Chapter 5

Paper 2: Avian Influenza H5N1 Transmission in Households, Indonesia.
About this chapter

Building on the findings from Chapter 4, this chapter explored AI H5N1 transmission patterns and risk factors for infection. Using the same data and household-study design as that reported in Chapter 4, this study quantified zoonotic and human transmission as well as the extent to which the virus was transmissible. Other transmission patterns assessed include household attack rates, secondary attack rates and disease intervals between cases in outbreaks.

This study was the first globally to assess transmission patterns for a large number of outbreaks. It found that most H5N1-cases were a result of exposure to zoonotic sources of virus. Strong support for human transmission of the virus was only found when a single large cluster was included in the transmission model. The reproduction number was well below the threshold for sustained transmission.

My role in this study was to design the study research question, extract the relevant data from the MOH existing surveillance system and analyze the data. I worked with a mathematical modeller to develop the disease transmission models. I wrote the paper for publication and obtained feedback from all the co-authors. The study was submitted to PLOS One and was published in January 2012.
Avian Influenza H5N1 Transmission in Households, Indonesia

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Abstract

Background: Disease transmission patterns are needed to inform public health interventions, but remain largely unknown for avian influenza H5N1 virus infections. A recent study on the 139 outbreaks detected in Indonesia between 2005 and 2009 found that the type of exposure to sources of H5N1 virus for both the index case and their household members impacted the risk of additional cases in the household. This study describes the disease transmission patterns in those outbreak households.

Methodology/Principal Findings: We compared cases (n = 177) and contacts (n = 496) in the 113 sporadic and 26 cluster outbreaks detected between July 2005 and July 2009 to estimate attack rates and disease intervals. We used final size household models to fit transmission parameters to data on household size, cases and blood-related household contacts to assess the relative contribution of zoonotic and human-to-human transmission of the virus, as well as the reproduction number for human virus transmission. The overall household attack rate was 18.3% and secondary attack rate was 5.5%. Secondary attack rate remained stable as household size increased. The mean interval between onset of subsequent cases in outbreaks was 5.6 days. The transmission model found that human transmission was very rare, with a reproduction number between 0.1 and 0.25, and the upper confidence bounds below 0.4. Transmission model fit was best when the denominator population was restricted to blood-related household contacts of index cases.

Conclusions/Significance: The study only found strong support for human transmission of the virus when a single large cluster was included in the transmission model. The reproduction number was well below the threshold for sustained transmission. This study provides baseline information on the transmission dynamics for the current zoonotic virus and can be used to detect and define signatures of a virus with increasing capacity for human-to-human transmission.

Introduction

The avian influenza (AI) H5N1 virus remains of international public health concern due to its pandemic potential. Based on analyses of AI H5N1 outbreaks during 2003 to 2009, most cases were sporadic and had documented exposure to zoonotic sources of the virus [1]. For clusters of AI H5N1 infection, the majority occurred in people who were genetically related to each other and most also had exposure to zoonotic (bird to human) sources of virus [1]. Studies suggest that human transmission of the virus occurred in a very limited way in some clusters [2,3]. However, the transmission patterns remain largely unknown.

Quantification of transmission patterns such as the probability of both human and zoonotic transmission of the H5N1-virus, the reproduction number \(R_0\), secondary attack rates (SAR) and the interval between case onsets are important parameters to inform preparedness and response measures to outbreaks, especially to signal events that indicate changed virus behavior [4]. It is also crucial that both zoonotic and human infection pathways are considered, and results are interpreted in the context of a zoonotic infection with limited transmission among humans [5,6]. Models that incorporate both the zoonotic and human transmission components are rare [5].

As of July 30, 2009, Indonesia had reported 139 outbreaks of avian influenza (AI) H5N1 infection in humans with a case fatality rate of 85% [7]. The epidemiology of Indonesia’s cases has been reported previously [8–10]. A recent study on the 139 outbreaks assessed the risk factors for household clustering of cases and the risk factors for who in the household is likely to become a secondary case of H5N1-infection [11]. The study found that the type of exposure to sources of H5N1 for both the index case and their household members impacted the risk of additional cases in
the household. The study also added evidence that H5N1 infection may be dependent on host genetic susceptibility since first-degree blood relatives to index cases were at greater risk of becoming secondary cases. However, this study did not assess the attack rates (AR), SAR or transmission parameters in those outbreak households.

To date, only one study has estimated the transmission patterns based on case data in Indonesia [4]. Estimates generated were solely based on one outbreak – a cluster of one probable and seven confirmed cases detected in North Sumatra in 2006. The study found statistical evidence of human-to-human transmission and estimated SAR at 29% and R0 at 1.14 [4]. Since data on the total persons exposed and individual factors such as exposure type were not fully available to that study, the transmission pathways were not investigated in detail. Also, since the model was fitted and transmission estimates generated based only on that one cluster, which is considered atypical due to its large size, the estimates are likely to be an over-estimate for outbreaks in Indonesia.

Since Indonesia’s cumulative AI H5N1 infection case count represents one-third of the world’s cases, the outbreak transmission patterns are of international importance. Building on previous findings about the epidemiology of H5N1 infection in households [11], we describe infection AR, infection SAR, risk factors for H5N1 infection and intervals between case illness onsets. We then estimate transmission parameters and quantify the relative contribution of zoonotic and human transmission as well as the extent to which the virus was transmissible between people (reproduction number). While international data suggest most transmission is zoonotic, there is also evidence of human-to-human transmission [2,12]. We fitted household models to the Indonesian data that allow for both zoonotic and human-to-human transmission to assess the extent of transmission from each source and to provide an estimate of the reproduction number in the case that human-to-human transmission occurs.

Results

A total of 139 outbreaks of human AI H5N1 infection were detected in Indonesia in the four year study period. There were 113 sporadic case outbreaks and 26 cluster outbreaks. The total number of cases was 177, with 64 cases in the 26 clusters. Only one cluster had over four cases; the North Sumatran cluster of 2006, which can be considered an outlier based on its large size of seven confirmed and one probable case. There were 535 household contacts to index cases in the study, of which blood relation was known for 94% (n = 503). Most of the 503 contacts were blood relatives (n = 303, 76%) and 120 (24%) were non-blood relatives. None of the non-blood related household contacts became secondary cases.

Household Study

For the 80 outbreaks for which household and contact data were available, the proportion of cluster to sporadic outbreaks increased as household size increased (Table 1). To highlight the impact of the outlier cluster on the AR and SAR, findings are presented both including and excluding that cluster. The overall AR was 17.8% (103 cases / 579 exposed) when the outlier cluster was excluded and 18.3% (111 cases / 607 exposed) when included. There was a stable SAR between 3.1–4.5% across household size (Table 1). However, inclusion of the outlier cluster inflated SAR for households with >15 persons to 12.5% (Table 1). These findings are consistent with predominantly zoonotic virus transmission. In the absence of human transmission, and with low levels of zoonotic transmission, the AR would be expected to decline with household size, while the SAR should remain roughly constant.

Cases (n = 177) and healthy contacts (n = 496) were compared to assess risk factors for infection (Table 2). Young age groups (≤30 years) were at increased risk of infection, where individuals between five and 17 years of age had 3.5 times the odds to be infected when compared with those >30 years of age [Adjusted Odds Ratio (aOR) = 3.44, 95% Confidence Interval (CI) 1.86–6.36]. Most cases (87%) and their healthy contacts (69%) had zoonotic exposure. However, direct exposure to zoonotic sources of AI H5N1 virus tripled the odds of infection (aOR = 3.08, 95% CI 1.54–6.13). Lastly, small households (1–5 persons) were significantly more likely to have cases than households with >5 people (Table 2). The final multivariate model with three variables had good fit (p = 0.17).

In cluster outbreaks, the median interval between the index case onset and secondary case onset of illness was 8 days (range 1–21 days, Figure 1A). The median interval between the onset of illness of a secondary case and the previous case in the same outbreak was 6 days (range 1–12 days, Figure 1B). Based on the investigation reports, eleven secondary cases had inconclusive exposure to a zoonotic source of virus. All of these had onset of illness at least two days after the index case’s onset of illness. For these 11 cases, the median interval between illness onset of serial cases was 8 days (range 2–11 days, Figure 1B).

Table 1. Household size and secondary attack rate for outbreaks of avian influenza H5N1 infection.

<table>
<thead>
<tr>
<th>Contact data</th>
<th>Household size</th>
<th>Outbreak size (confirmed and probable cases)</th>
<th>Total outbreaks</th>
<th>Proportion cluster</th>
<th>Total contacts</th>
<th>Secondary cases</th>
<th>SAR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 2 3 4 5 6 7 8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Available</td>
<td>1–5</td>
<td>1 0 0 0 0 0 0 0 0 0</td>
<td>32</td>
<td>0.16</td>
<td>152</td>
<td>9</td>
<td>0.059</td>
</tr>
<tr>
<td></td>
<td>6–10</td>
<td>25 6 2 0 0 0 0 0 0</td>
<td>35</td>
<td>0.24</td>
<td>219</td>
<td>8</td>
<td>0.036</td>
</tr>
<tr>
<td></td>
<td>11–15</td>
<td>8 3 0 1 0 0 0 0 0 0 1</td>
<td>12</td>
<td>0.33</td>
<td>85</td>
<td>3</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>&gt;15</td>
<td>0 2 0 0 0 0 0 0 0 1 3</td>
<td>1</td>
<td>1.00</td>
<td>69</td>
<td>9</td>
<td>0.130a</td>
</tr>
<tr>
<td>Sub-total</td>
<td>60 15 3 1 0 0 0 0 1</td>
<td>80</td>
<td>0.24</td>
<td>525</td>
<td>29</td>
<td>0.055a</td>
<td></td>
</tr>
<tr>
<td>Not available</td>
<td>53 5 1 0 0 0 0 0 0 1</td>
<td>59</td>
<td>0.10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>113 20 4 1 0 0 0 0 1</td>
<td>139</td>
<td>0.19</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

*aSAR declines to 0.047 when outlier cluster is excluded.

bSAR declines to 0.044 when outlier cluster is excluded.

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### Table 2. Comparison of cases (n = 177) and healthy contacts (n = 496) in outbreaks of avian influenza H5N1 infection.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases, n (%)</th>
<th>Healthy contacts, n (%)</th>
<th>Univariate OR (P-value)</th>
<th>Adjusted OR (P-value)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age groups (years)</strong>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–4</td>
<td>18 (10)</td>
<td>41 (9)</td>
<td>2.66 (0.004)</td>
<td>3.18 (0.004)</td>
<td>1.45–6.98</td>
</tr>
<tr>
<td>5–17</td>
<td>65 (37)</td>
<td>96 (21)</td>
<td>4.11 (&lt;0.001)</td>
<td>3.44 (&lt;0.001)</td>
<td>1.86–6.36</td>
</tr>
<tr>
<td>18–30</td>
<td>61 (35)</td>
<td>125 (28)</td>
<td>2.96 (&lt;0.001)</td>
<td>3.20 (&lt;0.001)</td>
<td>1.81–5.68</td>
</tr>
<tr>
<td>&gt;30</td>
<td>31 (18)</td>
<td>188 (42)</td>
<td>Reference group</td>
<td>Reference group</td>
<td>-</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>83 (47)</td>
<td>225 (47)</td>
<td>0.99 (0.94)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>94 (53)</td>
<td>258 (53)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Exposure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direct zoonotic</td>
<td>81 (46)</td>
<td>130 (26)</td>
<td>4.02 (0.002)</td>
<td>3.08 (0.001)</td>
<td>1.54–6.13</td>
</tr>
<tr>
<td>Indirect zoonotic</td>
<td>72 (41)</td>
<td>211 (43)</td>
<td>2.20 (&lt;0.001)</td>
<td>1.43 (0.29)</td>
<td>0.72–2.81</td>
</tr>
<tr>
<td>Inconclusive zoonotic</td>
<td>24 (13)</td>
<td>155 (31)</td>
<td>Reference group</td>
<td>Reference group</td>
<td>-</td>
</tr>
<tr>
<td><strong>Household size (persons)</strong>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–5</td>
<td>51 (46)</td>
<td>143 (29)</td>
<td>Reference group</td>
<td>Reference group</td>
<td>-</td>
</tr>
<tr>
<td>6–10</td>
<td>38 (34)</td>
<td>211 (43)</td>
<td>0.51 (0.009)</td>
<td>0.50 (&lt;0.001)</td>
<td>0.34–0.73</td>
</tr>
<tr>
<td>11–15</td>
<td>10 (9)</td>
<td>82 (16)</td>
<td>0.35 (0.001)</td>
<td>0.32 (&lt;0.001)</td>
<td>0.18–0.57</td>
</tr>
<tr>
<td>&gt;15</td>
<td>12 (11)</td>
<td>60 (12)</td>
<td>0.51 (0.07)</td>
<td>0.40 (0.16)</td>
<td>0.11–1.43</td>
</tr>
</tbody>
</table>

*Observations = 561, Goodness-of-fit test: P = 0.17, OR denotes odds ratio, CI denotes confidence interval. OR were adjusted for the inclusion of the three variables in the final multivariate model.

*Data missing for two cases and 46 healthy contacts.

*Data missing for 66 cases from the 59 outbreaks for which household data were not available.

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### Figure 1. Interval between onset of illness for cases (n = 34) in outbreaks of avian influenza H5N1 infection.

Panel A shows the interval between onsets of illness of index and secondary cases in outbreaks. Panel B shows the interval between onsets of illness of serial cases in outbreaks.

Black denotes cases not exposed to zoonotic sources of virus and white denotes cases exposed to zoonotic sources of virus.

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Transmission Model

To assess the exposure of secondary cases, Table 3 presents the transmission analysis comparing three model types: all transmission from zoonotic sources (Model A), all transmission was human transmission (Model B) and transmission was from both zoonotic and human sources (Model C). Two denominator populations are presented for comparison; all exposed individuals in outbreaks and all exposed blood-related household members to index cases. The final column of the tables shows the percentage support for the models, which can be interpreted as the probability that the model is the best among those considered. To highlight the impact of the outlier cluster on transmission parameters and model selection, findings for two datasets are presented; one with the outlier cluster included and one with it excluded.

Regardless of the denominator population or the dataset, there was much less support for Model A (zoonotic transmission only) than either Models B (human transmission only) or C (combination of zoonotic and human transmission) (Table 3). This was confirmed by a simulation-based test of model fit, which demonstrated significant differences between Model A and the data (p<0.01 for both). Despite significant evidence that human transmission occurred when the outlier cluster was included in the analysis, estimated human transmission rates were low with the reproduction number lying between 0.1 and 0.25, and the upper confidence bounds all below 0.4 for an exposed population of five individuals. Estimated zoonotic transmission rates ranged from 0 to 0.38 cases in an exposed population of five household members.

When the analysis excluded the outlier cluster (Table 3), similar estimates for the human transmission parameters and the reproduction number were found, but there was no longer significant evidence of human transmission. Indeed, the model with the strongest support was Model A (zoonotic transmission only), with 0.31 zoonotic cases infected in an exposed population of five household members. This suggests that the main evidence for human transmission comes from the outlier cluster. For all model types, both including and excluding the outlier cluster, use of blood-related household members as the denominator population provided better model fit. A test of the sensitivity of our results to the households in which contact data were missing found very little change to the transmission estimates, with estimates of zoonotic transmission parameters reduced by around 0.05–0.1 cases in an exposed population of size 5, point estimates of human transmission parameters largely unchanged, and a decrease in the upper bound of the human transmission parameter of 0.02–0.08 cases in an exposed population of size 5.

Discussion

This study is the first globally to examine AI H5N1 transmission patterns in households for a large number of outbreaks aimed at quantifying human-to-human transmission of the AI H5N1 virus. The study had three main findings. Firstly, most cases of AI H5N1 infection were a result of exposure to zoonotic sources of virus. In fact, the study only found strong support for human transmission of the virus when a single large cluster was included in the transmission model. Secondly, the overall SAR was 5.5% in the 80 outbreaks for which household contact data were available. This was much lower than previous estimates [4]. Thirdly, the study adds evidence that blood relatives are at greatest risk of becoming secondary cases in outbreak households. This adds support to the hypothesis that there is an element of genetic susceptibility to AI H5N1 infection [3].

The finding that the AI H5N1 virus does not transmit efficiently between humans and that infection remains primarily zoonotic impacts the interpretation of the interval between case onsets and the SAR. These parameters should not be interpreted as human-to-human transmission parameters. Rather, the interval between case onsets (median 6 days, range 1–12 days) represents observed timelines between human cases during an epizootic and indicates

Table 3. Transmission parameters for outbreaks of avian influenza H5N1 infection.

<table>
<thead>
<tr>
<th>Data</th>
<th>Denominator population</th>
<th>Model description</th>
<th>Mean human transmission cases a (95% CI)</th>
<th>Mean zoonotic infected cases b (95% CI)</th>
<th>AICc percent support c</th>
</tr>
</thead>
<tbody>
<tr>
<td>80 outbreaks (North Sumatra cluster included)</td>
<td>All exposed individuals</td>
<td>A) Only zoonotic transmission</td>
<td>-</td>
<td>0.276 (0.126, 0.476)</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>B) Only human transmission</td>
<td>0.172 (0.026, 0.322)</td>
<td>-</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C) Full model</td>
<td>0.115 (0.009, 0.315)</td>
<td>0.094 (0.000, 0.344)</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>79 outbreaks (North Sumatra cluster excluded)</td>
<td>All exposed individuals</td>
<td>A) Only zoonotic transmission</td>
<td>-</td>
<td>0.385 (0.185, 0.635)</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>B) Only human transmission</td>
<td>0.231 (0.082, 0.382)</td>
<td>-</td>
<td>42.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C) Full model</td>
<td>0.140 (0.004, 0.390)</td>
<td>0.157 (0.000, 0.452)</td>
<td>44.0</td>
<td></td>
</tr>
</tbody>
</table>

*Mean number of secondary cases infected by a single index case in an exposed population of size 5, CI denotes confidence interval.
1Mean number of zoonotic cases in an exposed population of size 5.
2AICc denotes Akaike Information Criterion adjusted for small sample size. This indicates the percent probability that the model is the best amongst those considered.
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the duration of risk of more cases being detected in association with the epizootic event. This information can guide the length of contact tracing needed to detect and prevent further cases during an outbreak. The findings from this study reinforce the WHO recommendation to trace and monitor case contacts for two weeks after the illness onset of the last case [13].

The SAR results add to the body of knowledge on typical outbreak size associated with the current zoonotic virus, where SAR remained approximately stable with household size. This provides important baseline information for future outbreak investigations and may help in the detection of changes in virus behavior. For a virus on the verge of efficient human spread, the household SAR should be compared to the current findings as well as SAR for other influenza viruses.

Although the SAR remained stable with household size, the proportion of outbreaks with more than one case increased with household size. This highlights an important distinction between individual and household risk for infection with the current zoonotic virus: a person in a large household is less likely to be infected than a person in a small household, but large households are more likely to have a secondary case than small households. Whether SAR was low due to virus and host characteristics or due to public health interventions such as prophylaxis of case contacts or isolation of cases was not explored in this study, but warrants further investigation. Importantly, the SAR could not be calculated for the remaining 59 outbreaks as contact data were not available to determine the household size. The missing data highlight the challenge in standardizing data collection for a new emerging disease. However, as the excluded outbreaks were typically smaller than those with full contact data (90% of excluded outbreaks were sporadic), it seems unlikely that inclusion of those outbreaks would increase the overall SAR or the transmission parameters. Our sensitivity analysis suggested that inclusion of these data would likely result in a slight decrease in the zoonotic transmission parameter, negligible impact on the point estimate of the human transmission parameter, and a slight decrease in the upper bound of the human transmission parameter.

Due to the limited sensitivity of public health surveillance systems, varied health-seeking behavior within the population and the potential for mild infections, it is possible that cases or clusters of H5N1 infection were missed and not included in the analysis. This affects our findings. If sporadic cases of H5N1 infection resulting from zoonotic transmission of the virus were missed, then our study likely over-estimates overall SAR and transmission parameters. If clusters of cases were missed, then our study may under-estimate these parameters. We speculate, based on our H5N1 case investigations, that clusters of disease are less likely to be missed than sporadic cases of infection since families and healthcare workers would raise alarms in the public health system about multiple cases of pneumonia in a single household. For mild cases, it is feasible that cases are missed, which suggests that our results would under-estimate transmission parameters. However, based on studies conducted amongst poultry workers exposed to H5N1 virus in the course of their work, mild and subclinical infections have been limited [14–16]. This is also mirrored in influenza virological surveillance findings conducted by countries affected by H5N1 virus such as Laos PDR, China and Cambodia, whereby these sentinel surveillance systems regularly detect seasonal influenza viruses circulating in the community and in hospital settings, yet they rarely detect cases of H5N1 virus infection [17–19].

The disease transmission model achieved a better fit when the exposed population was restricted to blood-related household members. The study also found that only blood relatives to the index case developed illness and that none of the 120 non-blood related household members (such as spouses and family-in-law) developed illness. Collectively, these findings add evidence to the hypothesis that there is a host genetic effect on susceptibility to AI H5N1 infection [11]. However, since genetic relationship and household membership are correlated, it is difficult to identify the mechanisms most responsible for household clustering. Thus, further research is needed to explore these findings.

Individuals at most risk of infection were those ≤30 years, especially children between five and 17 years. The young age pattern was also observed globally based on analysis of cases from 11 countries [1]. This suggests that young age groups have greater susceptibility to AI H5N1 infection; be it due to social, hygienic or biological factors. Potential reasons include that children are more likely to handle sick and infected birds or to be exposed to contaminated environment through play or through bird rearing. In Indonesia, anecdotal evidence suggests that bird rearing is delegated to young household members. Children are less conscious of hygiene and thus may have had unproctected interaction with sources of virus [20].

Household based studies exploring risk factors for infection are less likely to be affected by case-ascertainment bias [21]. However, since household data were not available for all outbreaks, our analyses and conclusions were based on a restricted dataset and should be interpreted with caution as the missing data limit the power of our study. Nevertheless, as discussed earlier, since 90% outbreaks lacking household data only had one case, our study likely over-estimated the transmission parameters and the SAR, indicating that human transmission rates were very low.

Overall, the study found that AI H5N1 human infection resulting from human transmission of the virus was very limited, and that the reproduction number was well below the threshold for sustained transmission. Case clustering does not always denote human transmission of the virus, but is often the result of household members’ shared exposure to zoonotic sources of the virus [22]. The study findings also suggest that there may be a host genetic effect on susceptibility to infection, but this warrants further investigation through epidemiological and immunological studies to untangle the correlation between household membership, shared exposures and genetics.

Materials and Methods

Ethics Statement

All data in this study were obtained from the case-investigation reports and the surveillance database at the Ministry of Health, which were collected as part of an ongoing public-health investigation. Permission to conduct the study and analyze the data was obtained from the data custodian (first author, Director-General for Disease Control and Environmental Health at the Ministry of Health, Republic of Indonesia). Data shared with international study collaborators, who were not involved in the case investigations, were de-identified to protect the confidentiality of the cases and their families, whereby names and addresses were removed. Ethics approval for the study was obtained from the Australian National University’s Human Research Ethics Committee.

Setting

The Ministry of Health AI H5N1 case database and detailed case investigation forms were reviewed and analyzed for cases detected in Indonesia between July 2003 and July 2009. The study conformed with the WHO definitions [13], whereby a cluster is a group composed of one confirmed case of H5N1 virus infection.
and additional confirmed or probable cases associated with a specific setting, with the onset of cases occurring within 2 weeks of each other. In households with a cluster of cases, the index case was defined as the one with the earliest symptom onset date amongst all the cases in that household. A sporadic outbreak was defined as one confirmed case of H5N1 virus infection. Case definitions for probable and confirmed cases were based on the WHO definitions described previously [23]. For both sporadic and cluster outbreaks, a household contact was a person who had at least four hours contact with a probable or confirmed case at home within the seven days prior or 14 days after the case’s onset of illness.

Data Collection
Field investigation teams investigated every outbreak. Teams interviewed cases when possible (since many cases died before investigation teams arrived), family members and key informants such as healthcare workers. As described previously [8,11], data were collected using a standardized H5N1-case questionnaire developed by the Ministry of Health based on WHO guidance [24]. The questionnaire collected data on the case’s household, clinical symptoms, healthcare facility attendance and potential zoonotic, human and environmental exposures to sources of H5N1-virus. Medical records from all healthcare facilities visited by cases during the course of their illness were reviewed and extracted to complete the questionnaire.

Contact tracing, clinical examination and testing of household contacts were done during the investigation. Serum samples were collected from all healthy household contacts to assess for H5N1 seroconversion using microneutralization test or haemagglutination inhibition test (with horse red blood cells). For household contacts with symptoms of H5N1 infection, nasal and throat swabs were collected and tested using real-time reverse transcriptase polymerase chain reaction (RT-PCR) test. All tests were conducted according to the WHO guideline on recommended laboratory procedures for H5N1 detection [25]. Healthcare workers from the nearest government primary healthcare centre were instructed to visit the household daily for two weeks to monitor and detect any additional cases.

Household Study
AR, SAR, risk factors for infection and intervals between case onsets were analyzed in a household-based study. Household size was the number of people in the household including cases. A household contact was a person who had at least four hours contact with a case at home within the seven days prior or fourteen days after a case’s onset of illness. AR was calculated for the 80 outbreaks (60 sporadic and 20 clusters) out of the 139 for which household data were available. Data on household contacts were missing for 59 outbreaks, of which 90% (n = 53) were sporadic case outbreaks and the largest outbreak involved three cases. AR was defined as the proportion of people who met the definition for confirmed or probable AI H5N1 infection in the outbreak (household). SAR was defined as the proportion of household contacts who met the probable or confirmed case definition after the onset date for the index case and within two weeks of the onset of symptoms of a prior household case. Two weeks was selected as the maximum follow up period based on WHO guidance [13]. The intervals (days) between onset of symptoms of index cases and subsequent cases in clusters, and the interval between serial cases in clusters were calculated.

Logistic regression models that accounted for household clustering using a cluster robust standard error for the coefficients were used to evaluate the risk factors for infection. Multivariate models were constructed using variables significant at p = 0.1 in the univariate analyses. A final model was achieved by sequentially discarding terms not significant at P = 0.05 starting with the ones with the highest P-values. We used the le Cessie-van Houwelingen-Copas-Hosmer unweighted sum of squares goodness-of-fit test to assess model validity, as advocated by Hosmer et al. [26–28]. Stata software version 10.0 (StataCorp) was used for this analysis.

Four variables were explored as risk factors for infection: age, sex, exposure type and household size. To simplify interpretation of results, age and household size were analyzed categorically. Categories were based on data spread; four groups for age in years (0–4, 5–17, 18–30 and ≥31) and four groups for household size (1–5, 6–10, 11–15 and >15 people). Exposure was defined as whether the individual had direct, indirect or inconclusive zoonotic exposure to a source of AI H5N1 virus. Direct zoonotic exposure referred to cases who handled sick or dead poultry, handled poultry products such as fertilizers, or who had poultry deaths in the home. Indirect zoonotic exposure referred to cases where poultry deaths were reported in the neighborhood, cases where healthy poultry were present in the neighborhood and cases who visited live bird markets. Inconclusive zoonotic exposure refers to cases where no zoonotic source of infection could be found despite investigation.

Transmission Model
To assess the potential for human transmission of the virus, we used final size household models to fit the human and zoonotic transmission parameters to outbreak data (household size, number of cases, blood-related household members to index case) in a manner similar to that described in van Boven et al. [29]. This approach allows for both human and zoonotic transmission, and enables comparison of different transmission assumptions. We used Akaike Information Criterion adjusted for small sample size ($AIC_{C}$) to select the most appropriate models. The $AIC_{C}$ percent support gives the probability that the model is the best model of those considered, but does not indicate how well the suite of models fit the data [30]. We used a simulation-based approach to compare the data with each of the model predictions, which allowed us to identify those models that differed significantly (P<0.05) from the data. Matlab (version R2010b) was used for this analysis. Our preliminary analysis indicated that density-dependent transmission [31] gave a better fit to the data than frequency-dependent transmission [29], and that assumptions concerning the distribution of the infectious period did not affect our results. Thus, our detailed analysis used a model with a fixed infectious period and density-dependent transmission. Under these assumptions, outbreak sizes will vary according to the exposed population, and we present results for an exposed population of size five (the median household size in the data).

Our estimation methods calculated the best-fit parameters to cluster data consisting of the number of exposed individuals, the number of index cases and the final outbreak size. In our initial analysis, we used all individuals exposed for a period of four hours or more in the household as the exposed population. In light of evidence concerning transmission of H5N1 to blood-related contacts [1,3], we also considered an alternative analysis in which the exposed population was restricted to all blood relatives exposed for a period of four hours or more in the household. Finally, we tested the sensitivity of our results to the inclusion of those households for which contact data were missing, by including the missing households into the data, assuming that they had 5 household members (the median household size in the data) and 4 blood relative contacts (again, the median in the data).
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References


Author Contributions

Conceived and designed the experiments: TYA GS KG KL PMK INK. Performed the experiments: GS RK WP M HS AB. Analyzed the data: GS AM ES VS KG. Contributed reagents/materials/analysis tools: HS VS ODS. Wrote the paper: GS TYA KG KL PMK.
Chapter 6

Paper 3: Chicken faeces garden fertilizer: possible source of human avian influenza H5N1 infection.
About this chapter

Chapters 4 and 5 explored the epidemiology of AI H5N1 in Indonesia based on the outbreak investigation reports and surveillance dataset at MOH. As part of the data used to explore the disease epidemiology, this chapter describes one specific cluster from the surveillance dataset. The cluster was detected in Indonesia in 2005 and comprises two cases: a 37 year old female and a nine year old male. The cluster is presented as a case report.

This case report is of importance as it was the first report globally to explore poultry faeces in garden fertilizer as a potential source for AI H5N1 human infection. The epidemiological investigation and supporting laboratory findings are presented. Even though the virological evidence does not provide conclusive evidence that the isolate from the H5N1-contaminated garden fertilizer was the source of the index case’s infection, it does highlight the importance of environmental investigation and laboratory testing to identify putative sources of infection for this emerging infectious disease.

I participated in the investigation and data collection for the outbreak reported in this chapter. In addition to collecting and analyzing the epidemiological data, I also coordinated with the virologists nationally and at a WHO Collaborating Centre for the virological analyses. I wrote the paper and obtained feedback from all the co-authors pre-publication. The published study has been reproduced here with permission from the publisher, John Wiley and Sons.
Chicken Faeces Garden Fertilizer: Possible Source of Human Avian Influenza H5N1 Infection

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Impacts
- Avian influenza A H5N1 infection in humans is generally associated with close contact with infected birds.
- This paper points to poultry faeces used in garden fertilizer as a potential source for human infection with avian influenza A H5N1 virus.
- Public education is needed to ensure safe handling of poultry faeces, especially in areas affected avian influenza A H5N1.

Introduction
Indonesia detected its first human case of avian influenza H5N1 in June 2005; nearly 2 years after the disease was detected in the local poultry population (World Health Organization, 2005). By end of June 2008, Indonesia had confirmed 135 human cases of H5N1, of which 110 were fatal (case fatality rate = 81%) (World Health Organization, 2008).

Each human case of H5N1 in Indonesia is thoroughly investigated by an epidemiological team to determine the likely source of infection and to determine whether the outbreak led to further human cases. This case report describes a confirmed human case where the investigation found that chicken faeces used as garden fertilizer was contaminated with viable H5N1 virus. We hope that this report will prompt investigators worldwide to consider this as a possible source of infection for H5N1 human cases and to incorporate testing of poultry products into their investigations.

Case Descriptions
A 37-year-old female from South Jakarta in the western part of Java developed fever and sore throat on 31 August 2005. She consulted a local medical clinic 3 days later because of persistent symptoms. By 6 September, she had also developed cough, shortness of breath and difficulty breathing and was admitted to a private hospital. Based on the clinical presentation, the hospital suspected influenza A H5N1 infection as a differential diagnosis. Throat and nasal swabs, as well as blood samples, were collected from the patient on 6 and 9 September. She developed respiratory distress and died on 10 September 2005.
Samples tested positive for H5N1 by RT-PCR on 11 September at the National Institutes of Health, Research and Development, and the results were confirmed on 16 September by two different WHO H5 reference laboratories (World Health Organization, 2006). A virus was isolated and sequenced, where it was found to be of a purely avian source.

A 9-year-old male, a nephew and blood-relative of the 37-year-old patient, developed headache and fever (40°C) on 4 September; 4 days after the onset of symptoms for the 37-year-old patient. A mild cough began 2 days later. He presented to a doctor on days 6 and 7 of illness due to his persistent symptoms. On 10 September, he was prescribed symptomatic treatments and antibiotics, but no antiviral drugs, as doctors did not suspect H5N1 infection. He did not develop shortness of breath or disabling symptoms. His white cell and platelet counts were 8800 cells/mm³ and 130 000 cells/mm³ respectively on 10 September, and 7100 cells/mm³ and 270 000 cells/mm³ respectively on 11 September.

As part of the contact tracing conducted for the 37-year-old index patient, sera and throat/nasal swabs were collected from the boy on 13 September. As the boy was mildly ill and had contact with a confirmed case of avian influenza, he was referred to hospital to be managed as a suspected H5N1 case. He was administered oseltamivir and remained under observation in the hospital even though his symptoms had subsided. Throat swabs collected on 17 September and 20 September from the boy tested positive by RT-PCR for H5N1, but a virus could not be isolated for sequencing. Samples collected after 20 September were found to be negative for the virus. Acute and convalescent sera were collected from the patient on 13 September and 7 October 2005, respectively. A micro-neutralization titre of 1 : 20 was observed. He survived and was discharged on 26 September.

Epidemiological Investigation

Family members were interviewed to assess exposure history, to cross-check timelines and to determine the mode of disease transmission for the cases, and serum samples were collected from relatives to assess serological evidence of H5N1 infection (below).

The index patient (37-year-old female) managed the family’s small printing business. The husband did not identify any occasions in the 2 weeks prior to the onset date where the patient slaughtered chicken or came into direct contact with sick animals. She also had not travelled to areas known to be infected with avian influenza nor was she in close contact with a person with influenza-like illness or acute respiratory illness. The husband reported that the patient never visited wet markets, preferring to purchase eggs and chicken pieces from the food stalls near her workplace and often purchased ready-to-eat fried chicken. The patient was known to be a keen gardener, and she used to purchase garden fertilizer (chicken faeces) in sealed bags from a gardening shop to use for her potted plants. She did not wear gloves or a mask whilst gardening.

The home and neighbourhood of the index patient were thoroughly inspected to assess the possible source of infection. The 37-year-old patient only kept pet fish and regularly purchased worms for them from a local pet shop. The pet shop owner reported no bird deaths in the 2 weeks preceding the index patient’s onset of illness. A bag of chicken faeces garden fertilizer was found at the patient’s home. The husband stated that the bag was purchased before the patient’s onset, but the exact dates of purchase or manufacture were unknown. The label on the bag indicated that the fertilizer came from a company in East Java (over 1000 km away). Through trace-back, we found out that the company purchased chicken faeces from a variety of collectors for inclusion in their product, and that the collectors sourced the faeces from numerous farms, as far as hundreds of kilometres away. Further trace back was deemed unreliable and was not conducted.

There were many caged birds, chickens and some swans in the index patient’s neighbourhood, but no reported deaths. There was also a backyard poultry slaughterhouse approximately 50 metres away from her home. The slaughterhouse was not located on the same road as the patient’s home, and could only be reached by entering small side lanes. The slaughterhouse did not keep cages or chicken flocks but received approximately 150 chickens from a neighbouring district to slaughter on a daily basis. Most slaughtered chickens were sold to the wet market 2 km from the patient’s home. It is unlikely that the patient had contact with these chickens as they generally arrived and were slaughtered before dawn to be available at the markets by sunrise. The patient did not purchase chicken meat from this slaughterhouse nor the wet market.

The 9-year-old nephew did not report any contact with chicken or birds in the fortnight preceding his illness. Yet, he visited the 37-year-old index-patient in her home 2 days into her illness (1 September). They met again at a large family gathering the next day.

Laboratory Investigation

Serum samples were collected from 119 contacts traced during the investigation to assess for H5N1 seroconversion. Samples were collected from 49 family members, 26 neighbours, 41 healthcare workers and 3 gravediggers. All
samples were collected at least 2 weeks after the index patient’s illness onset. The samples were tested by microneutralization assay, where none was positive for H5N1 antibodies. Nasal and throat swabs were collected from two individuals who reported recent history of influenza-like illness. None of these contacts was found to be infected with H5N1. All virus isolation, RT-PCR and microneutralization tests were carried out as described previously (Kandun et al., 2006).

Through the course of the investigation, 99 animal and environmental samples were collected from the 37-year-old patient’s home, relatives’ homes, nearby slaughterhouse and wet market. All of the samples were collected 17 days after the index patient’s onset of illness. This included animal samples (chickens, caged birds, swans) and environmental swabs (garden fertilizer from the patient’s home and water from the processing tubs at the slaughterhouse). All of the animals sampled appeared healthy at the time of specimen collection. To process environmental samples, 5 g of specimen was dissolved in virus transport medium. The suspension was then centrifuged and the supernatant was inoculated into 9- to 11-old-day specific-pathogen-free embryonated eggs for isolating virus. From the animal and environmental samples, one chicken sampled at a wet market 2 km away from the index patient’s home was H5N1 positive by RT-PCR, but the virus could not be isolated for sequencing. The chicken faeces (garden fertilizer) collected from the index patient’s home also tested H5N1 positive by RT-PCR. All other samples, including samples from the slaughterhouse, were negative.

For both, patient and fertilizer isolates, RNA extraction, cDNA synthesis and PCR were used as described previously (Guan et al., 2000). Sequencing was performed with the BigDye Terminator v3.1 cycle sequencing kit on an ABI PRISM 3700 DNA analyzer (Applied Biosystems) by following the manufacturer’s instructions. Sequence fragments were assembled with Lasergene (version 6.0; DNASTAR) and then aligned by using BioEdit, version 7, and residue analysis was performed with BioEdit, version 7. Phylogenetic trees were generated by neighbour-joining bootstrap analysis (1,000 replicates) by using the Tamura-Nei algorithm in MEGA, version 2.1. The percent nucleotide homology of HA genes of A/Indonesia/6/05 versus A/Indonesia/Environment/05 was calculated using MegAlign v8.0.2 (DNASTAR) and found to be 97.5% (Fig. 1).

Discussion

From the epidemiological aspects of this investigation, we found that the patient had direct and unprotected contact with the H5N1-contaminated fertilizer. Our results also confirmed the prolonged (>2 weeks) environmental stability of H5N1 virus in bags of chicken faeces garden fertilizer. However, from the virological aspects of this investigation, we could not conclude that the isolate from the H5N1-contaminated garden fertilizer was the source of infection for the index patient.

The phylogenetic tree highlighted that the virus isolated from the index patient was most similar to viruses isolated in the eastern parts of Java, the location of the fertilizer manufacturing company, as opposed to isolates located in the western parts of Java where the index patient resided. It is possible that the fertilizer bag which likely contained poultry faeces from many birds harboured multiple strains of H5N1 viruses, one of which led to her infection. The one we isolated from the fertilizer bag represented a different isolate. Based on information from the fertilizer manufacturer, it is plausible that the same batch of fertilizer may have been contaminated with different strains of H5N1 virus as faeces were sourced from various geographical areas in Java. We also cannot exclude the possibility that the patient was infected from other sources, such as the nearby slaughterhouse or wet market, due to their geographical proximity to the case. However, the interview with the patient’s husband suggested that she did not visit these places.

The finding of prolonged environmental stability of H5N1 virus in chicken faeces in this observational study is similar to observations made during the 1983–1985 Pennsylvania outbreak of avian influenza A H5N2, where viable virus was detectable up to 6 weeks in wet faeces (Benedictis et al., 2007). However, our finding contrasts with some studies, where avian influenza viruses in faeces were inactivated within a week at 25–32°C (Beard et al., 1984; Lu et al., 1984; Songserm et al., 2006). These studies suggest that sunlight and viral inactivating factors in the organic material of faeces contribute to more rapid inactivation of the virus. As suggested by Benedictis et al., survival of avian influenza viruses in faeces is likely to be influenced by a number of factors, including the virus strain, temperature, amount of organic material and the host animal type. Further systematic studies need to be conducted to assess factors that explain the conflicting findings.

The source of the 9-year-old male’s infection could not be ascertained. Nevertheless, he was considered epidemiologically-linked to the index patient. This is based on the disease timeline, his exposure to the same contaminated environment and close contact with the 37-year-old during her initial phase of illness. A number of hypotheses were considered to determine the source of the 9-year-old patient’s infection. Exposure to a discrete source of infection at his home or at another location was possible. However, based on inspection of his home, the surrounding
neighbourhood and a general assessment of his activities during the 2 weeks preceding the onset of illness, no independent environmental risk factor for infection could be identified. The second hypothesis was that he acquired infection from a continuing point source at the index patient’s home during his visit on 1 September. Details of his activities during that visit, including whether he handled the fertilizer or helped his aunt in gardening, could not be elucidated. The third hypothesis was that the patient acquired the infection directly from the 37-year-old patient either on 1 or 2 September. It is not possible to either support or discard either the second or third hypotheses, as the patient was exposed to both risk factors in a similar time period, and both are compatible with the incubation period for his illness. Limited human to human transmission could not be excluded in this cluster; how-

Fig. 1. Phylogenetic tree of the haemagglutinin of the H5N1 viruses isolated from the 37-year-old index patient (IDN/6/05) and the fertilizer sample found at her home (IDN/ENVIRONMENT/P3/05). Trees were generated with MEGA2 by using a Tamura-Nei (gamma) neighbour-joining analysis. The numbers at the nodes indicate bootstrap values from 1000 bootstrap replicates. Accession numbers for patient isolate and fertilizer isolate are EU146624 and EU146642, respectively.
ever no further cases were identified in the course of the investigation.

Even though the 9-year-old had clinical symptoms, and throat swabs collected up to day 17 of his illness were positive for H5 by RT-PCR, he did not mount a 4-fold rise in antibody titre. A potential reason for the lack of antibody response is the administration of Oseltamivir during his course of illness, however, this finding warrants further investigation and comparison to other surviving cases of avian influenza H5N1 infection.

Our study highlights the importance of timely and comprehensive environmental investigations to identify the likely source of infection for H5N1 cases. Health authorities need to devise sensitive criteria to detect suspect human H5N1 cases, have adequate resources to investigate them in a timely fashion and have functional coordination with the agriculture authorities to share and analyse the microbiological and environmental data. This is especially important when direct contact with infected animals or humans cannot be established, and where environmental contamination appears to be a likely source of infection; perhaps even a continuing source of infection. This study suggests that avian influenza H5N1 may be acquired from contaminated fertilizer or soil, similar to other pathogens like Legionella longbeachae, Chlamydia psittaci and Histoplasma capsulatum (Heymann, 2004).

Further research is needed to learn more about the modes of H5N1 transmission, but our findings suggest that untreated poultry faeces as fertilizer is a possible source of infection. Education efforts should include public education on avoiding direct contact with items that contain poultry sources like fertilizers, and for healthcare providers to recognize possible sources of infection for diagnosis. This is especially important in areas where the virus is endemic and where access to diagnostic facilities is limited. Figure 2 highlights some steps that can be taken to prevent transmission of the virus from contaminated fertilizer to humans. In addition, control measures should be in place to detect such contamination and reduce future spread.

The limitations of this study are common to epidemiological investigations for high case-fatality rate diseases: the lack of primary source data from the case(s) means that definite sources of contact cannot be elucidated. Samples from the fertilizer producer should have been tested for possible viruses that were similar to the cases, but this was not possible, given the lack of batch number on the fertilizer.

We have shown that poultry products such as fertilizer need to be assessed in the course of an investigation, as a possible source of transmission for H5N1. Public and healthcare worker education should warn of the risks in coming into unprotected contact with such products.

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**Fig. 2.** Steps to prevent transmission of H5N1 virus from contaminated fertilizer to humans in H5N1 endemic areas (Smith et al., 2005; Food and Agriculture Organization of the United Nations, 2006).

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**Conflicts of Interest**

None of the authors has a commercial or other association that might pose a conflict of interest.

**Financial Sources**

None.

**Previous Reporting of Findings at Meetings**

None.

**References**


Food and Agriculture Organization of the United Nations, 2006: FAO/OIE/WHO Consultation on Avian Influenza and


Chapter 7

Paper 4: Environmental sampling for avian influenza virus
A (H5N1) in live-bird markets, Indonesia
About this chapter

Chapters 4 to 6 explored the epidemiology of AI H5N1 in Indonesia based on the outbreak investigation reports and surveillance dataset for 2005-2009 at MOH. One of the main findings of those chapters was that most human cases of AI H5N1 infection resulted from zoonotic, including environmental, transmission of the virus. This highlights the importance of addressing the disease in the birds and the environment that can be contaminated with the virus.

One focus for AI H5N1 disease activity is the LBM setting, where live birds come into the market, are slaughtered and sold to consumers. As was seen in Chapter 4, LBM exposure was the putative source of infection for seven human cases. LBMs are considered a site of disease activity at the interface between birds and humans, thus have the potential for maintaining circulation of virus between birds as well as leading to human infections. Thus, the subsequent chapters in this PhD examine the epidemiology and disease control methods in the LBM setting.

The study reported in Chapter 7 identified environmental sites commonly contaminated by AI H5N1 virus and the risk factors for this contamination. The environmental sites most commonly contaminated are those in the slaughter and subsequent zones in LBMs. Slaughtering birds in LBMs was a risk factor for contamination, whilst daily solid waste removal and clear zoning between work processes such as holding, slaughtering and selling birds were protective.

My role in the study included designing the study, collecting and analysing the data. I was also responsible for writing the manuscript and incorporating co-author feedback. The published study has been reproduced in this chapter with permission from Emerging Infectious Diseases journal.
Environmental Sampling for Avian Influenza Virus A (H5N1) in Live-Bird Markets, Indonesia

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To identify environmental sites commonly contaminated by avian influenza virus A (H5N1) in live-bird markets in Indonesia, we investigated 83 markets in 3 provinces in Indonesia. At each market, samples were collected from up to 27 poultry-related sites to assess the extent of contamination. Samples were tested by using real-time reverse transcription–PCR and virus isolation. A questionnaire was used to ascertain types of birds in the market, general infrastructure, and work practices. Thirty-nine (47%) markets showed contamination with avian influenza virus in >1 of the sites sampled. Risk factors were slaughtering birds in the market and being located in West Java province. Protective factors included daily removal of waste and zoning that segregated poultry-related work flow areas. These results can aid in the design of evidence-based programs concerning environmental sanitation, food safety, and surveillance to reduce the risk for avian influenza virus A (H5N1) transmission in live-bird markets.

Food markets that offer both poultry meat and live birds either for sale or for slaughter are collectively referred to as live-bird markets (LBMs). LBMs are part of the supply chain and are essential for maintaining the health and nutritional status of rural and urban populations, especially in developing countries (1, 2). However, LBMs provide optimal conditions for the zoonotic transfer and evolution of infectious disease pathogens because they provide major contact points between humans and live animals (3, 4).

Studies in Hong Kong Special Administrative Region, People’s Republic of China; other areas of China; Indonesia; and the United States have shown that LBMs can harbor avian influenza viruses (AIVs), including highly pathogenic influenza virus A (H5N1), and have been associated with human infection (4–9). Continual movement of birds into, through, and out of markets provides opportunity for the introduction, entrenchment, and dissemination of AIVs. Most studies have focused on testing live birds rather than environmental sites in the LBMs (6, 7, 10). However, a study in New York, NY, that tested environmental sites for AIV (H7N2) found that virus could be isolated from samples from floors, walls, and drains from the poultry areas of LBMs (8). The study also found that despite the ongoing influx of infected birds into LBMs, the level of environmental contamination decreased with routine cleaning and disinfection. Another study in Hong Kong LBMs showed that AIV (H9N2) could be isolated at higher rates from poultry drinking water than from samples of bird fecal droppings (11). Environmental aspects of LBMs are needed for an avian influenza control program for 2 reasons. First, a contaminated environment can provide a continuing source of virus transmission, in which healthy birds coming into the market may become infected and persons working in or visiting the market may also be exposed. Second, ongoing surveillance programs in LBMs based on environmental sampling are more likely than those based on invasive bird testing to be acceptable to traders and stall vendors. Environmental sampling is also safer for public health officers and veterinary health officers than handling and sampling live birds that may be infected with AIV.

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In this study, we aimed to identify the environmental sites commonly contaminated by AIV (H5N1) in LBMs in Indonesia. Identifying these sites is the first step in the design of evidence-based environmental sanitation, food safety, and surveillance programs to reduce the risk for virus transmission and to develop environmental surveillance programs to monitor LBM contamination status.

Methods

Three provinces in the western part of Java Island in Indonesia participated in the study: Jakarta, Banten, and West Java (Figure). Eighteen districts in these provinces were selected on the basis of their proximity to the laboratory, high levels of avian influenza activity in farmed birds (Ministry of Agriculture, unpub. data), and high number of LBMs available for study (n = 300). The required sample size was 73 markets based on an estimated disease prevalence of 50% and a maximum error of 10% at 95% confidence. We based our assumption that 50% of LBMs would be contaminated with AIV (H5N1) on results from a previous study in US LBMs in 2001 (12). This study found that 60% of markets tested positive for AIV (H7N2) virus in areas in which the virus was endemic. To account for nonresponse, we increased the total sample size to 83 LBMs. We selected markets for inclusion in the study using systematic sampling. On the basis of a sampling frame of 300 markets, every fourth market (the sampling interval) was selected from a list of all the markets. A random numbers table was used to determine the starting point for selection of the 83 markets from the list. Diagnostic specimens and data were collected during October 2007–March 2008. These months have high rainfall and high AIV transmission according to data gathered during 2005–2007 about AIV (H5N1) outbreaks in farmed birds (Ministry of Agriculture, unpub. data).

A structured questionnaire containing 42 questions to assess risk factors for AIV (H5N1) contamination was developed. Responses to questions were obtained through visual inspection of each LBM and through an interview with the manager of the participating LBM. The questions sought information about volume of poultry in the LBM and the infrastructure in the delivery, holding, slaughter, sale, and waste-disposal zones of the market. These 5 zones reflect general demarcation of work flow and activities relating to poultry in LBMs (13). Questions about the sanitation and slaughtering practices were also included.

Questionnaire validation was conducted by members of a study advisory team. The team comprised 2 food safety/environmental health officers from the Ministry of Health, a communicable disease epidemiologist from the World Health Organization, a veterinary epidemiologist from the Food and Agriculture Organization, and 2 virologists from the Ministry of Agriculture in Indonesia. The questionnaire was tested in 3 LBMs in West Java province to ensure coherence, appropriate use of terminology, and high face validity. The same markets were also inspected to ensure that the questionnaire addressed all aspects of the poultry-related work flow in the 5 poultry zones and relevant infrastructure. Members of the study advisory team trained 3 study data collection teams in questionnaire administration and sample collection procedures.

To select the environmental sites to be sampled in each LBM, the study advisory team visually inspected 3 markets and reviewed the literature to identify LBM sites commonly contaminated with AIVs or similar pathogens. Sites sampled in previous studies for AIV included floors, drains, and water troughs (8,11,12). In this study, 27 sites were selected for environmental sampling (Table 1). The sites represented different poultry-related work activities: 3 sites related to delivery of birds into LBMs, 7 in the bird-holding zone, 9 in the slaughter zone, 6 in the sale zone, and 2 in the waste-disposal zone. Because of variation in LBM infrastructure and processes, each LBM did not necessarily have all 27 sites. Samples were collected from as many of the 27 sites as were available in each LBM.

For each of the 27 sites, 6 swab specimens were collected and pooled. Each pool (vial) consisted of a maximum of 3 swabs. The data collection teams were instructed to increase the representativeness of the samples by swabbing different locations for each environmental site. For
example, if the market had 6 poultry stalls, each with its own scale for weighing poultry, then teams collected 1 swab from each scale and pooled them into 2 pools of 3 swabs each. Swab specimens were pooled in the market, and swabs remained inside the vials until testing. The data collection teams were instructed to focus on visibly dirty, moist, or difficult-to-clean surfaces in an effort to increase the sensitivity of the sampling.

Sample collection, pooling, transportation, and storage were based on techniques used in previous studies (10,12). Each data collection team comprised 3 persons, 2 of whom collected samples and 1 administered the questionnaire. To reduce the risk for cross-contamination during sample collection, teams changed disposable gloves and shoe covers between each of the 5 LBM poultry zones. Sterile cotton-tipped swabs were used to collect all samples, and samples were placed in viral transport media and transported immediately back to the laboratory on frozen gel packs. The viral transport media consisted of Dulbecco modified Eagle medium (Sigma-Aldrich, St. Louis, MO, USA) with 1,000 IU penicillin and gentamicin, and 1% fetal bovine serum (14). Samples were stored in the laboratory at –70°C until tested.

RNA extraction, cDNA synthesis, and real-time reverse transcription–PCR (RT-PCR) were used as described (15). Virus isolation methods have also been described (16) but in general involved supernatants from a 1,000-μL sample homogenized by vortex and centrifuged at 2,500–3,000 rpm into 9- to 10-day-old specific pathogen–free eggs. Those positive in the hemagglutination assay were tested by hemagglutination-inhibition test with reference antisera (A/chicken/West Java/Hamd/2006).

The degree of association between AIV (H5N1) positivity in the 5 LBM poultry zones was determined by using Spearman rank correlation. To assess risk factors for environmental virus (H5N1) contamination, we estimated odds ratios (ORs) using multivariable logistic regression analyses, where variables with p<0.1 from the univariate analyses were included in the initial model. A backward stepwise variable–selection strategy was used to construct a final model with a significance level of p<0.05. The Hosmer and Lemeshow test and the residual χ² goodness-of-fit test were used to assess model stability. Microsoft Excel (Microsoft, Redmond, WA, USA), Epi Info (Centers for Disease Control and Prevention, Atlanta, GA, USA), and

Table 1. Environmental sites in LBMs contaminated by influenza virus A (H5N1) as detected by RT-PCR and virus isolation, Indonesia, 2007–2008*.

<table>
<thead>
<tr>
<th>Site no.</th>
<th>Environmental site</th>
<th>RT-PCR–positive/markets tested (%), N = 1,862</th>
<th>VI–positive/RT-PCR positive, n = 280</th>
<th>LBMs positive for zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delivery</td>
<td>1 Inside cages on truck</td>
<td>6/45 (13.3)</td>
<td>1/6</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>2 Floor in delivery area</td>
<td>6/49 (12.2)</td>
<td>0/6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 Water run-off in delivery area</td>
<td>4/38 (10.5)</td>
<td>0/4</td>
<td></td>
</tr>
<tr>
<td>Holding</td>
<td>4 Poultry cage floors</td>
<td>6/79 (7.6)</td>
<td>0/6</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>5 Holding area floor</td>
<td>8/80 (10)</td>
<td>1/8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 Water run-off</td>
<td>11/72 (15.3)</td>
<td>0/11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7 Poultry feeding bottle water</td>
<td>8/67 (11.9)</td>
<td>0/8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8 Poultry feeding basket food</td>
<td>6/72 (8.3)</td>
<td>0/6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9 Handles to poultry cages</td>
<td>9/79 (11.4)</td>
<td>0/9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 Inside of waste bins</td>
<td>10/59 (16.9)</td>
<td>0/10</td>
<td></td>
</tr>
<tr>
<td>Slaughter</td>
<td>11 Handles of knives used for slaughtering</td>
<td>8/75 (10.7)</td>
<td>1/8</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>12 Basket holding dying chickens</td>
<td>8/71 (11.3)</td>
<td>2/8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13 Floor in slaughter area</td>
<td>10/77 (13)</td>
<td>0/10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14 Chopping or slaughtering board</td>
<td>14/71 (19.7)</td>
<td>2/14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15 Processing table after de-feathering</td>
<td>15/70 (21.4)</td>
<td>0/15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16 Baskets holding poultry meat</td>
<td>14/70 (20)</td>
<td>1/14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>17 Drain path</td>
<td>12/75 (16)</td>
<td>0/12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18 Tap handles in slaughter area</td>
<td>7/65 (10.8)</td>
<td>0/7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>19 Waste bin</td>
<td>13/71 (18.3)</td>
<td>1/13</td>
<td></td>
</tr>
<tr>
<td>Sale</td>
<td>20 Chopping boards</td>
<td>15/80 (18.8)</td>
<td>1/15</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>21 Scales</td>
<td>12/57 (21.1)</td>
<td>0/12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>22 Knife handles</td>
<td>12/78 (15.4)</td>
<td>1/12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>23 Waste bins</td>
<td>10/60 (16.7)</td>
<td>1/10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>24 Wet cloths for cleaning surfaces</td>
<td>14/78 (17.9)</td>
<td>0/14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25 Tables for poultry display</td>
<td>19/80 (23.8)</td>
<td>0/19</td>
<td></td>
</tr>
<tr>
<td>Waste disposal</td>
<td>26 Area waste-disposal bin</td>
<td>15/78 (19.2)</td>
<td>1/15</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>27 Wet cleaning mops</td>
<td>8/86 (12.1)</td>
<td>0/8</td>
<td></td>
</tr>
<tr>
<td>Total positive</td>
<td>280 (15)</td>
<td>13 (4.6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*LBM, live-bird market; RT-PCR, reverse transcription–PCR; VI, virus isolation.
Stata version 10.0 (StataCorp, College Station, TX, USA) were used for the descriptive and statistical analyses.

Approval for the study was obtained from the Health Research Ethics Committee at the Indonesian Ministry of Health and the Australian National University Human Research Ethics Committee. Permission was obtained from LBM managers before participation in the study.

Results

LBM Demographics and Practices

All 83 LBMs selected participated in the study; 62 (75%) were located in urban and 21 in rural areas. LBMs were from 16 districts in 3 provinces: 31 (38%) from Jakarta province, 11 (13%) from Banten province, and 41 (49%) from West Java province (Figure). Most (49 [59%]) LBMs were retail markets, 10 (12%) were wholesale only, and 24 (29%) were a combination of retail and wholesale. Most (82 [99%]) LBMs operated daily, with the same vendors operating in the same stalls.

Most LBMs received their poultry from commercial farms (71 [86%]), and some also sourced poultry from small-scale holders (36 [43%]). Most (42 [51%]) LBMs had medium-sized poultry areas (11–50 poultry cages), and 21 (25%) had large poultry areas (>50 cages). LBMs had village free-ranging chickens (69 [83%]), fighting cocks (13 [16%]), broilers (67 [81%]), spent hens (24 [29%]), Muscovy ducks (48 [58%]), ducks other than Muscovy (32 [39%]), and pigeons (16 [19%]). Most (71 [86%]) LBMs generally kept live poultry in the market for a few days until sold, housing them overnight in cages.

Forty-eight (58%) LBMs reported monthly or more frequent visits from animal/human health personnel to inspect the poultry zones. Eight (10%) LBMs reported that live birds were tested periodically (less frequently than weekly) for AIV infection. For cleaning and sanitation, 80 (96%) LBMs reported washing poultry zones daily, and 55 (66%) applied detergent or disinfectant daily.

Laboratory Findings

Thirty-nine (47%) LBMs had evidence of contamination. For 17 (44%) of these, ≤5 environmental sites were positive for AIV (H5N1) by real-time RT-PCR. For each of 22 (56%) LBMs, ≥6 environmental sites were positive.

The environmental sites most heavily contaminated were in the slaughter and sale zones (Table 1). In the slaughter zone, the most contaminated sites were the poultry-processing tables (21%), baskets holding poultry meat (20%), and chopping boards (20%). In the sale zone, the most contaminated sites were the tables for carcass display (24%) and scales (21%). Another commonly contaminated site was the waste-disposal bin in the waste-disposal zone (19%). In most cases, this bin is not an enclosed bin but rather was a dedicated uncovered floor space where remnants are dumped daily and collected weekly by the local government rubbish collection team.

Thirteen viruses were isolated from LBMs, most frequently from the slaughter zone (7 of 13 viruses isolated, Table 1). All isolated viruses came from 6 LBMs, from which 1–4 viruses were isolated per LBM.

From the zones contaminated in each LBM (Table 1), we calculated correlations between different zones. Contamination in preceding LBM poultry zones correlated with contamination in the subsequent zones (Table 2). Correlations were high between holding and slaughter zones, slaughter and sale zones, and waste disposal zones.

Risk Factors for Contamination

We assessed risk factors for AIV (H5N1) contamination in LBMs. We compared exposures in 39 LBMs with a minimum of 1 contaminated environmental site to 44 LBMs with no contamination. From the univariate analyses, several exposures predicted AIV (H5N1) contamination in LBMs (Table 3). LBMs with wooden tables, Muscovy ducks, or ≥200 ducks other than Muscovy were at greater risk for AIV (H5N1) contamination, as were LBMs in West Java province.

Six other exposures approached significance, either as protective factors or as risk factors. LBMs that disposed and removed solid waste daily (OR 0.41, 95% confidence interval [CI] 0.16–1.09); had zoning that clearly segregated poultry delivery, holding, slaughter, sale, and waste disposal areas (OR 0.28, 95% CI 0.96–9.81); had stacked poultry cages vertically rather than side by side (OR 0.38, 95% CI 0.13–1.10) had less risk for avian influenza virus (H5N1) contamination. LBMs with pigeons (OR 3.06, 95% CI 0.96–9.81), mixed bird species in the same cages (OR 2.92, 95% CI 0.98–8.70), or slaughtered birds in the market (OR 3.53, 95% CI 0.89–13.93) were more likely to be contaminated.

None of the 9 other variables considered in the study were associated with AIV (H5N1) contamination in LBMs (data not shown). These included the LBM trading category (wholesale, retail, or combination), days operational per week, chicken population in LBM, source of chickens (small-scale backyard farmers, commercial farms, or commercial farms).
Sampling for Avian Influenza

From the univariate analyses, 10 variables were significant at p<0.1. However, the ducks other than Muscovy variable was removed from the multivariate analyses because of its collinearity with another variable (presence of Muscovy ducks, r>0.4). Nine variables were considered for the multivariate analyses. The final multivariable logistic regression model had 4 variables, of which 2 were independent risk factors for subtype H5N1 contamination in LBMs (Table 3). They were location in West Java province (adjusted OR [aOR] 6.83, 95% CI 2.01–23.19) and bird slaughtering in the LBM (aOR 6.43, 95% CI 1.01–40.82). Two variables were independent protective factors: zoning of poultry activities in LBMs (aOR 0.16, 95% CI 0.03–0.86) and daily disposal of solid waste (aOR 0.2, CI 95% 0.06–0.69).

Discussion

We have demonstrated extensive environmental contamination in LBMs with the AIV (H5N1) in Indonesia. Nearly 50% of LBMs in AIV (H5N1)—endemic districts were positive, with all 5 poultry zones affected. The study identified environmental points of contamination and protective and risk factors for contamination. This study provides baseline information for 2 aspects that can aid in control of AIV (H5N1) in LBMs: 1) development of routine monitoring and surveillance programs and 2) structural interventions and work flow modifications to minimize risk for contamination.

Our findings provide further evidence that environmental contamination with AIVs is not uncommon (8,14). Poultry water, drains, tabletops, cages, tablecloths, utensils, bins, and floors were all contaminated. Environmental sites most commonly contaminated were located in slaughter zones and zones where carcasses were taken after slaughtering, such as the sale and waste-disposal zones. This contamination can be expected because slaughtering generates droplets that may contain viral particles and exposes internal organs with potentially high viral loads. Even if slaughtering is conducted in a separate zone, contamination can spread to the sale and waste-disposal zone through the carcasses and through the process of evisceration usually conducted in both slaughter and sale stalls.

We found rates of contamination in water from poultry feeding bottles similar to those from the study in Hong

<table>
<thead>
<tr>
<th>Exposure</th>
<th>No. positive markets, n = 39</th>
<th>No. negative markets, n = 44</th>
<th>OR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ducks other than Muscovy in LBM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;11</td>
<td>8</td>
<td>11</td>
<td>Reference group</td>
<td></td>
</tr>
<tr>
<td>11–100</td>
<td>12</td>
<td>16</td>
<td>1.03 (0.32–3.35)</td>
<td>0.959</td>
</tr>
<tr>
<td>101–200</td>
<td>2</td>
<td>2</td>
<td>4.13 (0.16–11.95)</td>
<td>0.773</td>
</tr>
<tr>
<td>&gt;200</td>
<td>10</td>
<td>2</td>
<td>6.88 (1.17–40.38)</td>
<td>0.033</td>
</tr>
<tr>
<td>Muscovy ducks</td>
<td>28</td>
<td>20</td>
<td>3.05 (1.22–7.63)</td>
<td>0.017</td>
</tr>
<tr>
<td>Pigeons</td>
<td>11</td>
<td>5</td>
<td>3.06 (0.96–9.81)</td>
<td>0.059</td>
</tr>
<tr>
<td>Clear zoning in LBM</td>
<td>3</td>
<td>10</td>
<td>0.28 (0.07–1.11)</td>
<td>0.072</td>
</tr>
<tr>
<td>Wooden tables</td>
<td>23</td>
<td>34</td>
<td>3.83 (1.53–9.62)</td>
<td>0.004</td>
</tr>
<tr>
<td>Slaughtering in LBM</td>
<td>36</td>
<td>34</td>
<td>3.53 (0.89–13.93)</td>
<td>0.072</td>
</tr>
<tr>
<td>Daily solid waste disposal</td>
<td>24</td>
<td>35</td>
<td>0.41 (0.16–1.09)</td>
<td>0.075</td>
</tr>
<tr>
<td>Mixing of species in same cage</td>
<td>13</td>
<td>6</td>
<td>2.92 (0.98–8.70)</td>
<td>0.055</td>
</tr>
<tr>
<td>Cages stacked vertically</td>
<td>25</td>
<td>33</td>
<td>0.38 (0.13–1.10)</td>
<td>0.069</td>
</tr>
<tr>
<td>Province</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jakarta</td>
<td>23</td>
<td>8</td>
<td>Reference group</td>
<td></td>
</tr>
<tr>
<td>West Java</td>
<td>25</td>
<td>16</td>
<td>4.49 (1.62–12.46)</td>
<td>0.004</td>
</tr>
<tr>
<td>Banten</td>
<td>6</td>
<td>5</td>
<td>3.45 (0.82–14.47)</td>
<td>0.090</td>
</tr>
</tbody>
</table>

Multivariable analysis†

<table>
<thead>
<tr>
<th>Exposure</th>
<th>OR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear zoning in LBM</td>
<td>0.16 (0.03–0.86)†</td>
<td>0.030</td>
</tr>
<tr>
<td>Slaughtering in LBM</td>
<td>6.43 (1.01–40.82)†</td>
<td>0.048</td>
</tr>
<tr>
<td>Daily solid waste disposal</td>
<td>0.20 (0.06–0.69)‡</td>
<td>0.010</td>
</tr>
<tr>
<td>Province</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jakarta</td>
<td>Reference group</td>
<td></td>
</tr>
<tr>
<td>West Java</td>
<td>6.83 (2.01–23.19)†</td>
<td>0.002</td>
</tr>
<tr>
<td>Banten</td>
<td>2.94 (0.59–14.69)†</td>
<td>0.190</td>
</tr>
</tbody>
</table>

*LBMs, live-bird markets; AI, avian influenza; OR, odds ratio; CI, confidence interval.
†Final model with 4 variables, no. observations = 83, goodness-of-fit tests: residual $\chi^2$, p = 0.38; Hosmer and Lemeshow test, p = 0.45.
‡Adjusted OR.
Kong on AIV (H9N2) (11% and 7% markets with contamination respectively, \( p = 0.12 \)) (11). Even though AIVs were detected from poultry drinking water, our study suggests that other environmental sites are more efficient for monitoring AIV (H5N1) in markets. Processing tables and baskets holding freshly cut poultry meat in the slaughter area, as well as display tables and scales in the sale area, were positive in 20 (24%) LBMs surveyed.

The risk and protective factors we identified complement findings from previous studies. Daily disposal and removal of waste from the market is part of routine environmental cleaning and sanitation and eliminates AIV reservoirs (8). Segregating poultry-related activities into zones limits virus spread (17). Vertical stacking of cages can limit transmission because trays between layers of birds prevent the scatter of fecal matter. These results add evidence to the World Health Organization current recommendation that waste trays should be used to segregate stacked cages in markets to prevent cross-contamination (13).

LBMs in West Java province had a higher risk for contamination than did other provinces. This risk probably is due to greater AIV (H5N1) disease activity in the province. Surveillance activities during 2006–2008 showed that West Java had a 4.7% outbreak detection rate compared with rates in Banten (4%) and Jakarta (0.2%) (18). Furthermore, in West Java province chicken density is high: 14,000 birds/km\(^2\) compared with densities in the neighboring provinces Banten and Jakarta (3,900 birds/km\(^2\) and 400 birds/km\(^2\), respectively) (19). Poultry density data are commonly used as a proxy for disease activity where areas of high poultry density have the highest risk for an outbreak (20,21).

Several issues need to be considered regarding our finding of low virus isolation rates compared with real-time RT-PCR–positive rates. Virus isolation detects viable virus, whereas real-time RT-PCR detects small stretches of nucleic acid, even if the larger genomic RNA is inactivated. This makes real-time RT-PCR a more sensitive detection tool but does not provide information about virus viability. Samples obtained from the environment may be less suitable than animal samples for virus isolation techniques. Organic matter, duration and temperature of exposure, and humidity can all affect virus survival outside the animal host (22). Three studies conducted in LBMs tested environmental samples and bird samples by using virus isolation (8,10,23). Only 1 of these studies stratified the avian influenza detection rates by type of sample (bird vs. environment) (8); that study found that from 12 LBMs, 11 were positive for avian influenza in bird samples compared with only 5 positive in environmental samples. These results were based on a small sample of LBMs, and real-time RT-PCR was not conducted. Therefore, to determine the suitability of virus isolation for environmental samples, we recommend that future studies compare real-time RT-PCR–positive rates to virus isolation rates in both environmental swab and bird samples.

Risk and protective factors identified in this study, together with findings from other studies, can assist in developing environmental or behavioral interventions to reduce AIV transmission in LBMs. Previous studies have shown that regular cleaning with detergents, including free chlorine concentrations typically used in drinking water treatment, can rapidly decontaminate surfaces from AIVs (8,24). Previous studies also have shown that periodic market rest days coupled with thorough cleaning can minimize the reservoir of AIV in LBMs (4,12,25). These messages have been disseminated to LBMs throughout Indonesia and formed the basis of the Ministry of Health Decree in 2008 on building healthy food markets (26).

For a more systematic food safety monitoring system, this study will be used to develop a risk-based approach for AIV risk reduction in LBMs in Indonesia (27). The contamination sites and risk factors will be used to determine critical control points and critical limits for intervention. LBM operators, stall vendors, and other stakeholders (e.g., sanitarians and public health officers) will need to be provided with simple monitoring plans to reduce the risk for contamination. Such monitoring plans are expected to have an impact not only on AIV (H5N1) but also on other viruses and bacteria commonly associated with food safety for poultry products.

In addition to tools for disease control, the study findings can aid AIV (H5N1) surveillance activities in LBMs. Commonly contaminated environmental sites in LBMs can form the basis of an environmental sampling strategy for detection of AIV (H5N1) in LBMs. Environmental sampling is more beneficial than live-bird sampling because it is less time and labor intensive and eliminates the need to handle and restrain live birds. Environmental sampling reduces the potential for virus aerosolization and the risk for infection for persons collecting the samples or standing nearby. Further work is needed to assess the adequacy of environmental sampling for surveillance in LBMs under different conditions, especially because detection sensitivity will vary by AIV (H5N1) prevalence in farms supplying the birds.

A limitation of this study is that the observation of environmental contamination was based on a cross-sectional survey in which LBMs were sampled only once. We recommend that future studies observe persistence of the virus over time in the various environmental sites. Reports from market managers and vendors about inspection and cleaning practices in the LBMs were not verified during the course of the study. These activities may have been overreported because respondents may have wanted to report what they perceived interviewers wanted to hear. Be-
cause of the high cost associated with the field and laboratory work for such studies, studies should focus on a small number of markets and collect in-depth information about contamination trends and associated risk factors, as well as data on other indicator organisms, such as Escherichia coli or Enterobacteriaceae, that provide information about general market hygiene. Future work also should evaluate the effects of interventions in markets especially in low-resource settings because this would be of most benefit to low-income and middle-income countries.

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References


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