ANTIBODY LEVEL OF AVIAN INFLUENZA SUBTYPE H5 IN COMMERCIAL BROILER FARMS

RISA INDRIANI and N.L.P.I. DHARMAYANTI

Institute Centre Research of Veterinary
Jl. R.E. Martadinata No. 30, Bogor 16114
marisa_risain@yahoo.co.id

ABSTRACT

Highly Pathogenic Avian Influenza (HPAI) subtype H5N1 has been in Indonesia since the middle of 2003. Vaccination of chickens has become a routine practice in the countries of Asia (including Indonesia) where HPAI is endemic. This is especially conducted for layer and breeder farms, but the broilers are often left unvaccinated. To observe the innate immune antibodies against AI subtype H5, which derived from hens in young broilers, this experiment was conducted on commercial broiler farms in sector 3. Seventeen farms which located in the district of Sukabumi and Cianjur were chosen. Twenty serum samples were collected randomly from each farm by random at the time of broiler day old chicks (DOC), two weeks of age and at around four to five weeks of age or market ready. Serum samples were tested by using haemagglutination inhibition (HI) against three antigen isolates of AI subtype H5N1 and one antigen of subtype AI H5N2. Data from HI tests were analyzed as the geometric mean titre (GMT) and negative titre (< 16) was regarded as 4 for the calculation of GMT. Results of analysis of serum samples showed that DOC of broiler chickens had maternal antibody titre of AI subtype H5 were ≥ 16, then at 2 weeks of age the maternal antibody titre subtype H5 were < 16 and at the time market ready or at 4 – 5 weeks of age the maternal antibody titre AI subtype H5 has reached 0. It is concluded that maternal antibody against AI subtype H5 in broiler is no longer providing protection, and it is possible that broiler chickens could be infected with AI virus in the field.

Key words: Maternal Antibodies, Avian Influenza, Subtype H5N1, Broiler

INTRODUCTION

The H5N1 highly pathogenic avian influenza (HPAI) virus was initially detected in poultry farms in Central Java in August 2003 (Wiyono *et al.*, 2004). Although the source of the virus has been traced by sequence comparisons back to Hunan province in southern China (Wang *et al.*, 2008), the mechanism of the introduction has not been established. Although initially it was suspected to be via migrating birds, currently it is considered more likely that the introduction of the virus occurred through the transboundary movement of poultry and/or poultry products by the commercial sector (Kilpatrick *et al*., 2006). Once introduced the disease spreads rapidly, and by 2011 it had been detected in 32 out of Indonesia's 33 provinces. Since then the disease has become endemic, and from July 2005, has also caused sporadic zoonotic transmissions to humans (Samaan, 2007 Personal Communication). Initially the veterinary authorities in Indonesia attempted a stamping out policy, but when the extent of the spread of the disease became clearer, this strategy was changed to one of vaccination (Naipospos 2005; Siregar *et al.*, 2007). As this was considered to be only a temporary emergency measure, in keeping with OIE guidelines, a heterologous vaccine was recommended (H5N2). However, this vaccine was later supplemented by locally produced (and imported) homologous H5N1 vaccines.

While vaccination has been largely successful in controlling massive mortalities, there are a number of outstanding questions about its field effectiveness. Of particular concern is the extent to which genetic drift is occurring, and is responsible for vaccine failure. Swayne (unpublished) undertook a challenge study using three field strains and found one of these vaccines to be ineffective. Nevertheless, there are other reports of continued effectiveness of the vaccines, and this was supported by a field study in West Java demonstrating the effectiveness of the

vaccine in preventing disease (Bouma *et al.*, 2008).

Specifically, in the broilers, to determine the length of time maternal antibody provided protection or potential interference if vaccination was practiced. Due to the variety of vaccines being used, we recognised the need to undertake serological assays using antigen closely matched to the vaccine strain. This also provided us with a way to assess the extent to which the test practice of using a single antigen for post-vaccination monitoring-irrespective of the administered vaccine-was valid.

This study was conducted to obtain data of antibody level of avian influenza subtype H5 in broilers at farms.

MATERIALS AND METHODS

Study farms

The study was carried out in 17 broiler farms. All were located in either the district of Sukabumi or Cianjur in the province of West Java, Indonesia. These provinces have a well developed poultry industry, being well placed to supply the large Jakarta market. All farms were considered as Sector 3 under DGLS Indonesia, but their size varied considerably.

All farms were selected by officials of the local District animal health office. There was no deliberate selection for any production or health criteria, though implicitly participating farms tended to be co-operative and had good relations with the local animal health office. Although not a truly random sample of poultry farms, the farms were considered representative of broiler farms in the area.

All farms were visited three times. At the first visit for each farm, a questionnaire about basic health and production parameters was completed, including the AI vaccinations used on the farm, and (where known) the vaccination regime used in the breeder flocks.

Sampling

The broiler, farms were contacted to determine when a production cycle was due to commence, and a visit undertaken within 1 – 2 days of the arrival of the day-old-chicks (DOC). Ten DOCs were randomly selected and blood collected from either the jugular vein or heart. A second visit was undertaken when the birds were about 14 days old, and sampling performed on 20 randomly selected birds. A final sampling was done when the broilers were market ready, at around 4 – 5 weeks of age.

Serology

All collected blood samples were transported at room temperature to the Virology laboratory at Bbalitvet (Bogor) within 12 hours. The serum was then extracted from the syringe, and transferred to an 1.8-ml Eppendof tube, where it was stored at -20°C before the Haemagglutination Inhibition tests were performed. The method used followed closely the OIE Terrestrial Manual (OIE, 2009). In brief, 25 µl of serum was diluted two-fold starting at 1 : 2 to 1 : 2048 in PBS in U-bottomed microwell plastic plates and 4 HA units of antigen was added to each well. Following incubation at room temperature for 30 min, 25 µl 1% chicken RBC was added to each well, and the plates were incubated at 4°C for 30 – 45 minutes to allow the RBCs to settle. Plates were read after tilting, and the HI titre was determined as the value of the highest dilution of serum causing complete inhibition of the 4 HA units of virus. Lack of inhibition at the 1:16 dilution was considered a negative result.

The antigens used for each test corresponded to one that was considered homologous to the vaccine used in the hens. In addition to the homologous antigen, all sera were tested against three isolates AI subtype H5N1 and one the heterologous antigen (H5N2); there are “Atg Smi-Hamd” was selected as this was considered to be close to the common circulating strain in 2005 – 2006, “Atg CSLK” was selected as being similar to a strain which was determined to have undergone antigenic drift in 2006 – 2007 (the Purwakarta isolate). Common vaccines did not protect against this strain in a challenge study in 2007 (Swayne, 2007), and “Atg Konawe” was selected on the basis of antigenic cartography, where it was considered to have a good cross-reaction against a broad range of

other antigens. Atg H5N2 was selected as Low Pathogenic Avian Influenza (LPAI) antigen.

Statistical analysis

The geometric mean titre (GMT) was calculated as the antilogarithm of the mean of the logarithms of each value. Negative titres (< 16) were regarded as 4 for the calculation of GMT.

RESULTS AND DISCUSSION

HPAI vaccination

Despite its effectiveness, most countries in Asia have avoided using vaccination to control HPAI H5N1, and have instead resorted to eradication (Peyre *et al.*, 2009). Indonesia is an important exception where the disease is recognised as endemic, and industry initiated vaccination is used routinely. The Indonesian experience with vaccination has important lessons for countries if they are ever faced with acceptance that eradication is not feasible. Two types of AI vaccines are currently used: (i) inactivated whole AI vaccines, and (ii) live recombinant vaccines (Peyre *et al.,* 2009), Currently in Indonesia only the former are used. The efficacy of H5 inactivated vaccines (of varying N types) to protect against "Hong Kong" H5N1 was first demonstrated under experimental conditions by Swayne *et al.* (2001). This was later supported by field observations that the Mexico 232 H5N2 inactivated vaccine prevented transmission in an outbreak in Hong Kong in 2002 – 2003 (Ellis *et al.*, 2004).

Immune responses in broilers

Maternal antibody AI subtype H5 in broiler DOC have diverse titres and depend on level of antibody at the time of egg production by laying hens. The pattern of maternal antibody titre in broilers from DOC is shown in Figure 1; the immunity against HPAI will be discharged when broiler chicks are 2 to 4 weeks old.

The results of serologic study for antibodies to Avian Influenza subtype H5 in broilers are shown in Figures 2 and 3. Maternal antibody titres against AI subtype H5 in broiler chicks from 17 farms varied for the four kinds of antigen. Broiler DOC had mean titre of more than 4 log 2 (protective titre) in 12 of 17 farms using antigen Konawe, and the other remaining less then 4 log 2 (Figure 2). Broiler DOC which has mean titre more then 4 log2 were detected in 2 from 17 farms by using CSLK antigen (antigenic drift). Broiler DOC had mean titre more than or equal to 4 log2 against H5N2 antigen in 5 of 17 farms. The result of maternal antibody in broiler DOC has more then 4 log 2 titre against a specific antigen, and it can indicate that the hens had been

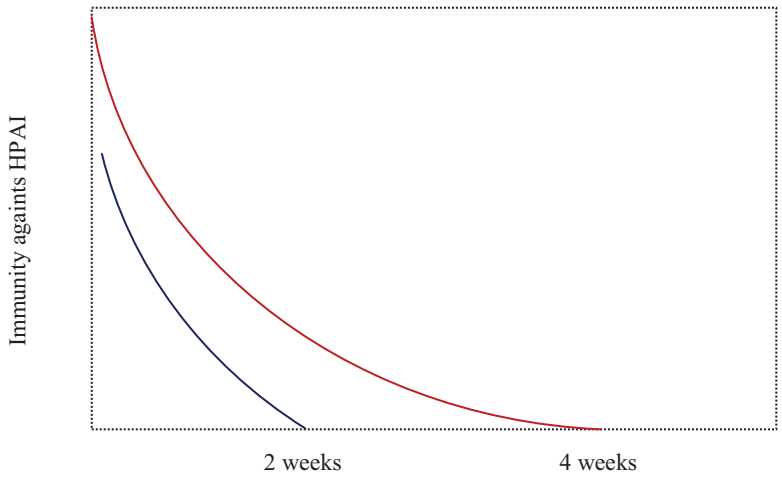

Figure 1. Lavel of maternal antibody in DOC

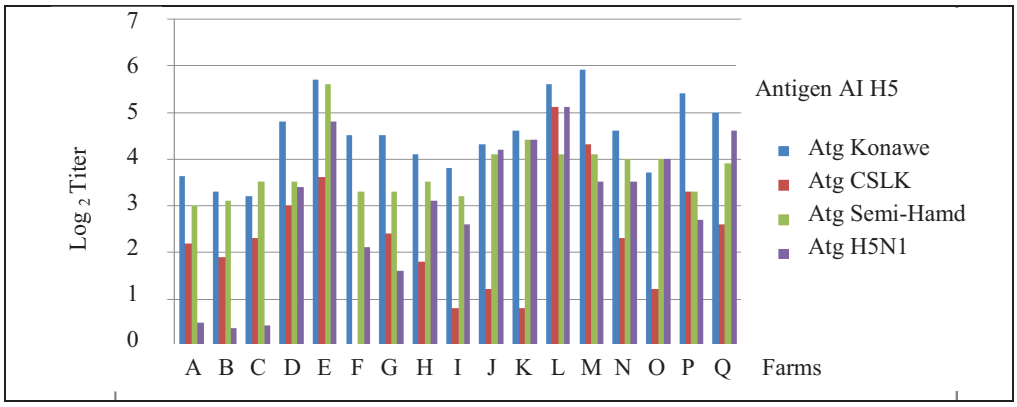

Figure 2. Titre of maternal antibody AI subtype H5 in broiler

vaccinated with that vaccine seed, such as a farm with initial L appeared to have high antibody titre against both antigen CSLK (antigenic drift) and H5N2 antigen.

Mean antibody titre against Avian Influenza subtype H5 in 2 weeks old broilers showed a sharp decline, and gradually disappeared by the time broiler chickens were 4 – 5 weeks old production (Figure 3). This is similar to the pattern of maternal antibody that has been described above (Figure 1). The data from the 17 farms in this study, all broilers are not vaccinated AI subtype H5. Broiler chickens produced without a sufficient AI antibody titre could be a threat for existence of field AI virus infection and spread of AI virus to the environment. A reason why is farms do not vaccinate against AI, because their life is short and the vaccination is not effective (Indriani and Dharmayanti, 2012; Lebdah and Shahin, 2010). Broiler chickens can be prevented from exposure of a field AI virus by good biosecurity on farms.

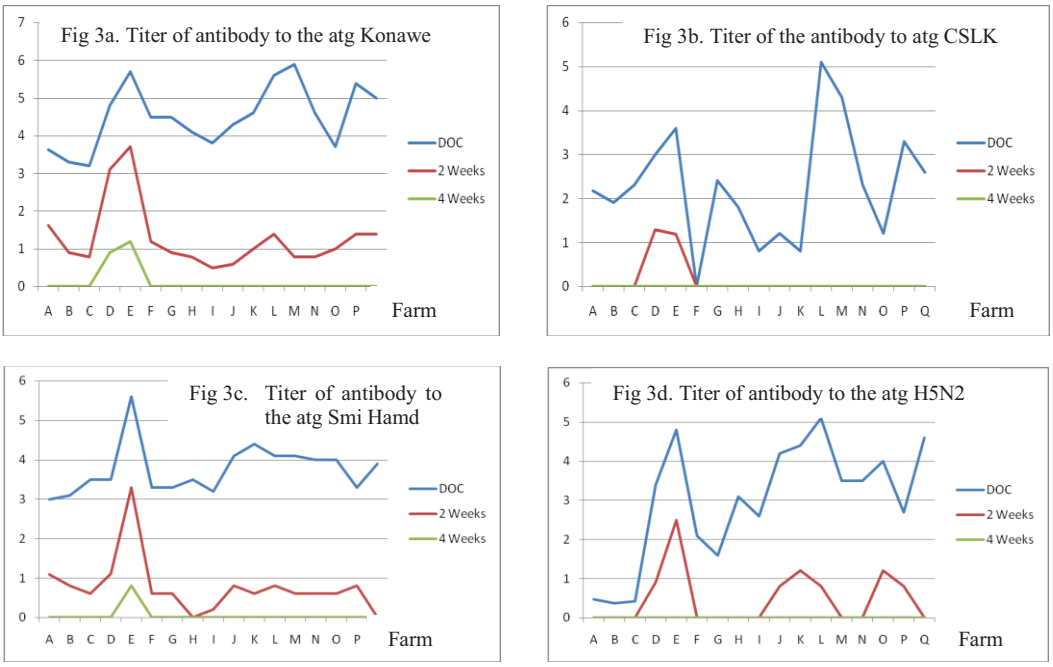

Figure 3. Curve of maternal antibody AI subtype H5 in broilers

CONCLUSION

This study concluded that antibody level for AI H5 in broilers on farms was not high enough titre to provide protection against infection, become risky when broiler were produced and could be a threat to existence of a field AI virus infection and spread to the environment.

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DISCUSSION

Questions:

What should farmers do to protect their birds, and what kind of vaccine to apply in farm to anticipate the varieties of virus?

Answers:

By a good biosecurity