SEROSURVEILLANCE OF AVIAN INFLUENZA VIRUS SUBTYPE H5N1 WITH HAEMAGGLUTINATION-INHIBITION ON WILD AQUATIC BIRDS IN PULAU DUA SERANG NATURAL RESERVES, BANTEN PROVINCE

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Abstract

Further detailed research is required to obtain deeper information on the role of wild birds on the distribution of Avian influenza in Asia. A research has been carried out on February–June 2007 focused on blood sampling (serosurveillance) of wild birds in Pulau Dua Nature Reserves (CAPD), Serang, Banten. The research is aimed to investigate the infection of AI virus sub-tye H5N1 on the studied wild birds. The blood samples were taken from studied aquatic birds, followed by HI (*haemagglutination-inhibition*) test. A total of 183 samples represent 7 water bird species were taken i.e Cattle egret *Bubulcus ibis,* Javan pond-heron *Ardeola speciosa,* Little egret *Egretta garzetta,* Intermediate egret *Egretta intermedia,* Black-crowned night heron *Nycticorax nycticorax,* Great egret *Casmerodius albus* and Grey heron *Ardea cinerea*. The result revealed that 41 (23.27%) samples showed the present of AIV antibodies serotype H5N1 which is identified as positive. Data showed 5 positive-test species, including *B. ibis* (29.27%), *E. garzetta* (29.27%), *E. intermedia* (4.88%), *Ardeola speciosa* (7.32%), and *N. nycticorax* (29.27%). A total of 41.46% were infected adult individual, whereas 58.54% were juveniles.

Keywords: Avian influenza virus, colonial aquatic birds, Pulau Dua Nature Reserve, serosurveillans

1. Introduction

Since the first Avian Influenza case was discovered in July 2005 until January 28, 2008, the number of humans infected by the virus in Indonesia has reached 124 people; 100 of them passed away. Thus, the death rate or case fatality rate (CFR) has reached 80.6%. Avian influenza virus (AIV) is an infectious disease caused by influenza viruses carried by fowl [1].

Systematically, influenza virus is a part of the *Orthomyxoviridae* family in the influenza genus. The virus' virion has a diameter of 80 to 120 nm in the form of filaments and consists of eight different segments of negative-stranded RNA [2]. AIV is one of the host-specific viruses, meaning that it has specific host to breed and has the potential to spread through a particular activity. The type of animal hosts for AIV (H5N1) is fowls [3].

AIV breeds in the digestive system of fowls. Infected birds will transmit the virus through its saliva, nasal secretions and feces. The spread of the virus happens from bird to bird and from bird to humans. So far, no evidence suggested that the virus can be transmitted from human to human (pandemic). The virus can also be transmitted through the air and water contaminated by AI virus subtype H5N1 from bird feces or fluids [2-3].

Various opinions related to the mechanism of AI virus subtype H5N1 transmission from birds to humans are still inconclusive. A variety of allegations including the role of wild birds and migratory birds in the spread of this virus has not been proven due to lack of intensive research. The mortality rate found in a number of wild aquatic birds on various countries have not yet managed to explain the cause of these deaths [4].

Data explaining whether the migratory birds flying through Indonesia are potential carriers of AI virus subtype H5N1 is not yet available. As already known, several locations in Indonesia are havens for migratory birds during migration. These areas are also usually the nest for these birds to breed and reside permanently. Some of the areas are protected by the government as biodiversity conservation area [5].

One of the conservation area protected by the government, which is an important breeding area for water birds is the Pulau Dua Nature Reserves (CAPD), located in the Bay of Banten, Serang regency, Banten Province. The area around the reservation is a vast area of mud uses by migratory aquatic birds as a foraging habitat, which includes ponds, paddy fields, grassy fields and plains of mudflat [6]. Thus, when these birds (migratory or settlers) are searching for food, frequent interactions between them and domestic fowls or humans occur.

Serologically, there are various ways to detect the existence of AI virus in fowls (serosurveillance), one of them is by doing a test called the haemagglutination inhibition (HI). HI test is a test that can be used to identify the presence of antibodies in the blood. In this test, the homologous antigen is used to bind antigen-antibody that can disable the virus capability in adhering or binding to the receptor membrane, thus preventing agglutination of red blood cells. One of the functions of HI test is as a mean to identify a specific type of antibody and to see its reaction against known homologous antigen. It is also used to determine the antibody titer by reacting a serum that has an antibody that needs to be identified with the identified standard antigen [7].

HI test has two methods, α and β methods. α method is used to test the type of antigen by diluting the antigen with the verified constant amount of antibodies. The advantage of this method is its ability to directly identify the HA antigen without testing it first. On the other hand, the β method is use to identify and quantify the titers of antibody. Testing is done by diluting the antibody with the constant amount of antigen. The advantages of this method are the use of fewer amounts of antibodies and the antibody titer is already known [7].

HI test can be done by either macro or micro titration, depending on the reagents being used. What distinguishes these two ways is only the volume of reagents and virus standards that is being used. The standard virus macro-titration uses 8 or 10 HAU (Haemagglutination units). Meanwhile, the standard virus micro-titration uses 4 HAU [7].

Due to the outbreaks of AI that infects human beings living around the Province of Banten and Jakarta, a surveillance of the condition of wild aquatic birds found in this area is needed in order to get clear information on whether the infection of AI virus subtype H5N1 has spread in this region and whether this region is one of the locations that has the potentials to spread the AI virus subtype H5N1.

This study aims to determine the presence of antibodies against H5N1 subtype of AI virus serologically (serosurveillance), as well as to obtain information on whether the wild aquatic birds inhabiting CAPD, Serangare the carriers of AI virus subtypes H5N1 around the Province of Banten and Jakarta.

2. Methods

This research consisted of field research and laboratory research. The field research was conducted in CAPD, Serang (Figure 1).

Figure 1. Pulau Dua Nature Reserves, Serang, Banten Province (Source: Google Map)

Blood sampling were carried out to the aquatic birds that inhabited the CAPD Area, Serang, Banten Province. Samples taken were blood samples and cloacae swabs. Tests on the samples were conducted in the laboratory of Medical Microbiology, Faculty of Veterinary Medicine, Bogor Agricultural Institute (IPB). Serological test carried out was HI test [8].

3. Results and Discussion

There were a total of 183 blood samples that were taken on February-June 2007. The number of samples based on the types of birds captured is as follows: *Bubulcus ibis* (60 samples), *Egrettagarzetta* (36 samples), *Nycticoraxnycticorax* (34 samples), *Ardeolaspeciosa* (31samples), *Egrettaintermedia* (20 samples), as well as one sample of both *Casmerodiusalbus* and *Ardeacinerea*. Based on the birds' individual age, the samples from CAPD were obtained from two categories: adult and juvenile. There were 98 adult samples and 85 juvenile samples (Table 1).

The HI test results to all of the blood samples from the wild aquatic birds showed that as much as 41 samples (23.43%) were tested positive, as the agglutination of the red blood cells did not occur. The agglutination of the red blood cells did not occur because the wild aquatic birds have developed antibody due to exposure to the Avian influenza (AI) virus type H5N1.

According to Stanley [9], on HI test the positive result is marked with no agglutination on the red blood cells, which is caused by the existing antibody towards AI virus in the sample serum. Antigen will be tied by the antibody, which would cause the antigen failing to tie with the red blood cells, so that the virus antigen cannot agglutinate the red blood cells. Meanwhile, negative results is marked by agglutination on red blood, which happened because the sample serum did not contain antibody towards the AI virus. Therefore, the virus antigen would join the red blood cells which would cause agglutination on the red blood cells.

Table 1. The Number of Samples based on the Age Difference Obtained and Showed Positive Results

Types of birds	English name	Adult	Juvenile
Bubulcus ibis	Cattle egret	40	20
Ardeola speciosa	Javan pond heron	17	14
Nycticorax nycticorax	Black-crowned night heron	15	18
Egretta intermedia	Intermediate egret	4	16
Egretta garzetta	Little egret	22	14
Casmerodius albus	Great egret	0	1
Ardea cinerea	Grey heron	0	1
		98	85

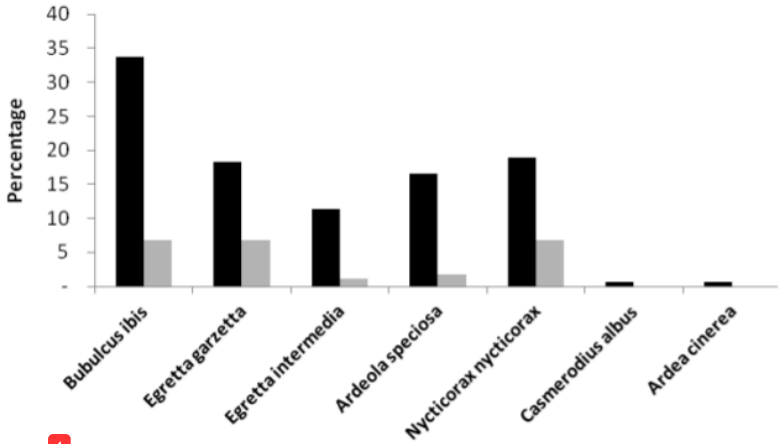

Figure 2. The Amount of Sample, Blood Samples (■), Positive HI Test (□)

The antibody that is formed in the animal's body reacted to the haemagglutininantigen that is located on the surface of the virus. The function of haemaglutinin is to initiate the infection mechanism that was done by the virus towards the target cell. This ability also applies to red blood cells (erythrocyte) so that it can cause agglutination. The resulting antibody is the manifestation of the immunology mechanism that is aimed to inactivate the virus or to lessen the amount of the virus that is still virulent to a certain benchmark so that it becomes no longer harmful for the animal body. The antibody exists in various body fluids, but the highest concentration and the easiest to be obtained in a huge amount to be analyzed is the one located in the serum [10].

The presence of antibody in the serum showed that the virus might still be inside the body so that the presence of antibody has the function to fight infections, or virus was no longer in the body because it has been eliminated by the antibody [11].

The positive result from HI Test showed that the wild aquatic birds in the CAPD have been exposed to AI virus subtype H5N1. The exposure may have been because the geographical position of the reserves made it one of the transit locations for migratory birds during migration period, and the migratory birds interacted with the birds that settled in Pulau Dua. This condition made the AI virus transmission easier. This result gave very useful information considering that wild aquatic birds that live in the CAPD often interact with migrating birds or domestic fowls such as ducks, chickens, and geese that are kept by the people.

The use of food source and the same feeding location on wild aquatic fowls and domestic aquatic fowls makes it possible for the two to interact on the feeding location around CAPD such as ponds, paddy fields, muddy grounds [12]. This can cause the spread of AI virus subtype H5N1. The result of Fang's [13] studies showed that the interaction happened between wild and domestic aquatic fowls can cause cross-infection whether from domestic fowls to wild fowls and vice versa. The interaction can happen in watery areas such as ponds, paddy fields, lake, and muddy grounds [14].

Direct interactions between wild aquatic birds that migrate and wild aquatic birds that settle happen during the migrating season. During that period, migrating aquatic birds will travel to its destination through Indonesian coastal areas. Direct contacts between wild aquatic birds and domestic fowls (ducks, geese) happen when the fowls search for food in the same location [15].

AI virus can travel fast throughout the fowl population, especially the AI virus subtype H5N1. The virus spread or transmission can happen through direct or indirect contacts. Direct transmission of AI virus subtype H5N1 can happen if the AI virus infects the water fowls or other animals without any media. Meanwhile, indirect transmission can happen through media such as water that becomes the drinking source for wild or domestic fowls that have been exposed to AI virus subtype H5N1 [16]. Indirect transmission can also happen through saliva and nose fluid secretion as well as feces through water medium that exists in the interaction place for both wild and domestic fowls [14].

The pollution of water by AI virus subtype H5N1 can happen because of the secretion of saliva or feces of the fowls that positively contains AI virus subtype H5N1, which usually happens when they search for food in the waters around CAPD. Water is a good medium for AI virus subtype H5N1. The suspension of the AI virus in the water can sustain the transmission power for more than 100 days on 17 ^{o}C. The AI virus can survive even longer in the temperature below -15 ^{o}C.

Based on the age difference of the samples captured, HI test results showed that 17 positive samples (41.46%) were adult individuals and 24 positive samples were juvenile (58.54%). The number of individual juveniles that showed the highest positive HI test results, which was 9 out of 12, were small egrets (*E. garzetta*), (Figure 3).

From 12 samples of night herons (*N. nycticorax*) that showedpositive results, 8 birds are juveniles. While the cattle egret (*B. ibis*) samples have shown positive HI testin nine adult birds, Yellow-billed egret (*E. intermedia*) and Javanese pond heron (*A. speciosa*), each has 2 HI-tested positive juveniles (Figure 3).

The presence of antibodies to AI viruses in adult wild aquatic birds is possibly caused by their interaction to the environment and other birds such as domestic fowls. Meanwhile, the formation of antibodies in nestlings (juveniles) may be caused by several factors such as: exposure to AI virus directly from interaction with the parents, the antibodies that were transferred from parents to the nestling (maternal antibodies) [18] or from food provided by the parents. This proves that the AI virus knows no age range in infecting the target animal.

The interaction between the parents that have been exposed to AI virus subtype H5N1 gives quite a big chance to transmit the virus to the nestling. The same thing happens during feeding time. The parents feed the nestling with food obtained from the waters around the nature reserves. If the waters in the area have been exposed to virus AI subtype H5N1, there is a big possibility that the nestlings will get an antibody towards AI virus [19]. Based on observation when the parents fed their nestlings, the parents would feed them

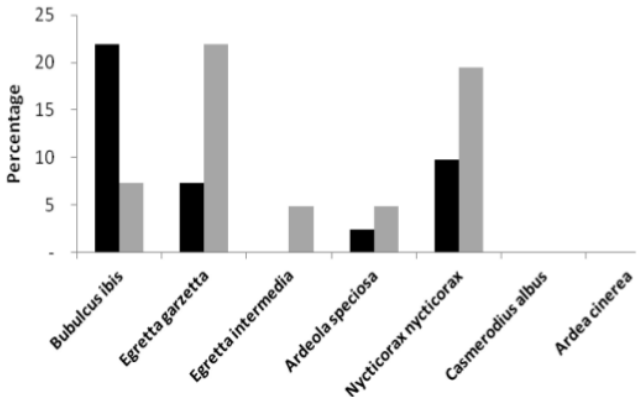

Figure 3. HI Positive Results based on Species and Age Sample Differentiation; Positive HI Test in Adult (■), Positive HI Test in Juvenile (□)

fish, worm, shrimp, or other small creatures from the waters around CAPD.

Although the derived antibodies from mother to nestling (maternal antibodies) in wild aquatic bird populations in CAPD is possible, the mechanism of this process needs further research.

4. Conclusion

The aquatic birds that live in the CAPD include the birds that have been exposed to AI virus subtype H5N1. The HI test result on the blood sample showed that 41 samples (23.43%) turned out positive. Based on the age difference of the 41 samples that showed the positive results, 41.46% are adult birds, while 58.54% are juvenile birds.

Acknowledgement

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SEROSURVEILLANCE OF AVIAN INFLUENZA VIRUS SUBTYPE H5N1 WITH HAEMAGGLUTINATION-INHIBITION ON WILD AQUATIC BIRDS IN PULAU DUA SERANG NATURAL RESERVES, BANTEN PROVINCE Dewi Elfidasari1*), Dedy Duryadi Solihin2, Retno Damayanti Soejoedono3, Sri Murtini3, and Yus Rusila Noor4 1. Programme Study of Biology, Faculty of Science and Technology, University of Al Azhar Indonesia, Jakarta 12110, Indonesia 2. Department of Biology, Faculty of Mathematic and Science, Bogor Agricultural University, Bogor 16680, Indonesia 3. Department of Animal Diseases and Veterinery Health, Faculty of Veterinary Medicine, Bogor Agricultural University, Bogor 16680, Indonesia 4. Wetland International, Indonesia Programme, Bogor 16161, Indonesia *)E-mail: d_elfidasari@uai.ac.id Abstract Further detailed research is required to obtain deeper information on the role of wild birds on the distribution of Avian influenza in Asia. A research has been carried out on February–June 2007 focused on blood sampling (serosurveillance) of wild birds in Pulau Dua Nature Reserves (CAPD), Serang, Banten. The research is aimed to investigate the infection of AI virus sub-tye H5N1 on the studied wild birds. The blood samples were taken from studied aquatic birds, followed by HI (haemagglutination-inhibition) test. A total of 183 samples represent 7 water bird species were taken i.e Cattle egret Bubulcus ibis, Javan pond-heron Ardeola speciosa, Little egret Egretta garzetta, Intermediate egret

Egretta intermedia, Black-crowned night heron Nycticorax nycticorax, Great egret Casmerodius albus and Grey heron Ardea cinerea. The result revealed that 41 (23.27%) samples showed the present of AIV antibodies serotype H5N1 which is identified as positive. Data showed 5 positive-test species, including B. ibis (29.27%), E. garzetta (29.27%), E. intermedia (4.88%), Ardeola speciosa (7.32%), and N. nycticorax (29.27%). A total of 41.46% were infected adult individual, whereas 58.54% were juveniles. Keywords: Avian influenza virus, colonial aquatic birds, Pulau Dua Nature Reserve, serosurveillans 1. Introduction AIV breeds in the digestive system of fowls. Infected Since the first Avian Influenza case was discovered in birds will transmit the virus through its saliva, nasal July 2005 until January 28, 2008, the number of humans secretions and feces. The spread of the virus happens infected by the virus in Indonesia has reached 124 from bird to bird and from bird to humans. So far, no people; 100 of them passed away. Thus, the death rate evidence suggested that the virus can be transmitted or case fatality rate (CFR) has reached 80.6%. Avian from human to human (pandemic). The virus can also influenza virus (AIV) is an infectious disease caused by be transmitted through the air and water contaminated influenza viruses carried by fowl [1]. by AI virus subtype H5N1 from bird feces or fluids [2-3]. Systematically, influenza virus is a part of the Various opinions related to the mechanism of AI virus Orthomyxoviridae family in the influenza genus. The subtype H5N1 transmission from birds to humans are virus' virion has a diameter of 80 to 120 nm in the form still inconclusive. A variety of allegations including the of filaments and consists of eight different segments of role of wild birds and migratory birds in the spread of negative-stranded RNA [2]. AIV is one of the host- this virus has not been proven due to lack of intensive specific viruses, meaning that it has specific host to research. The mortality rate found in a number of wild breed and has the potential to spread through a aquatic birds on various countries have not yet managed particular activity. The type of animal hosts for AIV to explain the cause of these deaths [4]. (H5N1) is fowls [3]. 179 Data explaining whether the migratory birds flying through Indonesia are potential carriers of AI virus subtype H5N1 is not yet available. As already known, several locations in Indonesia are havens for migratory birds during migration. These areas are also usually the nest for these birds to breed and reside permanently. Some of the areas are protected by the government as biodiversity conservation area [5]. One of the conservation area protected by the government, which is an important breeding area for water birds is the Pulau Dua Nature Reserves (CAPD), located in the Bay of Banten, Serang regency, Banten Province. The area around the reservation is a vast area of mud uses by migratory aquatic birds as a foraging habitat, which includes ponds, paddy fields, grassy fields and plains of mudflat [6]. Thus, when these birds (migratory or settlers) are searching for food, frequent interactions between them and domestic fowls or humans occur. Serologically, there are various ways to detect the existence of AI virus in fowls (serosurveillance), one of them is by doing a test called the haemagglutination inhibition (HI). HI test is a test that can be used to identify the presence of antibodies in the blood. In this test, the homologous antigen is used to bind antigen- antibody that can disable the virus capability in adhering or binding to the receptor membrane, thus preventing agglutination of red blood cells. One of the functions of HI test is as a mean to identify a specific type of antibody and to see its reaction against known homologous antigen. It is also used to determine the antibody titer by reacting a serum that has an antibody that needs to be identified with the identified standard antigen [7]. HI test has two methods, α and β methods. α method is used to test the type of antigen by diluting the antigen with the verified constant amount of antibodies. The advantage of this method is its ability to directly identify the HA antigen without testing it first. On the other hand, the β method is use to identify and quantify the titers of antibody. Testing is done by diluting the

antibody with the constant amount of antigen. The advantages of this method are the use of fewer amounts of antibodies and the antibody titer is already known [7]. HI test can be done by either macro or micro titration, depending on the reagents being used. What distinguishes these two ways is only the volume of reagents and virus standards that is being used. The standard virus macro-titration uses 8 or 10 HAU (Haemagglutination units). Meanwhile, the standard virus micro-titration uses 4 HAU [7]. Due to the outbreaks of AI that infects human beings living around the Province of Banten and Jakarta, a surveillance of the condition of wild aquatic birds found in this area is needed in order to get clear information on whether the infection of AI virus subtype H5N1 has spread in this region and whether this region is one of the locations that has the potentials to spread the AI virus subtype H5N1. This study aims to determine the presence of antibodies against H5N1 subtype of AI virus serologically (serosurveillance), as well as to obtain information on whether the wild aquatic birds inhabiting CAPD, Serangare the carriers of AI virus subtypes H5N1 around the Province of Banten and Jakarta. 2. Methods This research consisted of field research and laboratory research. The field research was conducted in CAPD, Serang (Figure 1). Figure 1. Pulau Dua Nature Reserves, Serang, Banten Province (Source: Google Map) Blood sampling were carried out to the aquatic birds that inhabited the CAPD Area, Serang, Banten Province. Samples taken were blood samples and cloacae swabs. Tests on the samples were conducted in the laboratory of Medical Microbiology, Faculty of Veterinary Medicine, Bogor Agricultural Institute (IPB). Serological test carried out was HI test [8]. 3. Results and Discussion There were a total of 183 blood samples that were taken on February-June 2007. The number of samples based on the types of birds captured is as follows: Bubulcus ibis (60 samples), Egrettagarzetta (36 samples), Nycticoraxnycticorax (34 samples), Ardeolaspeciosa (31samples), Egrettaintermedia (20 samples), as well as one sample of both Casmerodiusalbus and Ardeacinerea. Based on the birds' individual age, the samples from CAPD were obtained from two categories: adult and juvenile. There were 98 adult samples and 85 juvenile samples (Table 1). The HI test results to all of the blood samples from the wild aquatic birds showed that as much as 41 samples (23.43%) were tested positive, as the agglutination of the red blood cells did not occur. The agglutination of the red blood cells did not occur because the wild aquatic birds have developed antibody due to exposure to the Avian influenza (AI) virus type H5N1. According to Stanley [9], on HI test the positive result is marked with no agglutination on the red blood cells, which is caused by the existing antibody towards AI virus in the sample serum. Antigen will be tied by the antibody, which would cause the antigen failing to tie with the red blood cells, so that the virus antigen cannot agglutinate the red blood cells. Meanwhile, negative results is marked by agglutination on red blood, which happened because the sample serum did not contain antibody towards the AI virus. Therefore, the virus antigen would join the red blood cells which would cause agglutination on the red blood cells. Table 1. The Number of Samples based on the Age Difference Obtained and Showed Positive Results Types of birds English name Adult Juvenile Bubulcus ibis Ardeola speciosa Nycticorax nycticorax Egretta intermedia Egretta garzetta Casmerodius albus Ardea cinerea Cattle egret Javan pond heron Black-crowned night heron Intermediate egret Little egret Great egret Grey heron 40 17 15 4 22 0 0 98 20 14 18 16 14 1 1 85 Figure 2. The Amount of Sample, Blood Samples ( ), Positive HI Test ( ) The antibody that is formed in the animal's body reacted to the haemagglutininantigen that is located on the surface of the virus. The function of haemaglutinin is to initiate the infection mechanism that was done by the virus towards the target cell. This ability also applies to red blood cells (erythrocyte) so that it can cause agglutination. The resulting antibody is the manifestation of the immunology mechanism that is aimed to inactivate the virus or to lessen the amount of the virus that is still virulent to a certain benchmark

so that it becomes no longer harmful for the animal body. The antibody exists in various body fluids, but the highest concentration and the easiest to be obtained in a huge amount to be analyzed is the one located in the serum [10]. The presence of antibody in the serum showed that the virus might still be inside the body so that the presence of antibody has the function to fight infections, or virus was no longer in the body because it has been eliminated by the antibody [11]. The positive result from HI Test showed that the wild aquatic birds in the CAPD have been exposed to AI virus subtype H5N1. The exposure may have been because the geographical position of the reserves made it one of the transit locations for migratory birds during migration period, and the migratory birds interacted with the birds that settled in Pulau Dua. This condition made the AI virus transmission easier. This result gave very useful information considering that wild aquatic birds that live in the CAPD often interact with migrating birds or domestic fowls such as ducks, chickens, and geese that are kept by the people. The use of food source and the same feeding location on wild aquatic fowls and domestic aquatic fowls makes it possible for the two to interact on the feeding location around CAPD such as ponds, paddy fields, muddy grounds [12]. This can cause the spread of AI virus subtype H5N1. The result of Fang's [13] studies showed that the interaction happened between wild and domestic aquatic fowls can cause cross-infection whether from domestic fowls to wild fowls and vice versa. The interaction can happen in watery areas such as ponds, paddy fields, lake, and muddy grounds [14]. Direct interactions between wild aquatic birds that migrate and wild aquatic birds that settle happen during the migrating season. During that period, migrating aquatic birds will travel to its destination through Indonesian coastal areas. Direct contacts between wild aquatic birds and domestic fowls (ducks, geese) happen when the fowls search for food in the same location [15]. AI virus can travel fast throughout the fowl population, especially the AI virus subtype H5N1. The virus spread or transmission can happen through direct or indirect contacts. Direct transmission of AI virus subtype H5N1 can happen if the AI virus infects the water fowls or other animals without any media. Meanwhile, indirect transmission can happen through media such as water that becomes the drinking source for wild or domestic fowls that have been exposed to AI virus subtype H5N1 [16]. Indirect transmission can also happen through saliva and nose fluid secretion as well as feces through water medium that exists in the interaction place for both wild and domestic fowls [14]. The pollution of water by AI virus subtype H5N1 can happen because of the secretion of saliva or feces of the fowls that positively contains AI virus subtype H5N1, which usually happens when they search for food in the waters around CAPD. Water is a good medium for AI virus subtype H5N1. The suspension of the AI virus in the water can sustain the transmission power for more than 100 days on 17 oC. The AI virus can survive even longer in the temperature below -15 oC. Based on the age difference of the samples captured, HI test results showed that 17 positive samples (41.46%) were adult individuals and 24 positive samples were juvenile (58.54%). The number of individual juveniles that showed the highest positive HI test results, which was 9 out of 12, were small egrets (E. garzetta), (Figure 3). From 12 samples of night herons (N. nycticorax) that showedpositive results, 8 birds are juveniles. While the cattle egret (B. ibis) samples have shown positive HI testin nine adult birds, Yellow-billed egret (E. intermedia) and Javanese pond heron (A. speciosa), each has 2 HI-tested positive juveniles (Figure 3). The presence of antibodies to AI viruses in adult wild aquatic birds is possibly caused by their interaction to the environment and other birds such as domestic fowls. Meanwhile, the formation of antibodies in nestlings (juveniles) may be caused by several factors such as: exposure to AI virus directly from interaction with the parents, the antibodies that were transferred from parents to the nestling (maternal antibodies) [18] or from food provided by the parents. This proves that the AI virus knows no age range in

infecting the target animal. The interaction between the parents that have been exposed to AI virus subtype H5N1 gives quite a big chance to transmit the virus to the nestling. The same thing happens during feeding time. The parents feed the nestling with food obtained from the waters around the nature reserves. If the waters in the area have been exposed to virus AI subtype H5N1, there is a big possibility that the nestlings will get an antibody towards AI virus [19]. Based on observation when the parents fed their nestlings, the parents would feed them Figure 3. HI Positive Results based on Species and Age Sample Differentiation; Positive HI Test in Adult ( ), Positive HI Test in Juvenile ( ) fish, worm, shrimp, or other small creatures from the waters around CAPD. Although the derived antibodies from mother to nestling (maternal antibodies) in wild aquatic bird populations in CAPD is possible, the mechanism of this process needs further research. 4. Conclusion The aquatic birds that live in the CAPD include the birds that have been exposed to AI virus subtype H5N1. The HI test result on the blood sample showed that 41 samples (23.43%) turned out positive. Based on the age difference of the 41 samples that showed the positive results, 41.46% are adult birds, while 58.54% are juvenile birds. Acknowledgement This research received funding from the Basic Research Incentive of the Ministry of Research and Technology, Republic of Indonesia, fiscal year 2007 under contract No. 36/RD/Insentif/PPK/I/2007. We are very grateful for the support that has made this research went smoothly. References [1] R.D. Soejoedono, E. Handharyani, Flu Burung, Penebar Swadaya, Jakarta, 2005, p.68. [2] G.R. Whittaker, Intracellular Trafficking of Influenza Virus, http://www.expertreview.org/ 2001, 2011. [3] D.J.H. Post, K.M.S. Ramirez, J. Humberd, P. Seiler, E.A. Govorkova, S. Krauss, C. Scholtissek, P. Puthavathana, C. Buranathai, T.D. Nguyen, H.T. Long, T.S.P. Naipospos, H. Chen, T.M. Ellis, Y. Guan, J.S.M. Peiris, R.G.Webster, Proc. Natl. Acad. Sci., U.S.A. 102 (2005) 10682. [4] B. Mulyadi, Prihatini, Indonesian J. Clin. Pathol. Med. Lab. 12 (2005) 71. [5] Y.R. Noor, D. Sartono, S. Dana, Paparan Potensi dan Nilai Penting Cagar Alam Pulau Dua Serang Sebagai Kawasan Berbiak Burung Air, PKA/Wetland Interbational, Bogor, 2000. [6] D. Elfidasari, Pemanfaatan Lokasi di Sekitar Cagar Alam Pulau Dua Serang oleh Tiga Jenis Kuntul (Casmerodius albus, Egretta garzetta dan Bubulcus ibis), Widya Kesehatan dan Lingkungan VIII, 2005, p.260. [7] R.D. Soejoedono, Departemen Ilmu Penyakit Hewan dan Kesmavet, FKH IPB, Penuntun Praktikum Penyakit Infeksius, Bogor, 2006, unpublished. [8] World Organization for Animal Health [OIE], Avian Influenza, 2009. [9] J. Stanley, Essentials of Immunology & Serology, Delmar Thompson Learning Inc., New York, 2002, p.539. [10] I.R. Tizard, Vetenary Immunology an Introduct Seven Edition, Saunders, Philadelphia, 2004, p.494. [11] B.T. Akoso, Penyakit Menular pada Hewan dan Manusia, Penerbit Kanisius, Yogyakarta, 2006, p.108. [12] D. Elfidasari, Biodivers. 8 (2007) 266. [13] L.Q. Fang, S.J. Vles, S. Liang, C.W.N. Looman, P. Gong, B. Xu, L Yan, H. Yang, J.H. Ricardus, W.C. Cao, PloS One. 3/5 (2008) 1. [14] Convention on Migratory Species (CMS), Avian Influenza and Wild Bird, www.cms.int/avianflu/ cms_ai_brochure_oct06.pdf, 2006. [15] J.A.V. Gils, V.J. Munster, R. Radersma, D. Liefhebber, R.A.M. Founchier, M. Klassen, Hampered Foraging and Migratory Performance in Swans Infected with Low-Pathogenic Avian Influenza A Virus, 2 (2007) e184, http://www. plosone.org/article/info:doi/10.1371/journal.pone. 0000184, 2007. [16] W. Nazarudin, Avian Influenza pada Ungas, Pusat Kesehatan Hewan, http://www.vet-klinik.com, 2006. [17] K. Mohammad, Flu Burung, www.influenzareport. com/influenzareport_indonesia.pdf, 2006. [18] F.J. Fenner, E.P.J. Gibbs, F.A. Murphy, R. Rott, M.J. Studdert, D.O. White, Virologi Veteriner, Edisi Kedua, IKIP Semarang Press, Semarang, 1995, p.84. [19] Y. Si, T. Wang, A.K Skidmore, W.F. Boer, L. Li, H.T. Prins, Ecol. Soc. 15 (2010) 26.

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