Penelitian

Seroprevalence and Detection of H5N1 Avian Influenza Virus in Local Chickens in Tabanan Regency, Bali, Indonesia

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ABSTRACT

Avian Influenza (AI) is a zoonotic disease that causes death in poultry and humans. Monitoring the virus needs to be carried out continuously to prevent outbreaks of the disease. Seroprevalence and detection of H5N1 and H9N2 AI virus antigen were intended to monitor the presence of viruses in local chickens in Tabanan, a Regency of the Indonesian island Province of Bali. The research aims were to detect the presence of H5N1 AI virus, and measure the seroprevalence of this virus in Tabanan Regency of Bali Province. Research located in six districts of Tabanan regency namely Baturiti, Penebel, Marga, Kediri, Tabanan and Kerambitan. A total of 1,398 local chickens that never been vaccinated with AI were randomly sampled in this study. The samples collected were serum, cloacal and tracheal swabs. Serum samples were tested with hemagglutination inhibition (HI) assay. While samples of cloacal and tracheal swabs were isolated in 9-day-old germinated chicken eggs, followed by hemagglutination assay and RT-PCR test using H5N1 primer. AI seroprevalence in local chickens in Tabanan Regency was 1% with the distribution in each district as follows; Penebel 1.6%, Kerambitan 1.2%, Marga 1%, while Tabanan, Kediri, and Baturiti 0.7% each. H5N1 AI virus was detected in 11 samples, i.e. five in Marga district and three in Penebel district, two in Kediri, and one in Tabanan, while the H9N2 AI virus was not detected. These results indicate that H5N1 AI virus may still circulate in local chickens in Tabanan Regency of Bali Province, with 1% of prevalence.

Keywords: Avian influenza, Bali, H5N1, local chicken, seroprevalence

ABSTRAK


Kata kunci: Avian influenza, Bali, H5N1, ayam kampung, seroprevalensi
INTRODUCTION
Long-term and ongoing monitoring of avian influenza (AI) viruses is needed (Jonas et al., 2018; Li et al., 2004; Machalaba et al., 2015). Since 2002, the AI virus has spread to almost all parts of the world (Alexander, 2007; Chen et al., 2005; Ellis et al., 2004; Pantin-Jackwood and Swayne, 2007; Sturm-Ramirez et al., 2004) including Indonesia (Kandun et al., 2006). AI viruses are known to be endemic in some wild birds and infect domestic birds (Capua and Alexander, 2006). In Indonesia, this disease is classified as one of some infectious diseases in animals that is prioritized to be controlled (Santhia et al., 2009).

The H5N1 of AI virus epidemic in Indonesia began on Java in August 2003 which attacked domestic and commercial chickens (Santhia et al., 2009; Wiyono et al., 2004). Then the outbreak occurred in the Province of Bali began in October 2003 (Santhia et al., 2009). This case was first reported in Karangasem Regency, which was allegedly due to the entry of sick birds from Java. The same outbreak also occurred in Tabanan Regency and then spread rapidly to other districts in the island (Santhia et al., 2009). From October 2003 to September 2004, the highest percentage of AI infected villages in Bali was found in Bangli and Jembrana districts at 39.1% and 29.4% respectively. While the average outbreak rate was 20.4%, where the highest in Tabanan and Karangasem districts at 48.4% and 30.2% respectively (Santhia and Putra, 2004). More detail, Mahardika et al. (2018) reported that all districts in Bali Province have been infected with AI H5N1. AI antibodies as an indication of poultry have been infected with AI viruses detected from local chickens, ducks, thugs, geese and pigeons. It was also reported that seroprevalence of H5N1 AI virus infection in each district in Bali varied from 1.23% to 6.09% with the proportion of seroprevalence in local chickens (2.69%), waterfowl (9%), and various other poultry (8.06%).

AI outbreaks in Tabanan Regency caused fatalities and economic losses. In 2007, a resident who worked as a collector of poultry from Kediri District died because of AI infection (Lestari, 2009). In addition, due to the AI outbreak in 2012 hundreds of chickens died suddenly in Marga Subdistrict (Kaminsiah et al., 2014). The AI-H5N1 virus is very detrimental, including the reduced number of breeders, decreased income of poultry farmers, decreased supply, import and export of DOC for both broiler and layer, and the price of input and output of the poultry business.

As a result of the AI outbreak in Indonesia since 2004 ± 2008 caused loss of Rp. 4.3 trillion, excluding losses from lost job opportunities and reduced public protein consumption. FAO estimated that there were AI virus mutations in Indonesia that may cause a pandemic (Basuno, 2008).

Various factors are known associated with the H5N1 AI virus to be sustained in Tabanan Regency, namely poultry trade traffic between regions, buying and selling of poultry in traditional markets, unhygienic processing of poultry meat and the habit of people throwing dead chicken carcasses into rivers (Sartha et al., 2010). Seroprevalence and AI antigen detection study in local chickens in Tabanan are still limited, therefore the study of AI on household scale farms in the area is needed.

MATERIALS AND METHODS

Ethical Clearance
This research was approved by the Ethical Commission for the Use of Animals in Research and Education of the Faculty of Veterinary Medicine, Udayana University, Indonesia with Ref. No. 0034a/UN14.2.9/PD/2019.

Sample
The research samples were taken randomly from the Tabanan Regency of the Bali Province (Table 1). Sampling locations in six districts namely Kediri, Penebel, Baturiti, Marga, Kerambitan and Tabanan. Eight villages from each subdistrict were sampled, 3 sub-villages from each village, and from each sub-village 7-10 local chickens were sampled (Thrusfield, 2007). Samples consisted of serums, cloaca and tracheal swabs from the sampled chickens that were free to roam and had never vaccinated against AI virus (Sarker et al., 2017). A total of 1,398 samples used in this research.

The sampling location was determined based on the purposive sampling method. Meanwhile, in taking the sample used the Stratified Random Sampling method based on the data from reported by the Bali Livestock Service stated that the most cases of avian influenza occurred in Tabanan Regency, as many as 34 sub-village in 29 villages had contracted the virus (Lestari, 2009). Nine villages were selected in each district, and every village selected 3 sub-villages. Each sub-village sampled 10 chickens from the residents. The residents who being sampled must have more than 10 chickens which have not AI vaccinated record and the chickens were allowed to free roam. Three chicken were sampled in

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each resident. Sampling was considering the age of the chickens, which was above 3 months to eliminate the influence of maternal antibodies but ignoring gender, and body weight.

Sample Collection

All samples were taken according to the FAO procedure (FAO, 2014). Blood was drawn through the brachial vein using a 3 ml syringe. The serum was collected by centrifugation at a speed of 10,000 rpm for 5 minutes. The serum samples were stored at -18°C before being tested using Hemagglutination inhibition assay (HI) (Pedersen, 2014). The cloaca and trachea swabs were taken using cotton swabs and directly inserted into the transport media (which contains PBS + Penicillin and Streptomycin). The suspension of the cloaca and tracheal swabs was then made up to 10% inoculum, 5000 IU of Penicillin and 5 µg/ml of Streptomycin added. 0.1 ml of inoculum of each sample was isolated in 10-day-old hatched chicken eggs through the allantois chamber. The eggs were then incubated for 3 days at 37°C, and were observed every day.

All dead eggs were removed from the incubator, then put them into the 4°C refrigerator overnight. Allantois fluid was harvested for tested by a Hemagglutination assay (HA) (Killian, 2014) and confirmed by a molecular test using Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) (FAO, 2014; OIE, 2008).

DNA Isolation

DNA isolation process was done according to the Qiagen® DNeasy KIT. A total of 25 ml samples of each dead egg was extracted.

Table 1 Sample distribution in Tabanan Regency

<table>
<thead>
<tr>
<th>No</th>
<th>District name</th>
<th>Number of villages</th>
<th>Number of sub-villages</th>
<th>Number of samples per sub-village</th>
<th>Total sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Penebel</td>
<td>8</td>
<td>3</td>
<td>10</td>
<td>240</td>
</tr>
<tr>
<td>2</td>
<td>Baturiti</td>
<td>6</td>
<td>4</td>
<td>12</td>
<td>288</td>
</tr>
<tr>
<td>3</td>
<td>Marga</td>
<td>8</td>
<td>3</td>
<td>12</td>
<td>288</td>
</tr>
<tr>
<td>4</td>
<td>Kediri</td>
<td>9</td>
<td>3</td>
<td>10</td>
<td>270</td>
</tr>
<tr>
<td>5</td>
<td>Tabanan</td>
<td>6</td>
<td>3</td>
<td>8</td>
<td>144</td>
</tr>
<tr>
<td>6</td>
<td>Kerambitan</td>
<td>8</td>
<td>3</td>
<td>7</td>
<td>168</td>
</tr>
</tbody>
</table>

Total sample 1,398

Antigen and Primers

Antigen and standard serum for H5N1 and H9N2 AI viruses used in this research were originated from Pusvetma Surabaya. While the primers sequence used were H5-1B: 5'-GCCATCCACACCATACACC-3', H5-3B: 5'-CTCCCCCTGCTCATTGCTATG-3', N1-Fwd: 5'-TAGACTGATGAGGCCTTGCTTTG-3', and N1-Rev: 5'-CACCGTCTGGCCAAGACCAACCTA-3'(Network, 2005).

Polymerase Chain Reaction

The PCR mixture consisted of 2.5 µl (each) deoxynucleoside triphosphates (8 µM), 2.5 µl 10 X PCR buffer, 2.0 µl (1.5 mM) MgCl₂, 0.125 µl PE Amplitaq (5unit/ µl), 1.215 µl of forward and reverse primers (10 mM), 1 µl DNA sample and 14.5 ul dH₂O in a total 25 µl reaction mixture.

Amplification was performed with pre-denaturation condition at 95°C for seven minutes, followed by 39 cycles with the following reaction conditions: dena-turation at 94°C for 45 seconds, annealing at 52°C for 45 seconds, and polymerization at 72°C for one minute. At the end of the polymerase was added at 72°C for seven minutes (Mahardika et al., 2018). All PCR product run in the gel electrophoresis to find positive sample with 200 bp result.

Data Analysis

Data of serology and antigen test results were calculated statistically with SPSS versions 13 using the crosstabulation method, and continued with the Chi Square test (Arkkelin, 2014).
RESULTS AND DISCUSSION

Analysis of hemagglutination inhibition assay results on 1,398 samples of local chicken sera against the AI virus in Tabanan Regency is presented in Table 2. Almost all sampled regions in Tabanan Regency detected antibodies against AI virus in the chickens, and Penebel.

The latest research on AI seroprevalence in Bali found that the seroprevalence of AI virus infection in local chickens in Tabanan Regency was 1.79% averaged from the seroprevalence inMarga District 11.63%, and Penebel 1.59%, while other districts were negative (Mahardika, 2005). The span of nearly 13 years proves that until now Tabanan Regency is still suspected of having AI virus cycle, although the percentage of seroprevalence is lower than in 2005. The decrease in seroprevalence is probably due to the socialization from the government on prevention of AI in poultry to the public through a good poultry raising system is also believed to contribute to the decrease of the infection, as its implemented by the Government in Turkey (Edirne et al., 2011).

Even if the seroprevalence is decreased, when it compared to the previous data (Mahardika, 2005), the AI virus is still suspected spreading out in Tabanan Regency. This is presumably because local free roam chickens can easily come in contact with wild birds, and contaminated their feed by feces or secretions that may contain the virus (Spackman, 2009). Elfidasari et al. (2015) stated that chickens around the Serang Nature Reserve area of Banten were infected with AI virus due to the drinking water in the area was also consumed by wild water birds which may shed the virus in the water.

Pfeiffer et al. (2011) argues that the high frequency of AI virus transmission in several East and Southeast Asian countries is due to the high density of terrestrial and waterfowl populations supported by commercial livestock breeding and trade in poultry, which triggers antigenic drift. The presence of poultry slaughtering facilities in Marga subdistrict was suspected to be an important factor of the spread and propagation of the AI virus. Slaughterhouse owners tend to combine various types of poultry in one place (Suartha et al., 2010). While the slaughter process was also carried out without good biosecurity (Lohiniva et al., 2013). The spread of AI viruses tends to increase during the rainy season due to the migration of wild birds that occur in July to November (Halvorson et al., 1985).

Local chicken antibody titers against AI viruses detected in this study were classified as low (2^2-2^4 HI Unit) and its serologically unprotected against the virus. This can be caused by local chickens that never been vaccinated against AI which lead to the presence of very low antibody titers. Alternatively, it is likely due to natural infections from AI-contaminated environments. In this condition, the chickens will be susceptible to a virulent AI virus, with mortality can be reached up to 100% (Swayne and Suarez, 2000). This antibody variation titers can be influenced by several conditions including the health of chickens, the type and amount of virus that infects, as well as the difference period or the phase of infection when blood samples are taken (Darmawi et al., 2012).

From the swab samples tested for hemagglutination, we found the highest AI seroprevalence was in Marga subdistrict at 2%, Penebel and Tabanan was the same at 1% and other districts were negative (Table 3).

Table 2 Tabulation of seroprevalence antibodies against AI virus based on hemagglutination inhibition assay results from local chicken sera in Tabanan Regency

<table>
<thead>
<tr>
<th>District</th>
<th>Positive</th>
<th>%</th>
<th>Negative</th>
<th>%</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penebel</td>
<td>4</td>
<td>1.6</td>
<td>236</td>
<td>98.4</td>
<td>240</td>
</tr>
<tr>
<td>Tabanan</td>
<td>1</td>
<td>0.7</td>
<td>143</td>
<td>99.3</td>
<td>144</td>
</tr>
<tr>
<td>Kediri</td>
<td>2</td>
<td>0.7</td>
<td>286</td>
<td>99.3</td>
<td>288</td>
</tr>
<tr>
<td>Baturiti</td>
<td>2</td>
<td>0.7</td>
<td>270</td>
<td>99.3</td>
<td>270</td>
</tr>
<tr>
<td>Marga</td>
<td>3</td>
<td>1.0</td>
<td>285</td>
<td>99.0</td>
<td>288</td>
</tr>
<tr>
<td>Kerambitan</td>
<td>2</td>
<td>1.2</td>
<td>166</td>
<td>98.8</td>
<td>168</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>1</td>
<td>1,384</td>
<td>99</td>
<td>1,398</td>
</tr>
</tbody>
</table>
We found that 11 samples were positive of the H5N1 virus based on RT-PCR test (Table 4). These results indicate that the virus spread in Tabanan and it may be in accordance with previous reports where the Avian Influenza H5N1 subtype virus is still circulating in traditional poultry markets and farms (Dharmayanti et al., 2016; Hewajuli et al., 2017; Mahardika et al., 2018) in which it has been detected as AI virus subtype H5N1 clade 2.1.3 and clade 2.3.2 (Kusumastuti et al., 2015). Identification of the Avian Influenza virus using RT-PCR is very important to be conducted to assess the genetic mutations of various viral genomes, especially for them that can cause an annual epidemic or even occasional pandemic (Shao et al., 2017). In addition, the effect of the virus mutation to the pathogenic avian influenza can result in economic losses due to high morbidity and mortality in both poultry and humans (El-Shesheny et al., 2014).

Seroprevalence of AI virus in local chickens in Tabanan found at 1% spread out in all subdistricts in the regency. As much as 11/1,398 of H5N1 avian influenza virus in Tabanan regency observed where it spread out in the four districts, while no sample was detected for H9N2.

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“The authors declare that they have no competing interests”.

### REFERENCES


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