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Differences of antibody titer to avian influenza and hematology profile on local ducks in Central Java

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Abstract. Avian Influenza (AI) in poultry farms in Indonesia is still a threat to the sustainability of livestock and farmers. Duck is one of the most heterogeneous natural reservoirs and hosts of AI viruses. This study aims to detect antibody titer AI levels in blood serum and duck eggs as well as to know the different hematology profile of Tegal and Magelang ducks in Central Java. Research method was survey at farmer level. Farmer and duck samples were determined by purposive random sampling of 10 breeders for each region. Selection of respondents and ducks was based on the number of duck owners and duck age. The samples of farmers and ducks taken were determined randomly as many as 10 breeders in each area and from each breeder was selected 6 female ducks for their blood and 10 eggs to be analyzed using antibody titer against AI. The variables measured in this study were antibody titer against AI measured on blood serum and serum egg yolk, with inhibition hemagglutination test and haematological profile. The data obtained were analyzed using T-test. The results showed protective antibodies against AI on blood serum Tegal ducks (seropositive) 58% while Magelang ducks 46%. Titer of serum antibodies of Tegal duck in the range of 22-25 while Magelang ducks 22-26. Titer of egg antibody in Tegal ducks in the range of 20-210 while Magelang ducks 20-27. T-test on AI antibody titer on blood serum and serum of eggs of Tegal duck and Magelang ducks (which have different environment) showed no significant difference ($P > 0.05$). The result of T-test on leukocyte fraction profile showed that on heterophile, lymphocyte, and H / L both ducks showed a very significant difference ($P < 0.01$). Higher H / L values in Tegal ducks show stress due to heat stress. T-test on red blood cells, white blood cells, hemoglobin, packed cell volume (PCV), total plasma proteins, eosinophils, monocytes, fibrinogen and albumin showed no significant difference ($P > 0.05$). The study concluded that there was no difference of antibody titer level to AI and hematology profile in Tegal and Magelang ducks despite different maintenance environment. However, Tegal duck was indicative of higher stress with higher H / L values than Magelang duck.

Keywords - Avian Influenza, hematology, Magelang duck, Tegal duck, antibody titer.

1. Introduction

AI epidemic to the poultry declared in January 2004 in Indonesia has significantly decreased each year; 2,751 cases were reported in 2007 and it gradually declined up to 104 by the end of the year [1]. AI breakout ended in Tegal and Magelang in 2016 but vaccination among the breeders was rare. AI infection to ducks in a region may trigger antibody against AI. Ducks have a higher seroprevalence against High Pathogenic Avian Influenza (HPAI) H5N1 than chickens [2] [3]. Duck is one of the most



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heterogenous natural reservoirs and host for AI with the ability of shedding a mass of virus and mild pathological and clinical symptoms [4] [5]. Accordingly, ducks are concerned to transmit the virus to other ducks or even to other poultry.

In addition to detecting antibody titer to particular disease, examination on blood profile serves as a method to evaluate the physiological condition and health status of the poultry. It may be a good indicator for poultry in different environment and habitat [6]. This research, therefore, used survey method to investigate the in-situ antibody titer and blood profile of Tegal ducks and Magelang ducks from the native habitat in Tegal and Magelang.

1.1. The purpose of the research

The purpose of the research were to detect the level of antibody titer against AI in the ducks' blood serum and eggs, and to observe the different hematological profile between Tegal ducks and Magelang ducks in Central Java farming duck.

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2. Materials and methods

2.1. The material of the research

Research materials were two breeds of ducks and the eggs from the farmers, which were collected from duck breeding centers in Central Java that included "Berkah Abadi" farmer breeders group in Pesurungan Lor, Margadana, Tegal; and "Sido Rukun" farmers breeders group in Sawangan, Magelang. Ducks farmers in Tegal as the respondents had a minimum 600 ducks aged 8-15 months. Farmers in Magelang had 150 ducks with the same age range. The selection was based on the preliminary survey that observed a farmer in Tegal generally owned 400 adult female ducks while in Magelang 100 productive ducks. Ten farmers were randomly selected from every region. Blood sample was collected from six hens from each farmer, and 10 eggs were taken from each breeding center to analyze the antibody titer against AI.

2.2. Methods

Survey method was conducted among the farmers. Purposive random sampling was used to select 10 farmers and ducks in each region/respondents and ducks were selected based on the numbers of ducks in the breeders and ducks' age. The measured variables in the study were antibody titer against AI from the blood serum and egg yolk serum, and hematological profile i.e., erythrocyte count, leukocyte count and differential leukocyte, hematocrit value, hemoglobin, total plasma protein, albumin, fibrinogen and the H/L ratio. Serology test was performed to measure antibody titer against AI using hemagglutination inhibition (HI) test according to the procedure of Office International des Epizooties [7] to determine antibody titer against H5N1 virus. The obtained data were subject to T-test.

2.3. Egg yolk serum preparation procedure [8]

The eggs were cracked then the yolks were separated from the white. The egg yolks were paced in a petri dish and the yolk membrane was washed in aquadest. Then, the yolks were stored in a tube to measure the volume, added with Dulbecco solution twice as much as the yolk volume, then homogenized by stirring. As much chloroform as the yolk was added. The solution was stirred to homogenous and formed emulsion, then centrifuged at 1000 x g for 30 minutes at room temperature. Upon centrifugation, the solution was separated into three layers—orange liquid (lecithin) at the bottom, semi-solid yolk emulsion in chloroform in the middle, and the liquid phase of protein serum at the top. The top layer which contained IgY was stored at -20 °C until analysis was performed.

3. Result and discussion

3.1. Antibody titer against AI in Tegal duck in Tegal region and Magelang Duck in Magelang Region

The propagation pattern of AI virus is generally affected by the season; the prevalence of AI is higher in autumn, winter and rainy seasons. In Indonesia, AI breakout tends to increase during a rainy

season because the high humidity is the contributing factor to AI virus breeding and spread in the environment. Wild waterfowl such as Anseriformes (mallard, Muscovy and goose) and Charadriiformes (seagull, oceanic bird and wild bird) are the natural reservoir of type A influenza virus and play important role in the virus ecology and propagation.

Ducks breeding in the breeder centres in Tegal and a fraction of farmers in Magelang used an enclosed ducks house. This model has a lower risk of disease spread than the traditional or nomadic breeding. Nomadic behaviour and a considerably long-distant transporting increase the risk of the ducks as a carrier of AI from one region to another. Furthermore, feed poisoning risk during the free-range can be overcome by an enclosed breeding system [9] [10]. Antibody titer in Tegal duck in Tegal regency and Magelang duck in Magelang regency is presented in Table 1.

Table 1. Mean of antibody titer of blood serum and egg serum of Tegal duck in Tegal and Magelang duck in Magelang.

Breeder	Mean of antibody titer			
	Ducks' blood serum (2 ⁿ)		Ducks' egg serum (2 ⁿ)	
	Tegal	Magelang	Tegal	Magelang
1	4.2	3.5	1.6	2.1
2	3.8	3.9	1.1	1.5
3	3.6	3.4	2.2	2.4
4	3.8	3.7	1	1.7
5	3.9	3.7	1.1	1.5
6	3.7	3.7	2.2	2.4
7	3.5	3.7	1	1.7
8	3.9	3.4	1.1	1.5
9	3.7	3.8	2.2	2.4
10	3.7	3.5	1.1	1.6
Mean	3.80	3.63	1.43	1.86

The antibody is protective against AI when it inhibits the serum which is diluted 1 : 16 (2⁴) or log 2⁴ using antigen 4 HAU [7]. The antibody protective against AI in the blood serum of Tegal duck and Magelang duck was seropositive; 58% and 46%, respectively. The protective antibody titer in egg serum of Tegal duck and Magelang ducks was seropositive, 11% and 4%, respectively. Antibody titer serum in Tegal duck and Magelang duck was 2²-2⁵ and 2²-2⁶, respectively. Antibody titer egg in Tegal and Magelang duck was 2⁰-2¹⁰ and 2⁰-2⁷, respectively. The seropositive serum showed immunity in the ducks which may link to the previous exposure to AI in the region. On the other hand, the seropositive egg serum may due to the maternal antibody from the hen. However, the study shows that the majority of the ducks have low antibody titer against AI (Table 1).

Result of HI mean value showed that Tegal duck in Tegal had a slightly higher antibody titer compared to that of Magelang duck in Magelang. It may due to the coastal area of Tegal that allows a more frequent interaction between Tegal duck and the wild waterfowls than that of Magelang duck. Accordingly, several ducks with high immunity had a seropositive antibody titer against AI. However, the result of paired t-test on antibody titer AI on blood serum and egg serum did not show a significant effect. The possible reason for this was the absence of AI vaccine in both area; therefore, the ducks may not survive in the next AI breakout despite the fact that Tegal and Magelang had a high prevalence of AI.

Some contributing factors to the level antibody titer against AI are the history of AI incidence and AI vaccination. AI H5N1 vaccination increased antibody titer in ducks which is transferrable to the eggs produced and ducklings [11]. Infection case in a breeding centre without vaccination follow-up may lead to a poor distribution of antibody titer, i.e. some ducks have a very high antibody titer and the rest have the counterpart level.

A dense population and a free breeding system of waterfowl are the contributing factors to waterfowl as the potential reservoir of H5N1 virus in Indonesia [3]. An enclosed breeding system for Tegal ducks and Magelang ducks would decrease AI incidence compared to the backyard system (free-range around the cage) due to the minimum contact with the ducks or the other poultry [12], particularly for ducks with poor immunity or without AI vaccination. Furthermore, improvement of breeding management is important by grouping the ducks based on age to prevent the contagion [13].

High antibody titer was assumedly due to the body immune system in responding to the continuous, long-term AI exposure from the environment which, by no means, naturally vaccinated the poultry. This process will likely to occur in backyard system due to lack of biosecurity, so AI virus can easily transmit from one poultry to another through direct or indirect contact. Positive HI test showed that in the animal's body exist an antibody as a sign of early AI infection or exposure in the poultry. The gradual exposure may stimulate AI antibody but insufficient to induce the clinical symptoms [14].

3.2. Hematological profile of Tegal duck in Tegal and Magelang duck in Magelang

Hematological profile can be used to identify the clinical health status because blood plays an important role to measure the physiological, pathological and nutritional status of an animal. The change in blood constituent in animal physiological status may due to genetic and non-genetic factors such as age, sex, breeding and management system [15] [16]. The hematological profile in the present study (in situ) included erythrocyte, leukocyte, haemoglobin, packed cell volume (PCV), total protein plasma, heterophile, eosinophil, lymphocyte, monocyte, fibrinogen and albumin and H/L ratio. The surveyed hematological profile and the standard hematological profile are presented in Table 2.

Table 2. Mean of hematological profile of Tegal duck and Magelang duck in breeder centres in Tegal and Magelang.

Hematological profile	Mean		Standar (Scalm, 2010)
	Tegal duck	Magelang duck	
Erythrocyte (jt/ μ l)	3.048	3.243	2.01 \pm 0.4
Leukocyte ($\cdot 10^6 / \mu$ L)	9.489	9.567	23.4–24.8
Hb (g/dL)	10.893	10.987	11.4 \pm 1.6
PCV (%)	33.033	34.967	39 \pm 5.1
TPP (g/dl)	3.913 ^a	3.300 ^b	-
Heterophile segment (%)	36.83 ^a	28.23 ^b	29 \pm 1.4
Eosinophil (%)	7.1	7.07	0.2 \pm 0.17
Basophil (%)	0	0	2.2 \pm 0.3
Lymphocyte (%)	50.33 ^b	59.27 ^a	66 \pm 1.4
Monocyte(%)	6	5.4	2.5 \pm 0.3
Fibrinogen (g/dl)	0.460	0.460	-
Albumin (g/dl)	3.597	3.822	-
H/L	0.773 ^a	0.446 ^b	-

Note: Values bearing different superscript within column show a highly significant difference ($P > 0.01$) based on t-test

Table 2 shows the different blood parameter of Tegal and Magelang ducks compared to the normal standard [17]. The mean values of erythrocyte, leukocyte, haemoglobin, PCV and albumin in Magelang ducks were higher than those of Tegal ducks. TPP, heterophile, eosinophil, basophil, lymphocyte, monocyte and H/L ratio in Tegal ducks were higher than those of Magelang ducks (Table 2).

The result of t-test of erythrocyte, leukocyte, hemoglobin, packed cell volume (PCV), eosinophil, monocyte, fibrinogen and albumin did not show a significant difference ($P > 0.05$). it indicated that there was no difference in hematological profile in both types of ducks despite the different breeding

environment and nutrition in feed. The hematological profile was relatively similar because both ducks are derived from one species *Anas platyrhynchos*. A significant difference in blood profile is generally attributed to the variation in breed, hormone and nutrition [15]. Although Magelang ducks were given a lower protein feed (17,99%) than that of Tegal ducks (19,25%), the environment of Magelang region has supported the minimum requirement of growth and protein for the ducks. The result of this research was in line with [6] that the contributing factors to hematological value were breed, sex, age and reproductive status, heart rhythm, breeding procedure and nutritional status of the animals.

Table 2 shows that erythrocyte, hemoglobin and PCV of Magelang ducks are biologically higher than those of Tegal ducks. The influential factors may include the different breeding region; Magelang is a highland and Tegal is a coastal area. According to [18], erythrocyte count would increase in a lower temperature and decrease in a higher temperature. In addition, the heat stress in Tegal ducks may affect blood profile, particularly to erythrocyte, hematocrit, hemoglobin and leukocyte [19].

The result of t-test on hematological profile showed that the total protein plasma, heterophile, lymphocyte and H/L ratio of both types of ducks were significantly different ($P < 0.01$); the nutrient feed of Tegal ducks was higher than that of Magelang ducks. The different nutrient content profoundly affected the blood profile, particularly the blood protein synthesis [20]. The high protein feed resulted in a higher total protein than that of Magelang duck. Nutrition offered to Tegal ducks was rich in composition and variation, containing 19.25% crude protein and 2914 kcal/g energy, while Magelang ducks consumed 17.99% crude protein and 2801 kcal/g energy. However, the different nutrition did not significantly improve blood profile because of the potential heat stress for Tegal ducks.

A higher H/L ratio in Tegal duck showed a heat stress. The average temperature during the three-month observation (July - September 2017) in Tegal duck breeding centre was $30,53 \pm 1,38^{\circ}\text{C}$ while in Magelang was $28,32 \pm 1,00^{\circ}\text{C}$. The environment temperature of both regions is above the thermoneutral zone for ducks, i.e., $23\text{-}25^{\circ}\text{C}$ [21]. Heat stress was detected from the decreasing leukocyte count, particularly lymphocyte ($P < 0.01$) and the high H/L ratio compared to those of Magelang ducks which were below the Scalm standard [17]. Lymphocyte is the part of leukocyte that plays role in the immunity system. IgG is the main antibody produced by lymphocyte [20] [22]. The result of this research was in line with that of [22] that lymphocyte count decreased during the heat stress, as observed from the increasing H/L ratio. However, the effect of nutrition to Magelang ducks required a further investigation considering the low leukocyte level in the ducks compared to the Scalm standard [17].

4. Conclusions

Tegal ducks reared in Tegal region and Magelang ducks in Magelang region have been exposed to AI. The level of antibody titer to AI in blood serum and egg yolk was relatively similar. Also, Tegal ducks and Magelang ducks showed a relatively similar hematological profile and stress despite being reared in a different environment

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