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THE EEFECT OF COMBINATION OF CRUDE SALIVE GLAND EXTRACTE OF STOMOXYS CALCITRANS (DIPTERA: MUSCIDAE) WITH COLOSTRUM IMMUNOGLOBULIN-G ON IGG SERUM LEVEL OF YOUNG HORSES

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Abstract

This experiment was conducted to evaluate the influence of crude salive gland extracted from Stomoxys calcitrans applied to twelve young horses traditionally maintained. The treatment in study realized by injecting 0.5 ml of SGE to the foals compared to the control group of animals without SGE treatment. Each group was divided into two sub groups received colostrum and the other did notreceived any IgG colostrum. The IgG level was 60 gr/L colostrum and distributed to the foals with 9.6 gr IgG^{-1}J. The injection of SGE realized at the first day of experiment. The volume of blood collection was 3 ml through vena jugulaire at the 14th days after SGE injection. As soon as possible after collection, the blood was centrifuged and then its serum was placed in micro-tube to be observed. We did not found interaction of SGE and IgG on imunoglobuline-G serum (P>0.05) while the IgG serum level increased very significantly (P<0.001) as the influence of single treatment of SGE as described in data of A2B1 and A2B2, compared to the control without SGE.In other side this study showed an important relation between entomology and animal husbandry especially to the health care improvement for the young animals by using the antigen substance of the insect (Stomoxys calcitrans).

Key words: Entomology, Immunoglobuline,Stomoxys calcitrans.

INTRODUCTION

In entomology *Stomoxys calcitrans* (Diptera: Muscidae) flies are well known as stable flies, cosmopolite, economically are pests and able to affect the health of livestock. This insect can transmit infectious diseases in livestock and humans (Graczyk et al., 2001). Such insect can be a serious pest to livestock production, but in other side, this insect has antigen-5 accumulated in salivary gland that able to stimulate the synthesis of IgG antibodies of mammals (Ameri et al., 2008; Campbell et al., 2001).
Today, immunization studies using salivary protein from horn fly (*Haematobia irritans*) demonstrated the ability to reduce the size and to slow the progression of the eggs if this flies itself in immunized animal (Cuop et al., 2004). Ameri et al. (2008) showed that the content of the crude extract of salivary glands (SGE), *Stomoxys calcitrans* dominated by immunoglobulin binding protein or proteins called antigen 5 (AG5) with BM 27 kDa that give immuno-reactive response in cattle.
The problem underlying in this study was, whether SGE *Stomoxys calcitrans* provided immuno-reactive response or not in young horses having placenta of epitheliochorial.
In fact, horses which categorized as animal with epitheliochorial placenta, caused a high risk of failure of passive transfer of immunoglobulins. Therefore the use of crude of SGE could be an alternative solution for immunity enhancement of horses.

MATERIALS AND METHODS

The collection of *Stomoxys calcitrans* were carried out at the farm of 'Sentrum Agraris Lotta' (SAL). Twelve foals were used which were maintained in traditional farms in Minahasa region in North Sulawesi Indonesia. Identification of antigen proteins realized in Laboratoryof Immunology and Parasitology at the University of Salamanca, Spain.

Preparation of crude SGE

Salivary gland extract (SGE) of *Stomoxys calcitrans* obtained according to the procedure Swist et al. (2002). The dissection of *Stomoxys calcitrans* was placed in a Petri dish and placed nice. This dissection was realized under photonic microscope model Meiji EMZ-TR. We removed the head and the abdomen segment then we took carefully the part of salivary gland site in upper of the front legs in thorax, then transferred the salivary glands in the glass vessel filled with 1M Phosphate buffer solution with pH 6 and centrifuged at 5000 rpm for 10 min, then the supernatant obtained as saliva gland extract (SGE). After getting SGE extract, followed by identification with ND-100 spectrophotometer and separation by SDS-PAGE.

Research procedures

Each group divided into sub-groups: those who received the colostrums and other group did not received. The IgG content of colostrum utilised was $\pm$60g IgG/L of fresh colostrum. The treatment of colostrum IgG was distributed to animal with a consumption of 160 ml colostrum per hours(≈ 9.6g ofIgG-1J) in the first day after foaling. The treatment of SGE was delivered by injection subcutaneous on first hour of the experiment. Then blood samples were taken approximately 3 ml of venous jugular after 14 days of treatment. The blood was centrifuged immediately and then serum was collected in Eppendorf tube to prepare for the IgG analysis. Data were subjected to two ways ANOVA in completely randomized design in which groups were arranged in 2 X 2 factorial model. Factor A= crude of SGE (A1=0 ml SGE; A2=0.5mlSGE) which 0.5 ml equivalent to 100 µg. Factor B=Colostrum IgG (B1=0 IgG; B2=9.6 g IgG) each with three replication.

Statistical Analysis

To evaluate the effect of SGE crude and IgG colostrum on foal serum IgG level, the variance of data obtained statistically analysed according to Zar (1996).

Table 1. Group Treatment of SGE and Colostrum IgG

Repetition	SGE		Colostrum IgG	
R1	0 ml SGE	0.5 ml SGE	0 g IgG	9.6 g IgG
R2	0 ml SGE	0.5 ml SGE	0 g IgG	9.6 g IgG
R3	0 ml SGE	0.5 ml SGE	0 g IgG	9.6 g IgG
Total	(A1B1)	(A1B2)	(A2B1)	(A2B2)

Analysis of Serum IgG

The blood serum IgG level analysed by using *Single Radial ImmunoDiffusion* method, started with the following procedures: filling well 4 with 15 µl of standard, and then filled the trench (well) gel 15 µl sample of blood plasma. Then moved the plate into the incubator box at a temperature of 30-40^{o}C left for about 16 hours so that antibodies diffused in a gel containing anti-IgG antigen, after which the plate was filled with a solution of 2% acetic acid and incubated for one minute. The following stage was the drain plate and the gel rinsed twice using deionized water.

After that for the last time, the plate is filled with deionized water or distilled water and incubated for approximately ten to fifteen minutes. The next step was measurement the IgG content base on the radius precipitation according to the IDBiotech (2009).

RESULTS AND DISCUSSIONS

The identification of antigens protein of SGE showed a highest value of antigens proteins in the SGE substance collected from *Stomoxys calcitrans*. Through protein analysis using SDS-PAGE we identified several proteins belonged to SGE of stable fly which were similar results as reported Wang et al. (2009).

The effect of stable fly SGE and IgG colostrum treatment on serum IgG of young horses presented in Table 2.

Table 2. SGE and IgG Colostrum Treatment on Young Horses Serum IgG Secretion

Factor A (SGE)		Factor B (Colostrum IgG)			IgG serum level
		B1	0 g		
			$A1B1_1$	1	2.84 $g.L^{-1}$
			$A1B1_2$	2	3.51 $g.L^{-1}$
			$A1B1_3$	3	2.10 $g.L^{-1}$
A1	0µgAg5				
		B2	9.6 g		
			$A1B2_1$	1	4.72 $g.L^{-1}$
			$A1B2_1$	2	4.38 $g.L^{-1}$
			$A1B2_1$	3	3.26 $g.L^{-1}$
		B1	0 g		
			$A2B1_1$	1	6.08 $g.L^{-1}$
			$A2B1_2$	2	5.54 $g.L^{-1}$
			$A2B1_3$	3	6.24 $g.L^{-1}$
A2	100µg5				
		B2	9.6 g		
			$A2B2_1$	1	5.66 $g.L^{-1}$
			$A2B2_2$	2	6.92 $g.L^{-1}$
			$A2B2_3$	3	6.77 $g.L^{-1}$

The data showed that there had no interaction between treatment factors ($P>0.05$), although the serum IgG levels tended to be increased after treating with crude of SGE.

This performance linked to Swist et al. (2002) where substances dominated by proteins of 27 kDal, which played an important role as immune-reactive in cattle (Torrand Mangwiro, 2000).

The influence of IgG antibodies for passive transfer of antibodies was not significant ($P>0.05$) on IgG production in the body of foal. Lowest levels of serum IgG concentrations are obtained in animals without receiving IgGn or SGE injection.

All animals treated with SGE and colostrum IgG tended to have a higher concentration of IgG in the blood serum. This probably caused by the domination of antigen 5 protein as immunogen in SGE (Ueti et al., 2007).

Local foal breed with the traditional maintenance systems provided a very significant effect ($P<0.001$) on IgG antibodies in foal blood serum.

Antigen 5 treatment according Kresno (1996) classified the primary immune response in serum IgG which would peak 10-14 days after antigen exposure, which in the absence of antigen which cannot happen primary immune.

CONCLUSIONS

The role of salivary gland extracts, obtained from insect *Stomoxys calcitrans* can be used to improve the IgG antibodies circulated in young horses. However it will be important to continue the research to evaluate the role of the combination of colostrum IgG and SGE on specific IgG antibodies production in young horses.

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