Cross-Reaction of Duck and Chicken Sera against Avian Influenza H5N1 Virus Clades 2.1.3 and 2.3.2 Antigens by Hemagglutination Inhibition Test

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Received: June 2017, **Accepted:** October 2017

ABSTRACT

This study aims to determine the cross-reaction between the antigen of avian influenza (AI) H5N1 virus clades 2.1.3 and 2.3.2 in duck and chicken sera, which were vaccinated with inactivated AI H5N1 clade 2.1.3 vaccine against AI H5N1 clade 2.3.2 antigen and those vaccinated with inactivated AI H5N1 clade 2.3.2 vaccine against H5N1 clade 2.1.3 antigen. The sera tested were obtained from postvaccination and control (unvaccinated) chickens and ducks in the laboratory condition, and from AI H5N1 postvaccination ducks in the field condition. HI test was conducted by using AI H5N1 clades 2.1.3 and 2.3.2 antigens. The results of HI titer were analyzed by the geometric means and by ANOVA. The results show that cross-reactions in both chicken and duck sera after AI H5N1 clade 2.3.2 vaccination tested with AI H5N1 clade 2.1.3 antigen occurred with low antibody titers, whereas in chicken and duck sera postvaccination with avian influenza H5N1 virus clade 2.1.3 showed cross-reaction with high antibody titer against clade 2.3.2 antigen. The conclusion of this study, postvaccination sera of AI H5N1 clade 2.1.3 provide better cross-reaction compared to the postvaccination sera of AI H5N1 clade 2.3.2.

Keywords: sera, chicken, duck, AI H5N1, clade 2.1.3 and clade 2.3.2.

ABSTRAK

Studi ini bertujuan menentukan reaksi silang antara antigen (virus) avian influenza (AI) H5N1 *clade* 2.1.3 dan 2.3.2 di dalam serum ayam dan itik yang divaksinasi dengan vaksin inaktif AI H5N1 *clade* 2.1.3 terhadap antigen AI H5N1 *clade* 2.3.2 dan yang divaksinasi dengan vaksin inaktif AI H5N1 *clade* 2.3.2 terhadap antigen H5N1 *clade* 2.1.3. Serum uji diperoleh dari serum ayam dan itik coba pascavaksinasi dan kontrol (tidak divaksinasi) pada kondisi laboratorium dan serum itik lapang pascavaksinasi AI H5N1. Uji HI dilakukan dengan menggunakan antigen AI H5N1 *clade* 2.1.3 dan *clade* 2.3.2. Analisa hasil titer HI dilakukan dengan *geometric means* dilanjutkan dengan ANOVA. Hasil uji HI menunjukkan reaksi silang baik serum ayam maupun itik pascavaksinasi AI H5N1 *clade* 2.3.2 dengan antigen *clade* 2.1.3 terjadi dengan titer antibodi rendah, sedangkan serum ayam mupun serum itik pascavaksinasi AI H5N1 *clade* 2.1.3 menunjukan reaksi silang dengan titer antibodi tinggi terhadap antigen AI H5N1 *clade* 2.3.2. Simpulan dari studi ini, serum pascavaksinasi dengan vaksin AI H5N1 *clade* 2.1.3 memberikan reaksi silang lebih baik dibandingkan dengan serum pascavaksinasi dengan vaksin AI H5N1 *clade* 2.3.2.

Kata Kunci: serum, ayam, itik, AI H5N1, *clade* 2.1.3 dan *clade* 2.3.2.

INTRODUCTION

Avian influenza (H5N1) virus has spread to several countries in the world and caused a high mortality especially in poultry (Zessin, 2007). The H5N1 clade 2.1.3 virus, which causes many deaths in chickens and is economically disadvantageous, was firstly detected in Indonesia at the end of 2003 (Dharmayanti *et al*. 2004; Wiyono *et al.* 2004). From September to November 2012, there were many deaths of ducks in Central Java, Jogjakarta and East Java Provinces caused by AI H5N1 clade 2.3.2 virus (Wibawa *et al.* 2012). Wibawa *et al.* (2012) also reported that the AI H5N1 clade 2.3.2 virus is not a descendant of the AI H5N1 clade 2.1.3 virus in Indonesia, based on a low homology rate (91-93%) compared to viruses from clade 2.1.3.

Poultry (chickens and ducks) vaccinated with AI H5N1 can be avoided from the possibility of getting infected by the existing AI H5N1 field virus by either clade 2.1.3 or 2.3.2 (Indriani *et al*. 2014; Indriani & Dharmayanti 2015). The vaccine provides a high protective immune response generated by hemagglutinin (HA) and a small amount of neuraminidase (NA) present in the vaccine

antigen (WHO, 2002). Postvaccination immune responses in poultry can be measured by hemaglutination inhibition (HI) test (OIE, 2012). The current available AI H5N1 vaccines in the market are the AI H5N1 clades 2.1.3 and 2.3.2 vaccines (Ditjen PKH 2014). A study of the cross-reaction levels of AI H5N1 clades 2.1.3 and 2.3.2 vaccines in chicken and duck were carried out and discussed in this paper.

MATERIALS AND METHODS

Sera were obtained from groups of chickens and ducks vaccinated with AI H5N1 clades 2.1.3 and 2.3.2 in the laboratory (BB Litvet) and from ducks vaccinated with AI H5N1 vaccine in the field (farms).

The 3-week spesific pathogenic free (SPF) chickens were divided into 3 groups consisting of 20 chickens each namely; (1) vaccinated with inactivated AI H5N1 clade 2.1.3 vaccine, (2) vaccinated with inactivated AI H5N1 clade 2.3.2 vaccine and (3) unvaccinated (as the control). Three-week old local ducks (maternal AI H5N1 antibodies was negative) were divided into 3 groups of 20 each i.e.: (1) vaccinated with inactivated AI H5N1 clade 2.3.2 vaccine, (2) vaccinated with inactivated AI H5N1 clade 2.1.3 vaccine and (3) unvaccinated (as the control). Furthermore, the sera of six-week old chickens and ducks (3 weeks postvaccination) from each group were collected to be tested with HI test using AI H5N1 clade 2.1.3 and 2.3.2 antigens to detect antibodies prevaccination and postvaccination.

Forty sera from ducks vaccinated with AI H5N1 of unknown clade obtained from fields were tested with HI test using AI H5N1 clade 2.1.3 and 2.3.2 antigens to detect the antibody.

Preparing AI H5N1 clades 2.1.3 and 2.3.2 antigens

The antigen for HI test was prepared from the AI H5N1 virus clade 2.3.2: A/duck/Sukoharjo/ BBVW-1428-9 /2012 (BBVet-Wates-Jogja) and the AI H5N1 virus clade 2.1.3: A/Ck/Wj/PWT-Wij/2006 (BB Litvet). Each virus was propagated in the eleven-day old SPF eggs and inactivated with B-propiolacton by a ratio of 1: 2000 (Indriani and Dharmayanti, 2015).

Hemaglutination Inhibition (HI) Test

Before being used, the trial chicken and duck sera were inactivated for an hour at 56°C to remove specific inhibitors (Pedersen, 2008 and OIE, 2012) and duck sera were absorbed by red blood cells (RBC) to remove non-specific agglutinin (sometimes causing non-specific agglutination of chicken RBC) or to eliminate non-specific inhibitors of hemagglutination (Pedersen, 2008; OIE, 2012; WHO, 2002) prior to HI test. Briefly, a total of 25 µL of duck serum and 25 µL of 10% chicken RBC in PBS (OIE, 2012) in a V type microplate, incubated at 4 ° C for 1 hour and centrifuged at 800 g for 5 minutes to separate RBC. Around 25 µL was transferred into the first hole of a microplate containing 25 µL PBS. The duck and chicken sera were diluted at 1: 2and at 1:1respectively, thus the minimum detectable titers were 4 (2 log2) and 2 (1 log2) for the duck and chicken sera respectively. HI test was performed with 1% chicken RBC in PBS and used 4 hemaglutination units (HAU) antigen (OIE, 2012).

HI titers are expressed as reciprocal values of the highest serum dilution causing the complete inhibition of agglutination of 4 HAU antigens. HI titer of 16 (4 log2) is classified as positive for avian influenza antibodies, according to OIE guidelines (2012). HI test procedures follow OIE (2012) and INDRAINI *et al*. (2004).

HI test results were calculated by the geometric means titer (GMT) and analyzed by ANOVA for the significance ($P < 0.05$).

RESULTS

The sera of chickens vaccinated with inactivated AI H5N1 clade 2.1.3 against AI H5N1 clade 2.1.3 antigen shows an average titer of 5.6 log2 with confidence interval (CI) of 5.1 – 6. The cross-reaction against AI H5N1 clade 2.3.2 antigen shows an average titer of 4.9 log2 with CI of 4.3 - 5.3 (Figure 1). The sera of chickens vaccinated with inactivated AI H5N1 clade 2.3.2 vaccine against AI H5N1 clade 2.3.2 antigen shows an average titer of 6.8 log2 with a CI of 6.2 – 7.4, and the cross-reaction against AI H5N1 clade 2.1.3 antigen shows an average antibody titer of 2.8 log2 with CI of 2.4 – 3.2.

Meanwhile, the serum of unvaccinated SPF chickens shows a negative antibody titer against both AI H5N1 clades 2.1.3 and 2.3.2 antigens (Figure 1).

The sera of chickens vaccinated with AI H5N1 clade 2.1.3 and those sera of chickens vaccinated with AI H5N1 clade 2.3.2 show cross-reactions with AI H5N1 clade 2.1.3 and clade 2.3.2 antigens. HI test results show that the highest chicken anitbody titer occurred when sera were tested with homologous antigen (vaccine antigen is the same with HI test antigen). The cross-reaction titer showed significant different ($p < 0.05$) between the sera of chickens vaccinated with AI H5N1 clade 2.1.3 against clade 2.3.2 antigen and the sera of chickens vaccinated with AI H5N1 clade 2.3.2 againts clade 2.1.3 antigen.

The sera of ducks vaccinated with inactivated AI H5N1 clade 2.1.3 against AI H5N1 clade 2.1.3 antigen shows an average titer of 4.4 log2 with confidence interval (CI) of 3.7 – 5,1. The cross-reaction against AI H5N1 clade 2.3.2 antigen shows an average titer of 3.5 log2 with CI of 2.9 - 4 (Figure 2).

The sera of ducks vaccinated with inactivated AI H5N1 clade 2.3.2 vaccine against AI H5N1 clade 2.3.2 antigen shows an average antibody titer of 4,7 log2 with CI of 4-5, and the cross-reaction against AI H5N1 clade 2.1.3 antigen does not show the presence of the antibody (negative titer). The unvaccinated duck sera shows negative antibody titer against both AI H5N1 clades 2.1.3 and 2.3.2 antigens (Figure 2). The cross-reaction titer showed significant different ($p < 0.05$) between the sera of ducks vaccinated with AI H5N1 clade 2.1.3 against clade 2.3.2 antigens and the sera of ducks vaccinated with AI H5N1 clade 2.3.2 againts clade 2.1.3 antigen.

The antibody titer is influenced by the type of vaccine and antigen, the sera of chickens and ducks vaccinated with AI H5N1 show positive antibodies, while the control chickens (unvaccinated) sera shows a negative titer

The HI test results of sera collected from vaccinated ducks show the highest titer when tested with homologous antigen. The cross-reaction titers from the HI test show a significant difference ($p < 0.05$) between the titer of the ducks vaccinated with AI H5N1 clade 2.1.3 against clade 2.3.2 antigen and the ducks vaccinated with AI H5N1 clade 2.3.2 against clade 2.1.3 antigen.

The HI test results of sera collected from vaccinated ducks show that the highest titer obtained when being tested with homologous antigen. The cross-reaction antibody titers from the HI test show a significant difference ($p < 0.05$) between the titer of the ducks vaccinated with AI H5N1 clade 2.1.3 against clade 2.3.2 antigen and the ducks vaccinated with AI H5N1 clade 2.3.2 against clade 2.1.3 antigen.

The titer of field duck sera vaccinated with AI H5N1 vaccine tested against the AI H5N1 clade 2.1.3 antigen shows an average antibody titer of 4.3 log2 with CI of 3.5 - 5, and the cross

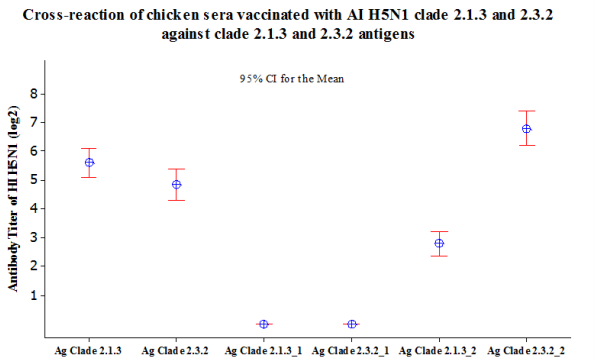

Figure 1. HI test results of chicken sera vaccinated with AI H5N1 clades 2.1.3 and 2.3.2 against AI H5N1 clade 2.1.3 and 2.3.2 antigens

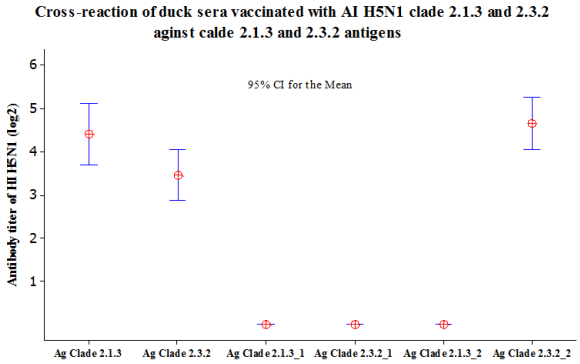

Figure 2. HI test results of duck sera vaccinated with AI H5N1 clade 2.3.1 and 2.3.2 against AI H5N1 clade 2.1.3 and 2.3.2 antigen.

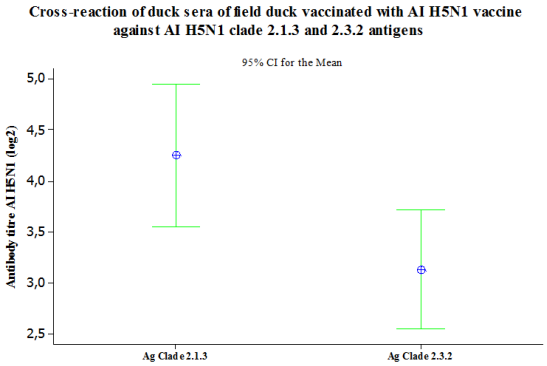

Figure 3. HI test results of ducks sera of field ducks vaccinated with AIH5N1 against AI H5N1 clade 2.3.1 and 2.3.2 antigens

-reaction titer against AI H5N1 clade 2.3.2 antigen shows an average antibody titer of 3.1 log2 with CI of 2.5 - 3.7 (Figure 3). The HI test results of field ducks vaccinated with AI H5N1 vaccine show high antibody titers when tested with AI H5N1 clade 2.1.3 antigen and good cross-reaction against AI H5N1 clade 2.3.2 antigen (Figure 3).

DISCUSSION

The avian influenza (AI) H5N1 clades 2.1.3 and 2.3.2 virus is highly pathogenic in poultry (OIE, 2013). Chickens and ducks infected by these viruses can result in illness and death. Vaccination can prevent ducks and chickens from H5N1 virus clades 2.1.3 and 2.3.2 infection, so that chickens or ducks have immunity by forming antibody against AI H5N1 viruses clades 2.1.3 and 2.3.2. Antibody titers in chicken and duck sera can be measured by HI test. The results of this study show that the antibody of ducks and chickens vaccinated with AI H5N1 clade 2.1.3 vaccine cross-reacts with AI H5N1 clade 2.3.2 antigen. This can be shown as presented by Indriani *et al.* (2014) and Indriani & Dharmayanti (2015); the protection level of Mojosari ducks and SPF chickens vaccinated with AI H5N1 clade 2.1.3 vaccine were 80% and 90% respectively against AI H5N1 virus clade 2.3.2 infection in the laboratory conditions, and the birds with the antibody titer of 6 log2 showed undetectable excretion of the infectious virus. This study also shows that chickens or ducks vaccinated with AI H5N1 clade 2.3.2 vaccinehad low cross-reaction antibody titers against AI H5N1 clade 2.1.3 antigen i.e. 2.8 log2 with CI of 2.4 – 3.2 in chickens and negative titer in ducks, which are still under the positive titer classified by OIE (4 log2) (OIE, 2012). Sera of chicken and ducks vaccinated with AI H5N1 clade 2.1.3 against AI H5N1 clade 2.3.2 antigenhad better cross-reaction compared to the sera of chickens and ducks vaccinated with AI H5N1 clade 2.3.2 vaccine against antigen of AI H5N1 clade 2.1.3. This can provide an overview in the selection of AI H5N1 vaccine for the prevention of infection in duck and chickenpopulation in the field. Also, the AI H5N1 clade 2.1.3 antigen showed superior compared to clade 2.3.2 antigen indicated by higher cross-reaction pattern, suggesting that the antigen generated from AI H5N1 clade 2.3.2 virus is better for diagnostic test. This is in agreement with previous study by Ducates *et al.* (2011) in which the cross-reactivity was evaluated for AI H5N1 clades 0, 1, 2.1, 2.2, 2.3, and 4 viruses for the selection of a prospective diagnostic reagent for AI H5N1 strains.

The sera of field ducks had high antibody titers againts AI H5N1 clade 2.1.3 antigen and lower titer against AI H5N1 clade 2.3.2 antigen; this may indicate that the sera tested in this study were obtained from the ducks vaccinated with AI H5N1 clade 2.1.3 vaccine.

CONCLUSION

It can be concluded that the sera of chickens and ducks vaccinated with a single AI H5N1 clade 2.1.3 vaccine show cross-reactions with high HI titers against AI H5N1 clade 2.3.2 antigen, compared to the sera of chickens and ducks vaccinated with a single AI H5N1 clade 2.3.2 vaccine that show low HI titers against AI H5N1 clade 2.1.3 antigen.

AKNOWLEDGMENTS

This research was supported by funds from BB Litvet DIPA in 2014. We thank Heri Hoerudin, Apipudin and all collaborators assisting the implementation of this study.

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