Yu, X., Pu, X., Yang, X., Kou, Y., Zhou, Y., Qian, X., Xie, L., and J. Pan, 2015: The diversity of avian influenza virus subtypes in live poultry markets before and during the second wave of A(H7N9) infections in Hangzhou, China. Emerg. Microbes Infect. 4, e14. doi:10.1038/emi.2015.14

Nasreen, S., Khan, S. U., Luby, S. P., Gurley, E. S., Abedin, J., Zaman, R.U., Sohel, B. M., Rahman, M., Hancock, K., Levine, M. Z., Veguilla, V., Wang, D., Holiday, C., Gillis, E., Sturm-Ramirez, K., Bresee, J. S., Rahman, M., Uyeki, T.M., Katz, J. M., and E. Azziz-Baumgartner, 2015: Highly pathogenic avian influenza A(H5N1) virus infection among workers at live bird markets, Bangladesh, 2009-2010. Emerg. Infect. Dis. 21, 629–637. doi:10.3201/eid2104.141281

Nguyen, D. T., Bryant, J. E., Davis, C. T., Nguyen, L. V, Pham, L. T., Loth, L., Inui, K., Nguyen, T., Jang, Y., To, T. L., Nguyen, T. D., Hoang, D. T., Do, H. T., Nguyen, T. T., Newman, S., Siembieda, J., and D. V Pham, 2014: Prevalence and distribution of avian influenza A(H5N1) virus clade variants in live bird markets of Vietnam, 2011 – 2013. Avian Dis. 58, 599–608. doi:10.1637/10814-030814-Reg

Phan, M. Q., Henry, W., Bui, C. B., Do, D. H., Hoang, N. V., Thu, N. T., Nguyen, T. T., Le, T. D., Diep, T. Q., Inui, K., Weaver, J., and J. Carrique-Mas, 2012: Detection of HPAI H5N1 viruses in ducks sampled from live bird markets in Vietnam. Epidemiol. Infect. 1–11. doi:10.1017/S0950268812001112

Pearson J.E., 2007: Regulatory constraints for the transport of samples and compliance with the World Organisation for Animal Health (OIE) standards for biosecurity and biocontainment. Dev. Biol. (Basel). 128, 59-68.

Rasbash, J., Steele, F., Browne, J, W., and H. Goldstein, 2012: A user's guide to MLwiN.

Scoones, I., 2010: The international response to avian influenza: science, policy and politics, in: Avian influenza: science, policy and politics (pathways to sustainability), pp. 1–28. Earthscan Publications Ltd., London, UK.

Snijders, T. A. B., and R.J. Bosker, 1999: Multilevel analysis: An introduction to basic and advanced multilevel modeling, 2nd edn. SAGE Publications Ltd, London.

Thrusfield, M., 2007: Surveys, in: Veterinary Epidemiology, 3rd edn. pp. 229–246. Blackwell Science Ltd, a Blackwell Publishing company, Oxford.

Trock, S. C., Gaeta, M., Gonzalez, A., Pederson, J. C., and D. A. Senne, 2008: Evaluation of routine depopulation, cleaning, and disinfection procedures in the live bird markets, New York.

Avian Dis. 52, 160–162. doi:10.1637/7980-040607-Reg
VAHIP-World Bank Group, 2015: Vietnam Avian and Human Influenza Control and Preparedness. Available at http://www.worldbank.org/en/results/2015/05/12/vietnam-avian-and-human-influenza-control-and-preparedness (accessed 24 June 2016).
Wan, X. -F., Dong, L., Lan, Y., Long, L. -P., Xu, C., Zou, S., Li, Z., Wen, L., Cai, Z., Wang, W., Li, X., Yuan, F., Sui, H., Zhang, Y., Dong, J., Sun, S., Gao, Y., Wang, M., Bai, T., Yang, L., Li, D., Yang, W., Yu, H., Wang, S., Feng, Z., Wang, Y., Guo, Y., Webby, R.J., and Y. Shu, 2011: Indications that live poultry markets are a major source of human H5N1 influenza virus infection in China. J. Virol. 85, 13432–8. doi:10.1128/JVI.05266-11
Wu, P., Jiang, H., Wu, J. T., Chen, E., He, J., Zhou, H., Wei, L., Yang, J., Yang, B., Qin, Y., Fang, V. J., Li, M., Tsang, T. K., Zheng, J., Lau, E. H. Y., Cao, Y., Chai, C., Zhong, H., Li, Z., Leung, G. M., Feng, L., Gao, G.F., Cowling, B. J., and H. Yu, 2014: Poultry Market Closures and Human Infection with Influenza A(H7N9) Virus, China, 2013–14. Emerg. Infect. Dis. 20, 1891–1894. doi:10.3201/eid2011.140556
Yu, H., Wu, J. T., Cowling, B. J., Liao, Q., Fang, V. J., Zhou, S., Wu, P., Zhou, H., Lau, E. H. Y., Guo, D., and M. Y. Ni, 2015: Impact of live poultry market closure in reducing bird-to-human transmission of avian influenza A(H7N9) virus: an ecological study. Lancet 383, 541–548. doi:10.1016/S0140-6736(13)61904-2
Zhang, Z., Parker, R. M. A., Charlton, C. M. J., Leckie, G., and W. J. Browne, 2016: R2MLwiN: A package to run MLwiN from within R. J. Stat. Softw. VV.
Zhou, X., Li, Y., Wang, Y., Edwards, J., Guo, F., Clements, A. C.A., Huang, B., and R. J. Soares Magalhaes, 2015: The role of live poultry movement and live bird market biosecurity in the epidemiology of influenza A(H7N9): A cross-sectional observational study in four eastern China provinces. J. Infect. 71, 470–479. doi:10.1016/j.jinf.2015.06.012

List of tables and figures

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

Table 2. Unconditional associations between the outcome variable (virus isolation positive) and the sixteen explanatory variables

Table 3. Estimated regression coefficients from a mixed-effects logistic regression model

Fig. 1. Location of the seven intervention- and non-intervention live bird markets described in this study: **a)** Research location – Thua Thien Hue province in center of Vietnam; **b)** Locations of the intervention- and non-intervention live bird markets in Thua Thien Hue province.

Fig. 2. Error bar plots showing avian influenza virus prevalence (and its 95% confidence interval) in the intervention- and non-intervention live bird markets over the two rounds of sample collection: **a)** Prevalence of avian influenza viruses in the intervention live bird markets; **b)** Prevalence of avian influenza viruses in the non-intervention live bird markets.

Fig. 3. Caterpillar plot showing the point estimate of the individual seller-level random effect terms (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 1st (August 2014)	64	1,078	1.00	Reference
The 2nd (December 2014)	49	551	1.55 (1.05 – 2.27)	0.03

Table 3. Estimated regression coefficients from a mixed-effects logistic regression model

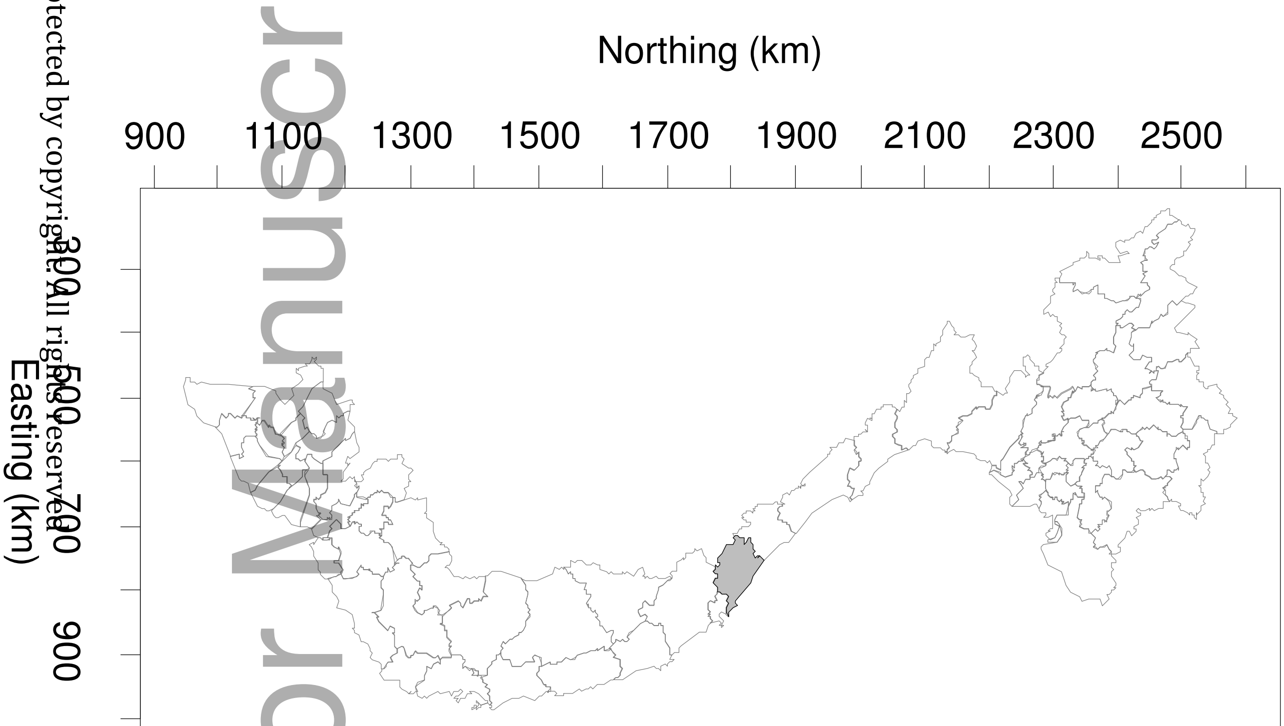
Explanatory variable	VI positive [a]	Total [b]	Coefficient (SE)	P-value	OR (95% CI)
Fixed effects					
Intercept	113	1,629	-2.5280 (0.4291)	< 0.01	—
District:					
Huong Thuy	48	606	Reference		1.00
Phong Dien	17	436	-0.6857 (0.7063)	0.33	0.50 (0.13 – 2.01) [c]
Phu Vang	48	587	0.0193 (0.6470)	0.97	1.02 (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.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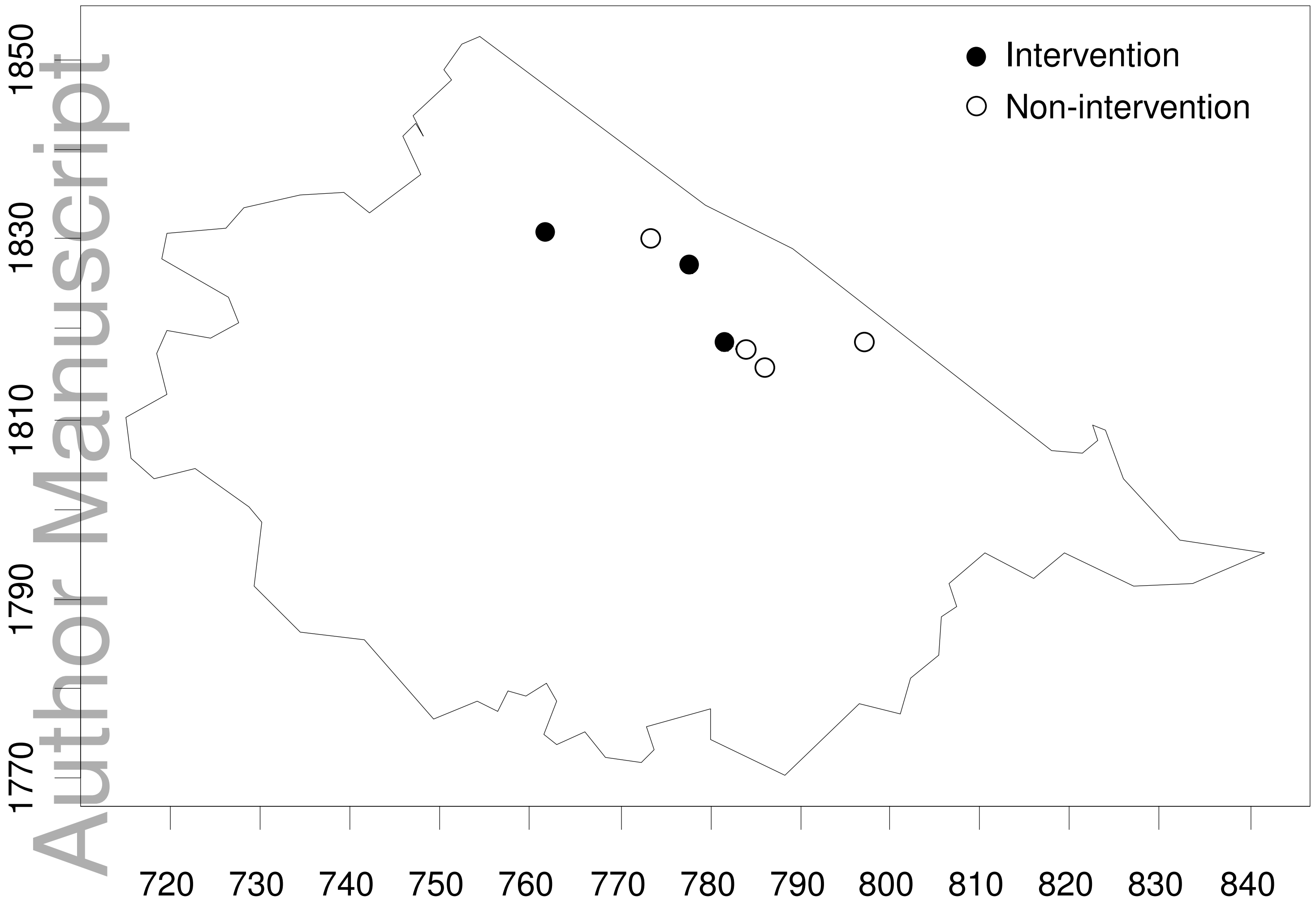

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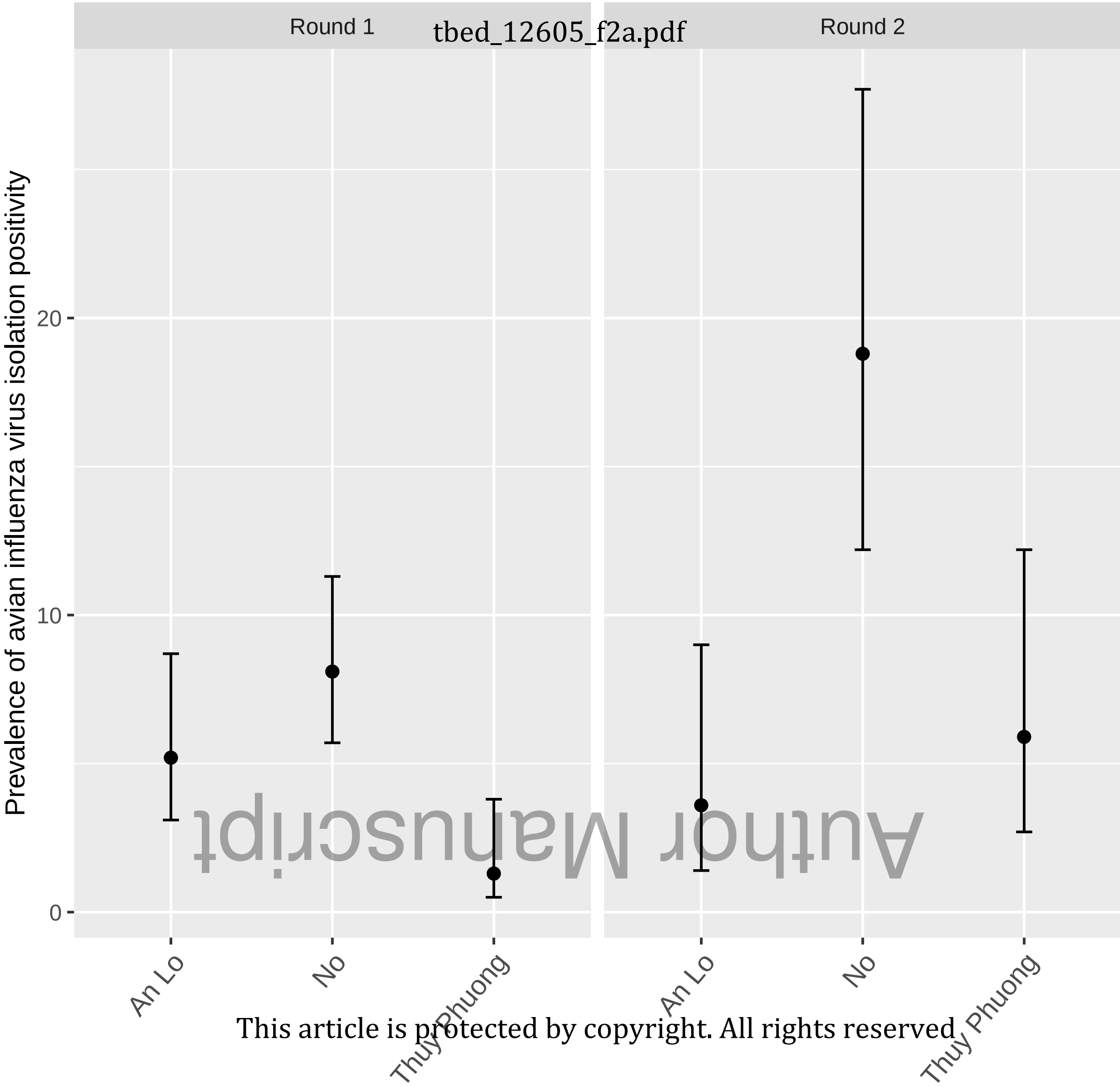

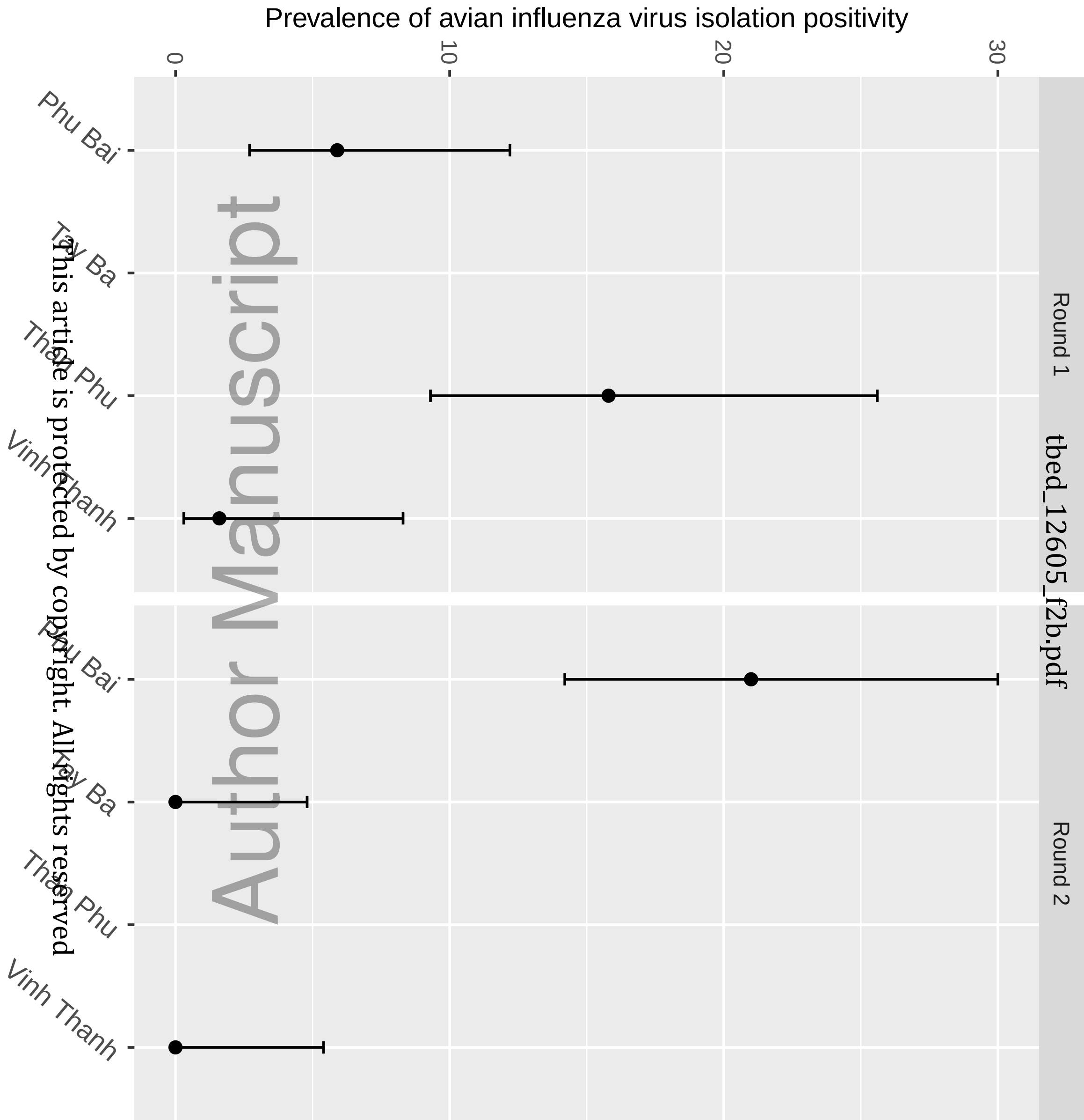

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