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Avian Influenza Virus Subtype H5N1 in Pigeons: A Conventional Serological Detection

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ABSTRACT
Prior to the novel coronavirus, the subtype H5N1 that emerged in Asia in 2003 and hit Indonesia from 2005 to 2010 has created fears with the public. Attention has not only been detections of domestic fowls but pigeons as well. This study aimed to detect the specific antibody to the avian influenza virus of the H5N1 subtype in pigeons. We collected 20 serum samples of unvaccinated pigeons and tested them using the haemagglutination inhibition test (HI-test). This conventional test is considered as a gold standard, with a specific antigen of the avian influenza virus and a one percent solution of chicken red blood cells as a pre-treatment. The results reported of 20 samples have no specific antibody to the H5N1 subtype of avian influenza. Therefore, It can be concluded that none of the pigeons was infected by the H5N1 subtype of avian influenza virus.

Keywords: *Avian influenza, Columbidae, Hemagglutination inhibition test, H5N1 subtype*

1. INTRODUCTION

The role of pigeons (*Columba livia*) as intermediate species and their closeness to humans cause them as a great potential host to spread and to transmit the bird flu virus to other wild animals [1].

The H5N1 is initially spread through wild birds which migrate periodically at every change of seasons [2]. Then, the virus spreads to poultry farms and various species of birds other than poultry. According to the surveillance results, poultries that are positive for Highly Pathogenic Avian Influenza (HPAI) in Indonesia are layers, broilers, native chickens, ducks, wild duck, geese, turkeys, ostriches, quails, pigeons, white peacocks, and turtles [3]. Diagnosis of avian influenza (AI) cases can be carried out by serological examination with haemagglutination inhibition (HI) test to determine antibody titers against the AI viruses [4,5].

In the HI test, the antibody titer is obtained from the binding reaction between the antigen and the antibody [5]. This antigen-antibody binding reaction is used as an indicator of infection or having been infected in poultry [6]. Therefore it is necessary to study the presence of specific antibodies against AI subtype H5N1 in pigeons (*Columba livia*) in Aceh..

2. MATERIALS AND METHODS

2.1. Sample Collection

In this study, 20 pigeons that had never been vaccinated were used. Samples were taken randomly from 10% of the population. Blood is drawn through the *vena brachialis* and *intra-cardiac*.

2.2. Haemagglutination (HA)

This test was carried out to determine the value of 4 HA units that can agglutinate erythrocytes optimally.

2.3. Haemagglutination inhibition (HI)

This test was carried out to determine the antibody titer from the serum of the pigeons after the HA test.

2.4. Back-titration

This test was carried out as same as the HA test [4].

3. RESULTS AND DISCUSSION

Serological identification of all samples showed that 20 samples (100%) were negative for specific antibodies against AI subtype H5N1, presented in Table 1.

Table 1. HI test result of pigeons serum sample

No.	Region	Sample (n)	Result	
			Positive	Negative
1	Neusu	6	0	6
2	Keutapang	5	0	5
3	Peunayong	3	0	3
4	Seutui	4	0	4
5	Kampung Ateuk	2	0	2
Total	20	0	0	20

The absence of antibodies in pigeon serum indicated that the bird had never been infected with the AI virus, either naturally or by vaccination. This is by results of the research conducted on 123 serum samples of pigeons in Germany, where it was found that the bird was not infected by the AI virus because none of the antibodies was formed against this virus. So, we can say that pigeons do not play a major part in the transmission of influenza viruses [7]. However, it is possible to the Pigeons to be infected and sensitive to the HPAI virus and could be a source of infection to other animals, including humans [8]. These results were also highlighted by Justin *et.al*, who said that "pigeons were resistant to the HPAI virus, requiring a high concentration of virus to produce infection or death" [9].

The principle of the HI test is based on the antibody titer in serum obtained from the highest dilution that inhibits haemagglutination after the control erythrocytes settled (Figure 1). The antibodies in the serum are antibodies that can bind and dissolve antigens. Each antigen will be coated with antibodies from the tested serum so that the haemagglutination process does not occur [6, 10,11]. A positive reaction is characterized by the deposition of erythrocytes at the V-bottom of the microplate as an indicator of antibodies in the serum [12,13,14].

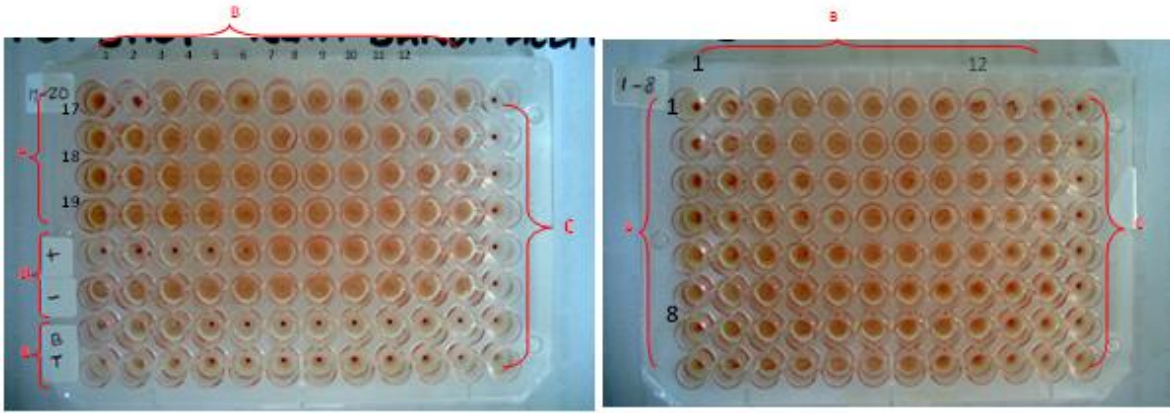

Figure 1 The results of positive serum control (+), negative serum control (-), and back titration test (BT)

Haemagglutination inhibition is a conventional serological test that is often used in many laboratories to detect the presence or absence of specific antibodies in serum samples of poultry or wild birds that have been infected by viral antigens. This is consistent with the statement of Beck *et al.* that the formation of specific antibodies for H5 in serum depends on the characteristics of the virus strain and the bird species of the infected animal [15].

In the HI test, antibodies bind directly to H and N proteins, the antibodies are directed against certain epitopes on the surface of the virus (HA and N) and can neutralize their infectivity, and can also act as opsonins that stimulate the uptake and destruction of viruses by macrophages [16,17]. Besides, antibodies can attach to viral antigens on the surface of infected cells and form complex antigen-antibodies [18,19].

Although this study got negative results, it has a possibility to the AI virus can infect pigeons. Since this virus can mutate at any time and any kind of animal species and human.

4. CONCLUSION

It can be concluded that these pigeons have never been infected by the avian influenza virus subtype H5N1 by using a conventional serological test (haemagglutination inhibition).

AUTHORS' CONTRIBUTIONS

SU, ROS, and AK are equally contributed to this work.

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