Post-Vaccination AI (Avian Influenza) Antibody Levels in Superior Native Chicken Balitnak (Kub Chicken) with Arab Chicken Aged 25 Days

**Abstract**

In Indonesia, native chickens are still in demand by the community. However, this type of chicken has low productivity compared to purebred chickens. At this time, there has been a type of native chicken that is Arab Chicken that has been widely developed in Indonesia because it has better endurance than other native chickens. In addition to Arabic chickens, there are also KUB chickens developed by the Bogor Livestock Research Institute to address the problem of low productivity of native chickens. But these two types of chickens remain susceptible to diseases, such as Avian Influenza (AI). To overcome these breeders conduct AI vaccinations using inactive vaccines. This study aims to find out the difference between post-vaccination antibodies between KUB chickens and Arab chickens. This research has been conducted from June to August 2018. The chickens used amounted to 40 chickens (20 KUB chickens and 20 Arab chickens). The chickens are kept from the age of one day and placed in two separate cages according to their breed. Intracardial blood sampling was pre-treatment (three days old) for the 40 chickens. At the age of four days, the chickens were injected with an inactive AI vaccine, and 21 days later blood sampling was again carried out (post-treatment). HI, tests are used to find out titer antibodies pre and post-treatment. The results of antibody titer readings were then analyzed using a t-test and Geometric Mean Titer test (GMT). T-test results showed that T value (6.364)>T table (1.734), so the results of this study showed that the titer antibodies formed are different (P<0.05). KUB chickens have higher antibody titers than Arab chickens.

**Keywords:** Arab chicken, KUB chicken, inactive vaccine, blood sample, titer antibodies

**A. Introduction**

Avian Influenza (AI) or better known to the public as "Bird Flu" is an infectious disease in poultry, caused by the avian influenza virus subtype A of the family Orthomyxoviridae (Easterday & Hinshaw, 1991). Most cases are caused by the Highly Pathogenic Avian Influenza (HPAI) subtype H5N1, causing systemic disorders followed by high mortality rates and varying organ...
lesions. The disease is listed as A by the Office International des Epizooties (OIE) because its rapid spread, covering borders between countries, is zoonotic because it has the potential to cause death in humans. In addition, it has an impact on international trade, especially poultry products and processed products (Alexander, 2000). In Indonesia, Avian Influenza has been spreading since mid-2003 in Java. AI continues to spread to other islands in Indonesia, including the island of Lombok. The disease can cause up to 100% death of poultry. Another disadvantage is the psychological effects on society, which significantly impact the country's economy, especially poultry and poultry products (Nataamijaya et al, 2003).

Avian Influenza is very worrying for poultry farms in Indonesia. In areas in Indonesia including Lombok, there are still many people who are interested in native chickens rather than thoroughbred chickens to be raised, even native chickens can be said to be one of the drivers of the rural economy. For example, native chicken is used as one of the family food sources, namely eggs, and meat. The other beneficial side is chickens can be used as 'savings' that can be cashed at any time (Iskandar, 2010). However, when viewed from egg production, the performance of native chickens is still low compared to other buras chickens, such as Arab chickens. Overcome the Bogor (Balitnak) has done a selection of chickens produced chicken Kampung Unggul Balitnak (KUB). The productivity of KUB chicken eggs is much better than that of ordinary native chickens, so it is expected to match the productivity of Arab chicken eggs. However, it is interesting to know the response of KUB chickens and Arab chickens to AI disease. This can be known by comparing the antibody titer of these two types of chickens obtained from the vaccination program.

Measuring the level of titer antibodies in poultry serum is one of the ways used to evaluate the success of the AI virus vaccination. It is commonly known that AI disease in poultry can be prevented by vaccination. The absence of regular AI vaccination programs will result in the easy spread of AI in poultry populations on a farm if there are cases of AI appearing on the farm. Moreover, if there has been a spread, it will cost a lot to control and control diseases, and there will also be a decrease in livestock productivity (Hanson, 1996). To be necessary as an effective measure in the prevention of AI diseases.

Vaccination is intended to stimulate the formation of antibodies (immune substances) by the type of vaccine to make chickens have high immunity to a particular disease (Sudaryani, 2013; Wibowo, 2006). The basic principle of the avian influenza vaccine is that the vaccine virus (master seed) must be homologous with subtype H or subtype H and N viruses from the field. According to OIE regulations, the vaccine master seed must come from the purified Low Pathogenic Avian Influenza (LPAI) virus isolate, have a stable genetic composition, perfect inactivation process (laboratory test), free of pollution of other infectious agents, contain high concentrations of antigens use high-quality adjuvants, have a high level of security, potential and effectiveness (Machdum, 2007).

Antibody detection can be done with serological tests such as hemagglutination (HA/HI) tests. In the HA test, there was a reaction between the antigen and the erythrocyte receptor. While in the hemagglutination resistance test (HI) antibodies bind directly with hemaglutinin so that red blood cell hemagglutination will occur by AI virus cells (Tabbu, 2000). Research on the level of titer antibodies in KUB chickens and Arabic chickens can be a means to understand more about KUB chickens and Arab chickens.

B. Methodology

1. Samples

This research used as many as 40 chickens consisting of 20 DOC KUB chickens and 20 DOC Arab chickens. All chickens are sampled as comparative data before vaccination.

2. Maintenance, Vaccination, and Sampling

Chickens samples that were used in this study are day-old chickens (DOC). KUB chickens and Arab chickens are grouped by breed into two cages. Both groups are nurtured (feeding and drinking) in the same way. On the third day of maintenance (chickens are three days old), all chickens are sampled by blood through jugular veins to be examined for pre-treatment antibody titer on the fourth day, the inactive AI vaccine is injected sub-cut into all chickens Three weeks (21 days) after vaccination, blood retrieval is re-examined for post-treatment antibody titer.

3. Serum Retrieval

The steps work as follows: Blood is taken through the heart (i0tracardial) in chickens aged 3 and 25 days using a 3ml syringe. The blood that has been taken is precipitated until the serum
separation occurs (given a little air space on the syringe), then the blood serum is transferred into a microtube tube. The clear serum is ready for use.

4. **Preparation of Red Blood Cell (RBC) Suspension**

The steps work as follows: Chickens are taken blood on brachialis veins (in the wing area) using a syringe, before the blood is taken, the area is cleaned with alcoholic cotton wool. Blood collection is carried out by Legeartis, so as not to cause damage to the veins and is not contaminated by other microorganisms. Blood is then immediately inserted into a tube that contains an anticoagulant. Blood is centrifuged for 15 minutes at a speed of about 2000rpm. Centrifugation results will separate between plasma and red blood cells that settle. The suspension of red blood cell stock was then diluted with PBS to obtain a 1% red blood cell suspension (Anonim, 2003). Then diluted with PBS to obtain a 1% red blood cell suspension (Anonim, 2003).

5. **Standard Virus Creation 4 HA Unit**

Before further identification in the HI test, the virus titer suspension must be diluted first with a PBS solution to obtain the 4H virus titer to test whether the standard 4HAU virus titer can be seen with the HA test.

6. **Hemagglutination Test / HA Test**

The methods of the hemagglutination test (HA Test) are as follows: Each hole in the microplate is filled with 25 μ PBS using a microplate. Plus the antigens to be tested are then shuffled using micropipes from holes 1-12. In each hole, 1-12 is added 25 μl PBS. Then in the hole is added 25 μ the 1% red blood cell suspension is then shaken for 30 seconds so that the suspension is mixed. It is then precipitated at room temperature and then observed the onset or not of red blood cell agglutination reaction is every 15 minutes (Anonim, 2003).

7. **Hemagglutination Inhibition Test / HI Test**

The methods of the hemagglutination resistance test (HI Test) are as follows: At holes 1-12 it is filled with 25 μPBS. Added serum which is then whisked with micropipes from holes 1-12. At each hole, 1-12 is filled with 25 μ antigen4HAU suspensions. So that the suspension is mixed with a microplate shaken for the next 30 seconds and precipitated at room temperature for 30mins. After 30 minutes at room temperature, add to each hole 1-12 every 25 μ suspensions of red blood cells 1% and microplate again shaken for 30 sec. Then the microplate is re-recorded at room temperature and continued with a reading every 15 minutes (Anonim, 2003).

8. **Data Analysis**

The data obtained from the results of the study were tabulated and tested with a t-test and Geometric Mean Titre (GMT).

C. **Result and Discussion**

1. **Result**

The results of antibody titer examination before and after AI vaccination conducted in the laboratory using the HI test showed that there are differences in titer in KUB chickens and Arab chickens (Table 1). Titer antibodies in pre-ai vaccination are 20. Titer antibodies after AI vaccination showed the occurrence of different antibody responses in Chicken KUB with Arab Chickens.
Figure 1. Comparison between antibody titer after AI vaccination in KUB chicken and Arab chicken.

The figure above showed that the increase in antibody titer formed after vaccination in Chicken KUB which is 24-25. While Arab chickens showed lower antibody titer after AI vaccination compared to KUB chickens which are 22-24 (Figure 1). From the results of the antibody titer examination above, a t-test was conducted to analyze the difference between titer antibodies shown by KUB chickens and Arab chickens. T-test results showed that T value (6,364)>T table (1,734) with p<0.05. So that it can be interpreted as there are differences that are significant, where the antibody titer produced by KUB chickens is higher than that of Arab chickens.

Geometric Mean Titre (GMT) tests were also conducted to further analyze the antibodies of both types of chickens to determine the uniformity of the level of protectiveness of titer antibodies shown by these two types of chickens. The result of GMT against Titer antibodies after AI vaccination of KUB chickens was 24.25046 while GMT titer antibodies post-vaccination Arab chickens were 6.9641. So from the results of this study, it can be said that KUB chickens that were sampled in this study have a degree of uniformity of post-vaccination protectiveness of AI (Coefficient of Variance / CV<35%). If CV<35% can be it is said that the antibodies titer of the chickens tested had a uniformity of post-vaccination protectiveness. Whereas if the CV >35% then it can be said that the uniformity of the level of protectiveness of the chickens tested varied per individual.

2. Discussion

This research was conducted on 40 chickens consisting of 20 chickens of Kampung Unggul Balitnak (KUB chicken) and 20 Arab Chickens. The results of this study showed that KUB chickens and Arab chickens have protective ai post-vaccination antibodies titer which is 24. Post-vaccination protective antibody titer according to OIE standards (Office International des Epizooties) is a minimum of 24. But KUB chickens showed the ability to produce better protective antibodies than Arab chickens. T-test results showed that T value (6,364)> T table (1,734) which means that the resulting antibody titer is different (P<0.05). Since the T value was larger than the T table, the alternative hypothesis was accepted and the nil hypothesis was rejected. This means that there is a difference in the level of antibody titer after AI vaccination (Avian Influenza) in KUB Chickens with Arab Chickens. KUB chickens produce higher levels of antibody titer than Arab Chickens.

This research has sought to minimize the bias that can be caused by maintenance management during research. All chickens in the first treatment were sampled as comparative data before vaccination. During the maintenance period, both breeds of chickens are given the same treatment (feed, feeding, and drinking time). Swayne & Suarez (2000) that chicken health was maintained by vitamin administration. The provision of vaccines has been done as well as possible ranging from maintaining the condition of the vaccine, how to bring it to the research
site using a storage box that has been filled with ice, storage at a temperature of 2-8°C, the use of sterile tools and paying attention to the dosage at the time of vaccination application. Good treatment during maintenance at the time of research, ranging from doc handling on the go, correct handling of vaccines, methods of administering vaccines to chickens as best as possible, the condition of chickens at the time of vaccination in a healthy state, and correct maintenance after vaccination against chickens were pursued in this study. According to Medion (2004), the correct treatment before and after the vaccination of chickens will provide good immunity as well.

The punctuality of post-vaccination antibody titer examination will also affect titer antibodies. In this study, vaccination was done using an inactive AI vaccine, so the sampling time for the test is 21 days after inactive AI vaccination. This is because the highest antibody titer post-vaccination inactive is formed at 21 days after vaccination. It is also in Akoso’s opinion (1993) states that to know the level of immunity achieved from inactive vaccines, the measurement of titer antibodies in chickens is 3 weeks after vaccination. Furthermore, Tabbu (2000) states that the peak of the antibody response will be achieved within 3-4 weeks after inactive vaccination.

KUB chickens have good immunity in line with the opinion of Sulandari et al. (2007) about chicken KUB. KUB chickens are more resistant to diseases and the taste of meat just like native chickens in general. Darma & Sitanggang (2002) said Arab chickens have fairly good disease resistance and easily adapt to the environment, but when viewed in terms of chicken farms KUB has several advantages over Arab chickens.

The formation of different antibody titers in the sample of Chicken KUB with Arab Chicken can be caused by several factors including stress levels in chickens and genetics. The administration of vaccines, especially by injection will cause stress in poultry in this case in KUB chickens and Arab chickens. Each vaccinated poultry individual has a different level of decreased stress levels. The faster the stress decreases, the better the titer antibodies are produced. KUB chickens obtained from the election results until the 6th generation seem to have a lower stress level than Arab chickens. So it gives a higher reaction to ai post-vaccination antibodies than Arab chickens.

Genetics also influence the formation of antibodies titer. KUB chicken and Arab chicken even though they come from the same class of Aves, but genetically can be different, this can be caused by the selection process of KUB chickens carried out until the 6th generation. Historically, at the time of the 2003 when AI outbreak, chickens and ducks had different responses to AI although both were from the same class (Aves). At that time the ducks seemed superior, the AI virus did not kill the ducks but the ducks simply became reservoirs. Although at this time ducks can also die from this virus, this is more due to the tendency that The virus has undergone mutations. So it can be said that the genetic side of Arab chickens and KUB is different, although it comes from the same class. So this is the reason why the antibody titer after AI vaccination between these two chickens is different.

**D. Conclusion**

Based on this research, it can be concluded that KUB chickens and Arab chickens that have been given an inactive AI vaccine have titer antibodies that vary. KUB chickens produce higher antibody titer compared to chickens Arab.

**E. References**


