Avian Influenza Surveillance in Nomadic Duck Flocks in Subang Indonesia

Etih Sudarnika\textsuperscript{1,\textcopyright}, Yusuf Ridwan\textsuperscript{b}, Abdul Zahid Ilyas\textsuperscript{1c}, Chaerul Basri\textsuperscript{1d}, Denny Widaya Lukman\textsuperscript{1c}, Ardlasunu Wicaksono\textsuperscript{1f}, Agus Sugama\textsuperscript{2g}, Patrick Hermans\textsuperscript{3h}, Arend Jan Nell\textsuperscript{4i}

\textsuperscript{1}Department of Animal Infectious Diseases, Faculty of Veterinary Medicine, Bogor Agricultural University
\textsuperscript{2}District Livestock Services, Subang District, West Java, Indonesia
\textsuperscript{3}Central Veterinary Institute, part of Wageningen University Research Centre, Lelystad, The Netherlands
\textsuperscript{4}Centre for Development Innovation, Wageningen University and Research Centre, Wageningen, The Netherlands

\textsuperscript{\textcopyright}Corresponding author:etih.sudarnika@gmail.com
\textsuperscript{b}yusufridwan67@yahoo.com
\textsuperscript{c}abdulzahid_ilyas@yahoo.com
\textsuperscript{d}chaerulbasri@gmail.com
\textsuperscript{e}dennylukman@hotmail.com
\textsuperscript{f}vetsunuedu@gmail.com
\textsuperscript{g}sugama.agus@yahoo.com
\textsuperscript{h}patrick.hermans@wur.nl
\textsuperscript{i}arendjan.Nell@wur.nl

Abstract. A surveillance were conducted to to estimate the prevalence of HPAI in nomadic duck flocks, to investigate the potential role of nomadic duck to harbour and transmit HPAI, and to describe the movements of nomadic duck flocks into, within and out of the sub-district during rice harvesting periods. Surveillance was conducted in fifty duck flocks and took place over two time periods: from April until June 2010 (period I) and from November 2010 until January 2011 (period II). Duck flocks were sampled by taking tracheal swabs, cloacal swabs and blood samples. Swab samples were analyzed with a M-PCR and a H5 PCR, and serum samples were was analyzed for antibodies directed against Influenza A using an ELISA test and for antibodies against H5 antigen using a HI test. Surveillance showed that 15\% during the first period and 6\% during the second period had antibody titres against H5 antigen. M-PCR test showed the positive result in 42\% of the duck flocks in the first period and 60\% in the second period. Movement of the duck flocks was confined within Subang District or to or from a neighbouring district and rarely moved to or from outside of West Java. A role of nomadic duck flocks in the epidemiology of HPAI cannot be confirmed. The presence of other Influenza A viruses in ducks is noteworthy although not totally unexpected and warrants further investigations.

Keywords: avian influenza, nomadic duck, surveillance

1. INTRODUCTION

Highly Pathogenic Avian Influenza (HPAI) continues to pose a considerable disease burden for the poultry industry in Indonesia. However, in the absence of adequate surveillance programs, reliable measures on the frequency of HPAI occurrence within the different poultry sectors are lacking. Nomadic duck was seen as of paramount importance because of the apparent role of nomadic duck flocks in the epidemiology of HPAI which was observed elsewhere\textsuperscript{1,2}.

Avian influenza surveillance in nomadic duck flocks were conducted to estimate the prevalence of HPAI in nomadic duck flocks, to investigate the potential role of nomadic duck to harbour and transmit HPAI, and to describe the movements of nomadic duck flocks into, within and out of the sub-district during rice harvesting periods.

2. METHODS

Surveillance was conducted in fifty duck flocks and took place over two separate time periods which coincided with the rice harvests: from the 15th of April 2010 until the 30th of June 2010 (period I) and from the 23rd of November 2010 until the 23rd of January 2011 (period II).
The sampling unit was the duck flock. A group of ducks was considered a flock if it arrived at and departed from a rice field as one group. The number of samples taken from a nomadic duck flock was based on the ability to detect a positive sample in a flock with a minimum expected prevalence of 5%, a test sensitivity of 95%, a test specificity of 100% and a 95% confidence level. Calculated sample sizes were dependent on flock size and ranged from a minimum of 10 samples (flock size equals 10) to a maximum of 60 samples (flock size equal to and larger than 400).

Nomadic duck flocks which were present within Cipunagara at the start of the surveillance period were sampled as soon as possible after the start of the study. Thereafter, all flocks were sampled which entered the subdistrict. From each selected animal, a tracheal swab, cloacal swabs and a blood samples was collected. Swab samples were analyzed with an M-PCR and a H5 PCR, and serum samples were was analyzed for antibodies directed against Influenza a using an ELISA test and for antibodies against H5 antigen using a HI test.

Questionnaires were used to collect additional data. Respondents were farm owners or workers. All questionnaires were administered by trained enumerators. Information pertaining to nomadic duck flocks, included flock characteristics such as flock size, breed, age, production type (i.e. egg, meat, dual purpose), movement records (where from, where to, date of movement, method of movement) and history of vaccination.

Data collected by questionnaire was entered directly into a Microsoft Access database and analyzed descriptively by generating frequency distributions of variables of interest. Laboratory results were entered into an Excel spreadsheet.

3. RESULTS AND DISCUSSION

Origin and Characteristic of the Duck Flocks

All the duck flocks which were sampled during the two surveillance periods were based in West Java Province when they were not moving around. The majority of the flocks during both surveillance periods were based in Subang district followed by Indramayu district.

The majority of duck flocks were raised for the purpose of egg production whereas only a few flocks were raised for meat. All the duck flocks were confined in cages or pens at night and the majority of these flocks (n=44; 88%), were confined on the rice fields which they grazed during the day. The remaining six flocks would be taken to a location away from the rice fields.

In general, the farmers did not vaccinate their duck flocks against any diseases. During the first surveillance period only one flock had been vaccinated against AI and ND and this had happened more than one year ago. During the second surveillance period, two duck flocks had been vaccinated against AI of which one flock was vaccinated during the last three months and of which one flock the date of vaccination was unknown.

![FIGURE 1.Districts of origin of nomadic duck flocks sampled during two surveillance periods in Cipunagara, Subang](image-url)
Movement of Nomadic Duck Flocks

Forty-three flocks left Cipunagara during surveillance period 1 after staying in Cipunagara for an average of 23 days (range 5 - 90 days) of which 26 flocks remained in Subang district, 15 flocks moved to the neighbouring district of Indramayu and for two flocks the destination was unknown. During the second surveillance period, 44 flocks planned to leave Cipunagara after staying an average of 19 days (range 2 - 90 days). Twenty-seven of these flocks remained in Subang, four flocks moved to Indramayu, one flock to Cirebon and six flocks planned to move to an unknown destination. The most common method for transporting the duck flocks into, within or out of Cipunagara was by pick-up truck.

Laboratory Analysis

The results of the M-PCR analysis of the pooled swab samples taken from nomadic duck flocks are shown in Table 1. During the first surveillance period, 21 (42%) nomadic duck flocks that were present in or entered Cipunagara had at least one pooled sample test positive for Influenza A. Whereas 2 (22%) flocks which were sampled when they left the district had one or more positive pooled samples. One of these two flocks tested negative at the first sampling and thus appears to have become positive during its stay in Cipunagara. During surveillance period 2, 30 (60%) flocks were found positive. The infection was more frequently detected in tracheal swab samples during the first surveillance period whereas in the second surveillance period pooled samples of cloacal swabs where more frequently positive. None of the samples which tested positive with the M-PCR gave a positive test result with the H5 PCR.

<table>
<thead>
<tr>
<th>Pooled swab samples</th>
<th>No. of positive pooled samples (%)</th>
<th>No. of positive flocks (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surveillance period 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tracheal swabs only</td>
<td>32 (6.3%)</td>
<td>5 (10.0%)</td>
</tr>
<tr>
<td>Cloacal swabs only</td>
<td>25 (4.9%)</td>
<td>8 (16.0%)</td>
</tr>
<tr>
<td>Both tracheal and cloacal swabs</td>
<td>7 (1.4%)</td>
<td>8 (16.0%)</td>
</tr>
<tr>
<td>Total</td>
<td>71 (14%)</td>
<td>21 (42.0%)</td>
</tr>
<tr>
<td>Surveillance period 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tracheal swabs only</td>
<td>27 (5.1%)</td>
<td>6 (12.0)</td>
</tr>
<tr>
<td>Cloacal swabs only</td>
<td>45 (8.6%)</td>
<td>9 (18.0%)</td>
</tr>
<tr>
<td>Both tracheal and cloacal swabs</td>
<td>28 (5.3%)</td>
<td>15 (30.0%)</td>
</tr>
<tr>
<td>Total</td>
<td>128 (24.4%)</td>
<td>30 (60.0%)</td>
</tr>
</tbody>
</table>

Serological test results of duck serum are presented in Table 2. The sero-prevalence of antibodies against Influenza A as measured by an Elisa test was above 80% during both surveillance periods resulting in a flock prevalence of 89.1% and 100% during the first and second surveillance period respectively. The analysis for H5 antibodies with the HI test resulted in a sero-prevalence of 1.7 % and 0.6% for first and second surveillance periods respectively. This meant that 15.2% of the flocks in the first surveillance period and 6% of the flocks in the second surveillance period had one or more samples with antibodies against H5. A test to determine if cross-reactions against the neuraminidase protein were occurring was not carried out.

<table>
<thead>
<tr>
<th>Surveillance</th>
<th>No. of positive samples and flocks</th>
<th>Elisa Test</th>
<th>HI Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period 1</td>
<td>No. of positive samples (%)</td>
<td>508 (84.8%)</td>
<td>10 (1.7%)</td>
</tr>
<tr>
<td></td>
<td>No. of positive flocks (%)</td>
<td>41 (89.1%)</td>
<td>7 (15.2%)</td>
</tr>
<tr>
<td>Period 2</td>
<td>No. of positive samples (%)</td>
<td>428 (85.6%)</td>
<td>3 (0.6%)</td>
</tr>
<tr>
<td></td>
<td>No. of positive flocks</td>
<td>50 (100%)</td>
<td>3 (6%)</td>
</tr>
</tbody>
</table>
CONCLUSIONS

There is no evidence that nomadic duck flocks play a role in the epidemiology of HPAI in Cipunagara. However, longitudinal surveillance studies carried out over extended periods of time are needed to confirm this. There is evidence for the presence of other Influenza A virus in nomadic duck flocks. Further virus isolation and sequencing is required to elucidate the nature of these viruses. Nomadic duck flocks movements travelled relatively short distances seem to play only a limited role in virus transmission over large distances.

ACKNOWLEDGEMENTS

We thank Indonesian–Dutch Partnership Program on Highly Pathogenic Avian Influenza Control for funding this activity. We also thank all the poultry farmers, field coordinators, enumerators, Subang Livestock Services, students of IPB and Utrecht University, the staff of IPB and Cikole Provincial Laboratory.

References