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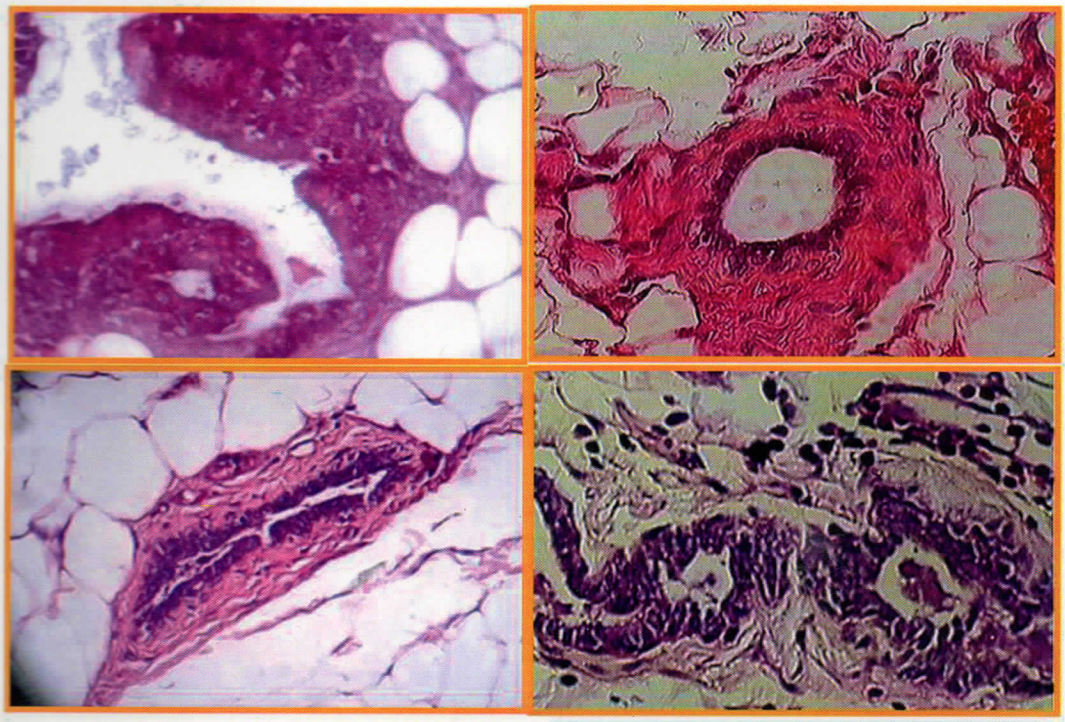

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THE STUDY OF CROSS REACTIVITY BETWEEN H5 CLADE 2.3.2 SERUM AND H5 CLADE 2.1.3 VIRUS BY USING SERUM NEUTRALIZATION TEST

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ABSTRACT

The aim of this study was to find the effectiveness of H5 clade 2.3.2 vaccine to H5 clade 2.1.3 and 2.3.2 virus. The study was located at Avian Influenza Research Center (AIRC) University of Airlangga in the BSL-2 Laboratory. Study was conducted from June to July 2014. Serum of H5 clade 2.3.2 that be used had the titre of 2^7 by Haemaglutination Inhibition (HI) assay. Serum was tested with both viruses by using serum neutralization (SNT) assay in the MDCK cell culture. The data was analyzed by Reed and Muench Formula. Tissue Culture Infectious Dose 50 ($TCID_{50}$) of H5 clade 2.1.3 was 1.78×10^8 $TCID_{50}$/ml and H5 clade 2.3.2 was 5.6×10^7 $TCID_{50}$/ml. The SNT result showed that 1:100 dilution of serum was able to neutralize H5 clade 2.3.2 virus and protect at least 50% of culture cell. The serum was not able to neutralize H5 clade 2.1.3 virus.

Keywords: *Avian influenza, H5N1 clade 2.3.2 Vaccine, Serum Neutralization Test (SNT), Cross Reactivity*

Introduction

Avian influenza (AI) or bird flu is a viral disease that attacks the avian. It caused by type A influenza viruses of the family Orthomyxoviridae. The disease is also classified as zoonoses because it can infect mammals including humans. According to World Health Organization (WHO) until 24th January 2014, there were total 195 human cases with 163 death in Indonesia, while there were total 650 cases and 386 death in the world (WHO, 2014).

According to data released by the Komite Nasional Pengendalian Flu Burung dan Kesiapsiagaan Menghadapi Pandemi Influenza (Komnas FBPI) (2008), the outbreak of highly pathogenic avian influenza and pandemic has beer spread in several countries in Asia, China, South Korea, Taiwan, Vietnam, Cambodia, Thailand, Laos, Pakistan and Indonesia, since the beginning of 2003. In our country highly pathogenic avian influenza (AI) in poultry has reached 31 provinces of 33 provinces in Indonesia, and endemic in 298 of 484 districts / cities in

Indonesia. Since that time the mortality recorded as many as 4.7 million of chicken, even until the end of February 2004, the death of birds recorded 6.2 million head (Raharjo and Nidom, 2004).

The new clade of AI outbreaks became evident at the end of 2012. in September- November 2012, high mortality of ducks in Central Java, East Java and Yogyakarta has been reported and identified as the cause of AI outbreaks subtype H5N1 clade 2.3.2 (Wibawa et al, 2013).

The H5N1 clade 2.3.2 is a new outbreak of AI virus in Indonesia. According to research conducted by Dharmayanti and Hartawan (2013), H5N1 clade 2.3.2 virus has 97-98 %

nucleotide similarity with VietNam clade 2.3.2 virus and 91-94 % with Indonesian Clade 2.1 virus. This is different from the case of AI that infected chickens in Indonesia that caused by the virus of H5N1 clade 2.1.3 before.

In order to control the avian influenza outbreak, the government has released nine strategic policies. Nine of the strategic policies through Decree of the Director General of Livestock Production No. 17/Kpts/PD.640/F/-02.04 on guidelines for prevention, control and eradication of animal diseases transmissible avian influenza in poultry. The core of the strategic policy are: (1) the application of strict biosecurity; (2) selective depopulation in infected areas; (3) vaccination; (4) traffic control (5) surveillance and tracking of cases; (6) public awareness; (7) charging back (restocking) poultry cages; (8) stamping out in newly infected areas and; (9) monitoring, reporting, and evaluation (Komnas FBPI, 2008)

Vaccination can be used as control because it can reduce the mortality and morbidity (Raharjo and Nidom , 2004). Vaccination in the veterinary pursues four goals: (i) protection from clinical disease, (ii) protection from infection with virulent virus, (iii) protection from

virus excretion, and (iv) serological differentiation of infected from vaccinated animals (so-called DIVA principle) (Kamps*et al* , 2006)

In the field, neither commercially nor experimentally tested vaccines have been shown to fulfil all of these requirements so far. The results of laboratory studies of Balai Besar Penelitian Veteriner (BBALITVET) (2014) showed that commercial local isolate vaccine (H5N1 AI virus clade 2.1.3) is less effective against H5N1 clade 2.3.2 AI in ducks. The vaccine can prevent mortality but the shedding of virus still detected.

Based on the research background above, the research about "Cross Reactivity Between H5 Clade 2.3.2 Sera And H5 Clade 2.1.3 Virus By Using Serum neutralization Test" will be conducted

Materials and Methods

Research was held at Avian Influenza Research Center (AIRC) University of Airlangga in laboratory BSL-2. Study was conducted from June to July 2014. Research was carried out by taking sera antibody titres which has 2^7 of the HI test. Furthermore, $TCID_{50}$ values of the viruscalculated by using the Reed-Muench method. Then proceed with the SNT method on both of virus, H5N1 clade 2.1.3 to see the cross-reaction and H5N1 viruses to neutralization capability. The SNT method calculated in accordance Reed-Muench calculation.

Reed-Muench Formula

$$PD = \frac{\text{(percentage positif above 50\%)} - 50\%}{\text{(\% positive above 50\%)} - \text{(\% positive below 50\%)}}$$

Log 50% endpoint =

(log dilution above 50%) - (PD x log dilution factor)

Substance and Equipments of Research

The substance needed for this study were 2^7 antibody titre of H5 clade 2.3.2 serum, H5N1 clade 2.1.3 virus (A/Ck/-Ind/114/08), H5N1 clade 2.3.2 virus (A/Dk/Ind/433/12), 0.5 % RBC, PBS, MDCK cells, MEM, MM, Crystal Violet Stain, Trypsin EDTA.

The equipments needed for this study were BSC, gloves, mask, pippete, vacuum pipette, tray, 1,5 ml eppendorf tube, incubator, yellow tips, blue tips, 96 well "V" microplate, 48 well microtittre plate, 96 well microtittre plate micro-pipette, laboratory shaker, centrifuge, autoclave, waterbath, microscope, T-75 Flasks.

Research Result

Calculation of Virus Titre

The $TCID_{50}$ of H5 virus clade 2.1.3 (A/Ck/Ind/114/08) and clade 2.3.2 (A/Dk/ind/433/12) result was showed in the table below :

$TCID_{50}$ of H5N1 virus (A/Ck/Ind/-114/08) is 1.82 x 10^8 $TCID_{50}$/ml. The result was calculated by Reed and Muench formula (Table 4.1a).

$TCID_{50}$ of H5N1 virus (A/dk/Ind/-433/12) is 5.62 x 10^7 $TCID_{50}$/ml. The result was calculated by Reed and Muench formula (Table 4.1b).

Serum Neutralization Test

The result of H5 clade 2.3.2 serum to H5 clade 2.3.2 virus is positive (+). The serum was able to neutralize the virus and protect cell culture. The 50% endpoint of neutralization of the serum

Table 4.1a. $TCID_{50}$ result of H5 clade 2.1.3

Virus Dilutions	CPE		Total		Ratio (+)	% positive (+)
	+	-	+	-		
10^{-1}	3	0	20	0	20/20	100
10^{-2}	3	0	17	0	17/17	100
10^{-3}	3	0	14	0	14/14	100
10^{-4}	3	0	11	0	11/11	100
10^{-5}	3	0	8	0	8/8	100
10^{-6}	3	0	5	0	5/5	100
10^{-7}	2	1	2	1	2/3	67
Control	-	3	-	-	-	-

Table 4.1b. $TCID_{50}$ result of H5 clade 2.3.2 virus

Virus Dilutions	CPE		Total		Ratio (+)	% positive (+)
	+	-	+	-		
10^{-1}	3	0	19	0	19/19	100
10^{-2}	3	0	16	0	16/16	100
10^{-3}	3	0	13	0	13/13	100
10^{-4}	3	0	10	0	10/10	100
10^{-5}	3	0	7	0	7/7	100
10^{-6}	3	0	4	0	4/4	100
10^{-7}	1	2	1	2	1/3	33
Control	-	3	-	-	-	-

Table 4.2 SNT result of H5 clade 2.3.2 serum to H5 clade 2.3.2 virus

Serum dilution		CPE		Total		Ratio	%	%
Quantitatve	Log_{10}	+	-	+	-	(+)	(+)	protective
1:10	10^{-1}	0	4	0	20	0/20	0	100
1:20	$10^{-1.3}$	0	4	0	16	0/16	0	100
1:40	$10^{-1.6}$	0	4	0	12	0/12	0	100
1:80	$10^{-1.9}$	0	4	0	8	0/8	0	100
1:160	$10^{-2.2}$	2	2	2	4	2/6	33	67
1:320	$10^{-2.5}$	2	2	4	2	4/6	67	33
1:640	$10^{-2.8}$	4	0	6	0	6/6	100	0
Control		0	4	-	-	-	-	

is 1:100. It means that the serum can protect at least 50% of the cells by neutralizing the virus at the 1:100 dilutions. The result was calculated by Reed anfMuench formula (Table 4.2).

The result of H5 clade 2.3.2 serum to H5 clade 2.1.3 virus is negative (-). The serum was not able to neutralize the virus.

Discussion

The result of SNT showed that the serum were able to neutralize and protect at least 50% of cell culture at the 1:100 dilution to H5 clade 2.3.2 virus. It is consistent with the research result of Risqiawan (2011) that said the antibody titre of the serum which able to neutralize and give protection if it has 2^7 of HI titre. While serum tested with H5 clade 2.1.3 virus on SNT, it was showed that the serum was not able to neutralize the virus. This result have shown that the absence of cross-reactions between H5 clade 2.3.2 serum and H5 clade 2.1.3 virus.

The SNT result shows that H5 clade 2.3.2 vaccine was able to neutralize H5 clade 2.3.2 virus (A/Dk/Ind/433/12). But it was not able to neutralize H5 clade 2.1.3 virus (A/Ck/Ind/114/08). It can be happen because of the compatibility of the seed vaccine and the virus. The H5 clade 2.3.2 seed vaccine is more compatible with H5 clade 2.3.2 virus (A/Dk/Ind/433/12) than clade 2.1.3 virus (A/Ck/Ind/114/08).

A vaccine will be effective as if meets several requirements, that were antigenic match (Compatibility to field virus) and antigenic mass (high titre). According to Sudarisman (2006) and Wibawan (2012), the persistence of AI virus in the field was caused by the use of vaccine strains that have difference in antigenic match or low in serological homology from the outbreak virus. So that for the selected vaccine should have high degree of homology to the virus that circulating in the field.

Conclusion

The serum of 2^7 HI titre of H5 clade 2.3.2 was able to neutralize H5 clade 2.3.2 virus. It can neutralize and protect at least 50% of culture cell in the SNT at 1:100 dilutions. It was not able to neutralize H5 clade 2.1.3 virus in the SNT.

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