

## **Isolation and Identification Avian Influenza A non-H5 Virus from Muscovy Duck (*Cairina moschata*) at Two Live Bird Markets in Surabaya**

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### **ABSTRACT**

The aim of this study was to isolate and identify Avian Influenza A non-H5 virus from muscovy duck at two live bird markets in Surabaya. Muscovy duck is the natural reservoir of Avian Influenza virus, in which all of the 16 HA subtypes and 9 NA maintained. The Avian Influenza virus replicates in intestinal tract of the reservoirs, causing the high amount of virus shed in the faeces. This study is an observational descriptive study, using non random sampling method of determined samples. The method used in this study were *Hemagglutination Inhibition* (HI) test and *Enzyme Linked Immunosorbent Assay* (ELISA) test. Avian Influenza A non-H5 virus was identified 19.23% (5 samples out of 26) in PS1 and 23.34% (7 samples out of 30) in PS2. This finding shows that Avian Influenza A non-H5 virus could be isolated and identified from muscovy duck at two live bird markets in Surabaya.

**Key words:** Avian Influenza, Muscovy duck, Influenza A non-H5, Live bird markets.

### **INTRODUCTION**

*Avian Influenza* is a viral disease in poultry that is infectious and zoonotic, and can even cause death. *Avian Influenza* is categorized as *Office International des Epizooties* (OIE) in list A, because it spreads rapidly across national borders. *Avian Influenza* outbreak also has an impact on socio-economic, public health and international trade, especially poultry products and their processed products (Alexander, 2000).

Waterfowl such as ducks, enthogs, and geese are natural hosts for the *Avian Influenza* virus, where the influenza A virus is in a balanced state and does not cause disease. Of all the 16 HA

subtypes and 9 NA subtypes present in the waterfowl population, in these birds the virus replicates, especially in the digestive tract, so that viruses with high titer are present in feces. (Mahardika et al, 2005).

The principle of waterfowl as a natural reservoir, the *Avian Influenza* virus is sometimes transmitted to other animals, including mammals and domestic farms causing infection transmission and outbreaks, through adaptation of viral mutations or genetic reassortment (de Jong and Tran, 2006).

Live poultry markets, especially those that are permanent in nature, where unsold poultry

will stay overnight in the market, has been known to be able to strengthen and store the *Avian Influenza* virus in these birds. Virus isolation rates in such markets are generally high compared to those detected in commercial poultry farms, so this market is an ideal setting for *Avian Influenza* virus surveys (Peiris, 2007).

The process of buying and selling live poultry in traditional markets in Indonesia is not much different from markets in neighboring

countries, even if the poultry that is sold is not known about the origin of the husbandry or the health status of the poultry. Live poultry sellers have the habit of mixing multi-species birds in one place, making the spread of the *Avian Influenza* virus easy. This makes traditional markets one of the critical points for the spread of the *Avian Influenza* virus in Indonesia (Jaelani, 2008).

## METHODS

The tools used in this study were bunsen burners, refrigerators, vortex mixers, tweezers, scissors, suction pipettes, pasteur pipettes, autoclaves, sterile microtubes, aluminum foil, beaker glasses, erlenmayer flasks, venoject tubes, petri dishes, 1 ml syringes and needle, test tube rack, centrifuge tube, centrifuge, incubator, egg candler, ice box, shaker, conical tube, microplate "V", microplate ELISA, micropipette multichannel yellow tips, blue tips, gloves and masks, cotton buds and ELISA Reader.

The materials used in this study, 9-11 days of age TAB are SAN (Specific Antibody Negative), H5N1 antigen and antiserum (chicken) and H5N2 antiserum (duck) from the Virology Laboratory, Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Airlangga University, Surabaya. , Streptomycin Sulfate, Penicillin-G, PZ 0.9% or physiological NaCl, chicken erythrocytes 0.5%, sterile aquadest, tepol, alcohol 70% Formaldehyde PA 37%, Etylen Diamine Tetra Acetic Acid (EDTA)

Carbonate Buffer, Washing Buffer , Anti-duck Conjugate, Buffer Substrate.

Samples were randomly selected enthog cloaca swabs. The swab taken can come from an enthog that is anchored at the market or from outside the area, namely faeces in the shipping box in a car or truck. The cloacal swab was performed using a sterile cotton bud and then inserted into a microtube containing PZ containing antibiotics.

### Virus Isolation

Virus isolation begins with the preparation of incubated TAB aged 9-11 days, then all TABs are disinfected with 70% alcohol. The virus suspension resulted from 0.1-0.2 ml cloacal swab is injected with a syringe into the allantoic liquid through a hole that has been made in the air cavity area about 1 cm deep and then covered with a plaster. Eggs were incubated at 37° and observed daily for embryo mortality. Allantoic fluid from eggs whose embryos died were tested with the HA test, if positive, where at the highest dilution it can still agglutinate erythrocytes, i.e. at titer greater than and equal to 24, allantoic fluid is

harvested. Furthermore, virus identification was carried out using the HI test (OIE, 2008). Isolates whose test results were negative were followed by the indirect-ELISA test.

### **Inhibition Test Hemagglutination (HI) Microtechnic**

The steps in the microtechnical HI test, starting with inserting 0.025 ml of PZ into each "V" microplate well, then adding 0.025 ml of AI/H5N1 antiserum into the first hole and homogenizing it, after that transfer 0.025 ml to the second hole and homogenize, continue until the hole. Finally, remove 0.025 ml from the last hole, then add 0.025 ml of the 4 HA unit viral antigen to each hole and leave it for 30 minutes at room temperature. The next step is to add 0.05 ml of 0.5% chicken erythrocyte per hole, shake gently and leave for 30 - 40 minutes at room temperature. HI titer is stated positive if there is agglutination barrier at serum dilution greater than and equal to 1/16 or 2<sup>4</sup> (OIE, 2008).

### **Elisa Indirect Test**

A total of 100 µl 1 HA units of inactivated Avian Influenza virus from the isolates to be examined and the Avian Influenza virus (BL-3) as positive control antigens and negative controls were derived from allantois liquid TAB

which was SAN, attached to each microplate well using carbonate buffer pH 9, 6 and incubated at 4° C for overnight (18 hours). Plate wells were washed three times with washing buffer (NaCl-Tween) and then blocked with 200 µl of buffer blocking, and incubated at 37° C for one hour. Next, the plates were washed and then added with 100 µl of duck antiserum from the vaccination with Avian Influenza H5N2 virus which had been diluted 1:100 with solvent buffer and incubated at 37° C for one hour. As a comparison, a positive control antiserum was used as a result of the H5N2 Avian Influenza virus vaccination and negative controls. After that, washing was done, added with 100 µl of goat anti-duck conjugate labeled with AP enzyme with a dilution of 1/2500 in the solvent buffer at each well, and incubated at the same temperature and time. After incubation, washing was carried out again and added with p-NPP substrate 10 mg p-NPP in 10 ml buffer substrate and added in each well as much as 100 µl and incubated in a dark room at room temperature 37° C for 30 minutes. The infiltration was then read on an ELISA reader with a wavelength of 405 nm (Kick et al., 1993; Rowe et al., 1999).

## **RESULTS AND DISCUSSION**

Table 1. Percentage of Isolation and Identification Results of Cloaca Enthog Swab Samples Sold in Two Live Poultry Markets in Surabaya with HI Test and Elisa Test

Pasar	Total Sampel	HI Positif		HI Negatif		Elisa Positif		Elisa Negatif	
		∑	%	∑	%	∑	%	∑	%
PS1	26	13	50	13	50	5	19,23	8	30,76
PS2	30	14	46,67	16	53,34	7	23,34	9	30
Total	56	27	48,21	29	51,78	12	21,42	17	30,35

Information :

PS1: market 1; PS2: market 2; ∑: total; %: percentage

Table 1. The results showed that 13 of the total 26 samples of PS1 tested positive for HI, so that the percentage of 50% of Avian Influenza H5 virus was obtained, while the results of the HI test were negative as many as 13 samples with a percentage of 50%. Elisa test results for PS1, there were 5 positive samples and 8 negative samples with the respective

percentages of 19.23% and 30.76%. PS2 14 samples from 30 positive samples of Avian Influenza H5 virus with a percentage of 46.67%, and 16 samples of HI negative with a percentage of 53.34%, the elisa test results for PS2 7 samples were positive with a percentage of 23.34% and 9 samples were negative and the percentage is 30%.

Table 2. Percentage of AI / H5, AI / Non-H5, and Non-Influenza Virus Isolates from Cloacal Entothog Swab Samples Sold in Two Live Poultry Markets in Surabaya

Pasar	Total Sampel	AI/ H5		AI/ Non-H5		Non-Influenza	
		$\sum$	%	$\sum$	%	$\sum$	%
PS1	26	13	50	5	19,23	8	30,76
PS2	30	14	46,67	7	23,34	9	30
Total	56	27	48,21	12	21,42	17	30,35

Information :

PS1: market 1; PS2: market 2;  $\sum$ : total; %: percentage

Table 2 shows that PS1 can be isolated and identified as many as 13 samples of the Avian Influenza H5 virus from a total of 26 samples with a percentage of 50%, while the samples showing positive Avian Influenza A non-H5 are 5 of the total 26 samples with a percentage of 19.23%. PS2, there were 14 positive samples of Avian Influenza H5 with a percentage of 46.67% and 7 samples of positive Avian Influenza A non-H5 samples from a total of 30 samples, so the percentage was 23.34%. The non-influenza samples from PS1 were 8 samples with a percentage of 30.76% and PS2 were 9 samples with a percentage of 30%. The total in Surabaya was 48.21%, namely 27 samples from 56 positive samples of Avian Influenza A H5, 12 samples from 56 positive samples of Avian Influenza A Non-H5 viruses with a percentage of 21.42%, and 17 samples

from a total of 56 samples including non-virus Influenza with a percentage of 30.35%.

This study proved that in two live poultry markets in Surabaya, non-H5 Avian Influenza A viruses could be found, with a percentage of 19.23% in PS1 and 23.34% in PS2. Referring to WHO (2004) and OIE (2008), the HPAI virus is not only H5 but also H7, while H9 is a notifiable influenza virus that needs to be monitored. Avian Influenza virus subtype H9 has spread throughout Asia and the Middle East, this virus subtype is LPAI, but if it occurs in combination with other pathogens it can cause severe respiratory disease in livestock (Alexander, 2000).

Isolates whose HI test results showed negative results were continued using the indirect-ELISA test. According to Pourbaksh (1999) specific virus types against antibodies can be detected using the indirect-ELISA test by

detecting the presence of nucleoproteins (NP) and Matrix (M) contained in the influenza A virus envelope, so this test is to determine that those that show negative HI test results include in the influenza A virus. Isolates that show negative results in both the indirect-ELISA and HI tests, means that these isolates are not Avian

## CONCLUSION

Based on the results of the research that has been done, it can be concluded that in the two live poultry markets in Surabaya, 5 samples of Avian Influenza A Non-H5 virus were isolated

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and identified from PS1 with a percentage of 19.23% and 7 samples from PS2 with a percentage 23.34%.

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