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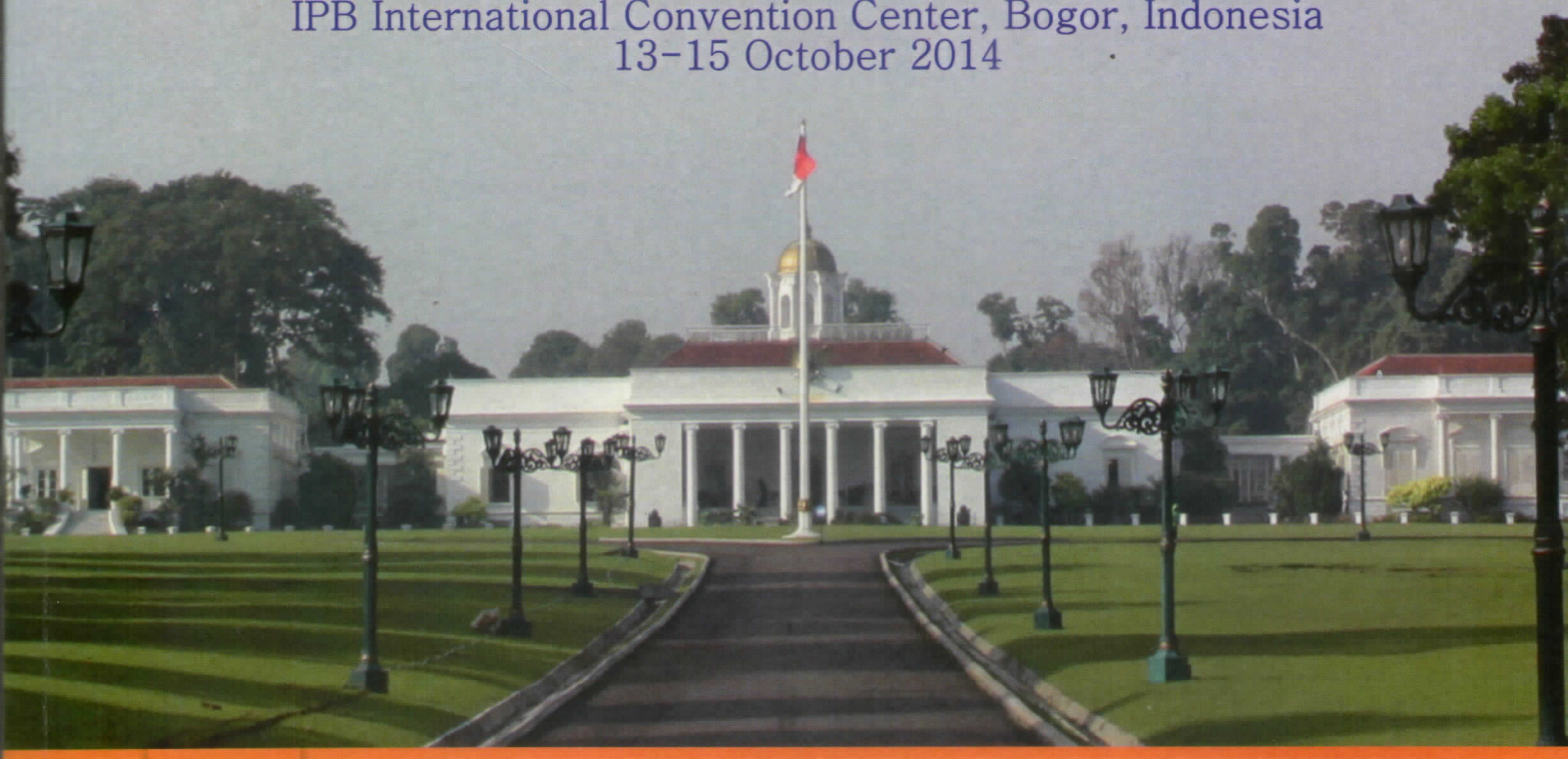
THE 14TH ANNUAL WORKSHOP OF THE REGIONAL NETWORK ON ASIAN SCHISTOSOMIASIS AND OTHER HELMINTH ZOONOSIS

THE 5TH ANNUAL MEETING OF SOUTH EAST ASIA VETERINARY SCHOOL ASSOCIATION

THE 3RD SCIENTIFIC MEETING OF INDONESIAN VETERINARY SCHOOL ASSOCIATION

IPB International Convention Center, Bogor, Indonesia
13-15 October 2014



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Effect of pH on the Stability of Anti Avian Influenza H5N1 IgG from Colostrum of Cows Vaccinated by H5N1

Anita Esfandiari[1]*, Fajar Kawitan[2], Sri Murtini[3], Sus Derthi Widhyari[1]

[1]Department of Veterinary Clinic, Reproduction, and Pathology, Faculty of Veterinary Medicine Bogor Agricultural University; [2)] Veterinary Practitioner; [3]Department of Veterinary Diseases and Public Health,Faculty of Veterinary Medicine, Bogor Agricultural University
*Corresponding author: esfandiari1962@gmail.com

Key words: bovine colostrum, IgG, H5N1, pH

INTRODUCTION

Since the first outbreak of avian influenza in Indonesia in 2005, it was reported 193 cases with 161 dead casualties [1]. Until now there was no avian influenza (AI) vaccine allow to be use for preventing the case in human [2]. The use of medication such as Tamiflu shows a number of limitation such as to generate resistency and tend to affect only at the early stage of infection (up to 48 hr post-infection). Therefore, the passive immunization approaches by the administration of hyperimmune colostrum may be as an alternative to control the AI.

Colostrums have a potency or prospect as a "biological factory" to produce antibody against AI for passive immunotherapy purposes on controlling avian influenza [3]. However, the oral application of immunoglobulin G (IgG) indicates a number of constrains, since IgG very sensitive to the intestine environment condition. IgG stability very much affected by intestine environment such as lower pH which destroy the protein molecule. This experiment was designed to study the oral application of colostrum contained anti AI (H5N1) IgG with emphasis in the potency of anti H5N1 IgG in the bovine colostrum in various pH conditions.

MATERIALS AND METHODS

Colostrum samples which contained anti AI H5N1IgG were collected from Holstein cows vaccinated with H5N1 inactive vaccine at the last trimesters of pregnancy. Colostrums were collected after partus and treated according to [3]. Detection of colostral anti H5N1 IgG used the enzyme linked immunosorbent assay (ELISA) technique, in-direct method. Acid and alkaline buffer were exposured to the colostrum samples to create low and high pH condition used method developed by [4].Titers of colostral anti H5N1 IgG before and after the exposure to low and high pH were measured using hemaglutination inhibition (HI) test, beta method.

RESULTS AND DISCUSSIONS

Results of the experiments had indicated that anti H5N1 IgG titers before the exposure to low and high pH condition were 2^8 HI Unit. Anti H5N1 IgG titers were declined following their exposure to low and high pH (Table 1). The anti H5N1 IgG titers following the exposure to pH 5 decreased at amount of 3.75% and 16.25%, respectively following 30 and 60 minutes incubation. However, anti H5N1 IgG titers following the exposure to pH 4 were 12.5% and 16.25% lower than standart. The exposure of high pH (9-10) caused the decrease anti H5N1 IgG until 100%. Moreover, the decreased of pH from 5 to 4 following by the increased incubation time from 30 to 60 minutes had shown a little effect on the decrease of anti H5N1 IgG titers.

Table 1. Anti-H5 IgG titers of colostrum before and after treatment to low and high pH

Anti-H5 IgG titers prior to treatment (pH 6,5)	Anti H5N1 IgG titers following treatment to acid and alkaline pH							
	4		5		9		10	
	Incubation time (minutes)							
	30	60	30	60	30	60	30	60
2^8	2^7	$2^{6,7}$	$2^{7,7}$	$2^{6,7}$	2^0	2^0	2^0	2^0

The decrease of IgG titers at the acid conditions indicated the degradation of IgG. Antibody, similar to other proteins, very sensitive to pH changes which cause physical and chemical degradation. Exposure to acid (pH below 4) may cause isomeration, hydrolysis, and fragmentation. On the other hand, very low pH (below 3), affect the conformation of tertiary structure of antibody [5].

Isomerization on protein might be done on a number of amino acids such as aspargine, aspartate and glutamine. The activity of antibody could be affected when isomerized aspartate involves in the antigen-antibody reaction [5]. *Humanized monoclonal antibody* anti-HER2 (Herceptin®) may reduce Asp 120 activity up to 80 - 90% on the heavy chain of antibody isomerized and become isoaspartate [6].

Fragmentation is a process which done as a result of hydrolysis on the *peptide backbone*. Amide chains Asp-Gly and Asp-Pro are chains sensitive to hydrolytic cutting, while other subject such as Asn-Ser also known as subject of hydrolysis [7]. According to [8], a peptide chain is susceptible to hydrolysis that particularly takes place in the domains interface close to or at the antibody loop structures. Hydrolysis often proceeds at the hinge region, the most flexible area of antibody [9]. If compared to intact antibody, fragmented antibodies are more fragile against protease enzyme in the intestine and have shorter half-life. Therefore, fragmentation on theurapeutic antibodies reduce their capacity to bound antigen and affected their pharmacokinetic properties [5].

Exposure to high pH on colostrum destroyed the IgG (titer 2^0). This result was different with the IgG profile in serum which exposed to high pH. In the case of serum IgG, high pH (pH 9-10) exposured did not destroy of IgG. This result indicated that there was difference between colostral IgG and serum IgG in high pH condition. The damage in colostral IgG might be a result of change of pH solution, where the normal range of colostrum pH is 6.00 to 6.61 [10]. When alkaline solution is added, pH were changed and resulted on the damage of IgG. According to [5], pH changes reflected the chemical stress resulted on antibody degradation.

The titer of colostral IgG following treatment decreased at the pH 4 and 5 with ranging from 2^6 to $2^{7.7}$. Neutralization test need to be performed to test the efficacy. As comparison, Anti virus IgG H5 from rat serum with the titer of 2^8 be able to netralize 100% of virus with the titer 10^4 EID_{50} [11].

The reduced activities of IgG caused by low and high pH condition have indicated the importance of specific treatment of colostral IgG on the formulation of antibody against H5N1. The oral application of immunization is the easiest way, however, it need a special treatment to cope up with extreem pH environment as well as enzymatic reaction in the intestine.

CONCLUSIONS

The exposure of colostral IgG to the low (4-5) and high (9-10) pH condition decreased the anti H5N1 IgG titers. Anti-H5 IgG from bovine colostrum is degraded in acid and alkaline conditions.

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