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Antibody Protein Hemagglutinin Subunit Pili with MW 49,8 kDa *Shigella dysenteriae* can inhibit *Shigella dysenteriae* adhesion on Mice Enterocyte

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***Abstract*—Among four species of Shigella, Shigella dysentriae (S. dysenteriae) is the most virulent species and often isolated from cases of shigellosis. The initial step in the development of colonization by S. dysenteriae is its ability to adhere toward mucosal surface by using adhesion molecule. Adhesion proteins of bacteria found in the pili and cell wall was evident as the hemagglutinin protein (HA). Until now there is no effective vaccine to Shigellosis in accordance with the expectations formalized by the WHO.**

This study aimed to prove that HA protein subunit pili with MW 49.8 kDa of S. dysenteriae antibody can inhibit the adhesion of S. dysenteriae on enterocyte of mice by using dose response and immunocytochemistry test (IT) .

Dose response method with the implementation of dose dilution of 1/400, 1/800, 1/1600, 1/3200, 1/6400, 1/12800 and 0 (control). While IT used with the implementation dose dilution HA protein sub-unit pili MW 49.8 kDa of 1/500, 1/1000, 1/2000, 1/4000, 1/8000, 1/16000 and 0 (control).
ANOVA analysis results of dose response method showed that HA protein subunit pili with MW 49.8 kDa S. dysenteriae antibody test with showed a significant difference of the adhesion index among treatment dose (p value = 0.000). It can be seen that lower dose of HA protein subunit pili with MW 49.8 kDa of S. dysenteriae antibody could increase adhesion index. HA protein sub-units pili MW 49.8 kDa Antibody dilution has a close relationship and a significant influence on the adhesion index (r = -0.724, p = 0.000, R square = 0.52, p = 0.000).

Furthermore, Kruskal-Wallis analysis results of IT showed that HA protein sub-units pili MW 49.8 kDa of S. dysenteriae antibody test by indicated that there was a significant difference among treatment doses (p = 0.000). Dilution dose of HA protein sub-unit pili 49.8 kDa has a close relationship and influences significantly on the adhesion index (r = 0.627, R square = 0.393, p = 0.000). HA protein sub-unit pili MW 49.8 kDa of S. dysenteriae antibody is an adhesion molecule antibody that can inhibit the adhesion of S. dysenteriae on enterocytes of mice.

Based on the results of research it can be concluded that antibodies of HA protein subunit pili with MW 49.8 kDa S. dysenteriae as an adhesion molecule is an antibody which is evidenced through the method of dose response and IT

***Keywords*—adhesion molecule, antibody, dose response, immunocytochemistry, S. dysenteriae.**

I. BACKGROUND

It is estimated that 1.1 million people die from *Shigella spp* infections per year and out of that number 60% of death occurs in children under 5 years old [1]. Among four species of *Shigella, S. dysenteriae* is the most virulent species and often isolated from shigellosis cases [2]. *S. dysenteriae* that causes bacillary dysentery, has a very complex pathogenesis. The first step of the pathogenesis is the attachment of bacteria to the receptor. After the attachment, colonization occurs and during the colonization production of material metabolism that causes shigellosis can be found [3,4,5]. Without the attachment there will be no colonization and shigellosis. Protein that plays a role in the attachment bacteria is known as an adhesion molecule. Bacterial adhesion protein found on the pili and cell wall was shown as a hem agglutinin (HA) protein [6]. HA protein is a protein that can cause clumping of red blood cells, especially in mammals.

Prevention of infectious diseases such as diarrhea can be done by improving sanitation / hygiene, antibiotics and vaccinations. However the suitable vaccine for shigellosis has not yet formalized by the WHO. *Bordetella pertussis* can cause pertussis and the function of infanryx vaccines circulated world wide is to prevent pertussis. Infanryx can also be found in Indonesia. The components of the vaccine are Pertussis Toxin (PT), filamentous Hemagglutinin (FHA) and Pertactin (PRN) so the component in fact contains the HA protein. HA protein component can also be found in cholera vaccine candidates. The candidate vaccine contains adhesion molecules of protein sub-unit pili of 37.8 kDa *Vibrio cholerae* [7]. Avanita (2011), in an attempt to obtain shigellosis vaccine candidates, has isolated the HA protein 49.8 kDa sub unit pili *S. dysenteriae* as an adhesion molecule. Further explanation on the HA protein antibody protection sub-unit of 49.8 kDa pili *S. dysenteriae* has not been done and it needs to be explained. Sumarno (2012) has studied the molecular identification of 48 kDa *Salmonella Typhi* adhesion by using immunocytochemistry method [8].

II. RESEARCH METHODS

Culture of Shigella spp.

The bacteria used was *S. dysenteriae* from Reseach of Laboratory Health East Java Indonesia. The Medium used was Thiaproline Carbonate Glutamate (TCG) in order to enrich the growth of pili *S. dysenteriae*. This medium contains 0.02% thioproline; 0.3% NaHCO 3, 0.1% mono sodium 1-glutamate, 1% bactotryptone; 0.2% yeast extract, 0.5% NaCl, 2% bacto agar and 1 mM β amino-ethyl ether-N, N, N 'n',-tetra acid (EGTA) Ehara [8]. Isolation of *Shigella dysenteriae* pili refers to the research carried out by Sumarno with modification. Bacteria pili cutting used pili bacterial cutter and was carried out for 30 seconds at a speed 5000 rpm, while the second to four cuttings used same speed. The isolation of pili fraction by centrifugation of cutting product as carried out at 12,000 rpm by using a temperature 4^0 C. The supernatants containing the bacterial pili are stored at a temperature of 4^0 C [9].

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Monitoring the molecular weight (MW) by SDS-PAGE was done by applying Laemmli method . Protein sample was heated in 100^0 C for 5 min in buffer solution containing 5 mM Tris pH 6.8, 5% 2-mercapto ethanol; 2.5% w / v sodium dodecyl sulfate, 10% v / v glycerol with bromophenol blue tracer color . 12.5% of a mini slab gel with 4% tracking gel was selected. Electric voltage used was 120 mV. The color material used was a coomassie brilliant blue and protein markers using fermentas[10].

Isolation of S. dysentriae protein hem agglutinin pili

Research method referred to Sumarno (2011). The results of pili collection was carried out electrophoretically by SDS-PAGE method. The product of electrophoresis in the form of gel was cut straight at the desired molecular weight. Then the pieces were cut perpendicularly so each piece will contain three protein bands. The resulted pieces of band above were collected and then inserted into the tube of dialysis membrane by using electrophoresis running buffer fluid. Electroellusion used a horizontal electrophoresis apparatus at 125 mV power for 25 minutes. The dialysis was performed on the product of electroellusion with PBS pH 7.4 buffer fluid as much as 2 liters during 2 X 24 hours. Dialysis fluid was replaced three times. Dialysis fluid in membrane dialysis as a result of electroellution of SDS-PAGE band was ready for hemagglutination test [7].

Hemagglutination test

Dilution of samples was prepared at a concentration of ½ on microplate volume V in which each well was 50 μl. At each wel a suspension of red blood of mice with a concentration of 0.5% was added with the same volume as much as 50μl. Then it was shaken with rotator plate for 1 minute. Subsequently it was placed at room temperature for 1 hour. The titer was determined by observing the agglutination of red blood on the lowest dilution [11].

Isolation of enterocytes of mice.

Isolation of enterocytes used Weiser method taken from Nagayama. Intestinal tissue of mice was cut with a size of 5 cm for each piece For each piece, lumen intestine was open with transverse cutting method. Intestinal contents were cleaned by using a fluid-containing PBS pH 7.4 dithiothreithol 1mm. Subsequently they were incorporated into the solution containing 1.5 mM KCl, 9.6 mM Na Cl, 2.7 mM Na-citrat, 8 and 5 mM KH2PO4, 6 mM Na2HPO4 pH 7.3. Then they were incubated at 37 ° C and shaken slowly for 30 minutes. Fathermore the liquid was removed and replaced with PBS pH 7.4 fluid containing 1.5 mM EDTA and 0.5 mM dithiothreitol pH 7.4, incubated at 37^0 C and shaken violently for 20 minutes. Then they were washed with PBS pH 7.4 with centrifugation using the speed of 1000 rpm for 5 minute at 4^0 C. Washing was performed three times. Subsequently the intestinal tissue was suspended in PBS pH 7.4. The suspension was shaken slowly, then the cloudy part of liquid was taken with a sterile pipette and placed in sterile tubes. Enterocytes cell concentration in the liquid was made into 10^6 per ml. Furthermore the enterocytes were kept at 4^0C until they were used for adhesion tests [12].

Production of polyclonal antibodies.

Antigens used were HA protein subunits pili with MW 49.8 kDa *S. dysenteriae*. The antigen in the syringe was emulsified with Freud's Complete Adjuvant (CFA) intraperitoneally with a dose of 100 mg in 100 ml PBS 10. Booster injection was performed in week two, three and four by using antigen emulsified with Incomplete Freud's Adjuvant (IFA) with the same dose. Serum will be taken one week after the last booster [13].

Serum collection method

Blood was collected from the heart. Blood was taken from five mice, after that it was collected in sterile tubes and placed into the incubator with temperature of 37^0C in a tilted position for 30 minutes. Then it was stored in a refrigerator with temperature of 4^0C for 10 min and then centrifuged at 10 000 rpm for 5 minutes. Clear liquid zone was separated from blood clots, put in sterile tubes and stored at-20^0 C [13].

Relation of dose response of protein HA antibody protein subunit MW 49.8 kDa S. Dysenteriae with adhesion index

Shigella dysenteriae was grown in BHI broth for 24 hour at 37^0C before harvested and suspended in PBS containing 1% BSA with a concentration of about 10^8/ ml. 100 μl of bacterial suspension was mixed with 100 μl antibody of hemagluitinin protein that has been isolated in PBS containing 1% BSA then it was mixed with 100 ml suspension of 10^6 ml per mouse enterocytes. The mixture was allowed to be incubated at 37^0 C for 30 min with gentle shaking, then the bacteria that were not adhesion (attached) were removed by applying repeated washing with PBS containing 1% BSA. Enterocytes were collected by 1500 rpm centrifugation for 2 min and suspended in 300 μl PBS. 20 microliters of the suspension was extracted and put on a glass slide to form a smear. Smears were stained with Gram and adhesion index was calculated by microscopic observation [14].

Gram staining

Staining was perform to gain main description of the morphology of enterocytes and bacterial adhesion of *S. dysenteriae* on cell enterocytes. Slide was protected using the crystal violet for 1 min and rinsed with water. Later lugol was dropped for 1 minute followed with 96% ethyl alcohol washing. Furthermore safranin was dropped for 30 seconds and the slide was rinsed with water. The observation can be done under a microscope with a magnification of 1000x.

HA antibody protein subunit pili with MW of 49.8 kDa S. dysenteriae with IT

For sample preparation protein adhesion subunit pili with MW 49.8 kDa S. dysenteriae in cells of mice enterocytes had been fixed with methanol, and then the process was continued by washing with PBS pH 7.4 for 3 x 5 minutes, inducing with hydrogen peroxide (H2O2) 3% for 15 minutes, and finally, washing with PBS pH 7.4 for 3 x 5 minutes. After that blocking was done with triton (triton X-100 (0.25%) in BSA blocking buffer) for 1 h at room temperature, then washed with PBS pH 7.4 for 3 x 5 minutes. Subsequently incubation with primary antibodies (polyclonal antibody protein adhesion subunit pili with MW 49.8 kDa S. dysentriae) was performed with ratio of primary antibodies: solvent (blocking buffer BSA) = 1: 50 for overnight at a temperature of 4^0C, and the process ended with sterile PBS washing for 3 x 5 minutes. For the next stage incubation with secondary antibody anti-mouse IgG for 1 h was performed with a ratio of 1:200 mL, before it was washed with sterile PBS for 3 x 5 minutes. After that SA-HRP (1:500) of 100 mL was poured for each slide and incubated for 40 submarines minutes, followed by washing with sterile PBS for 3 x 5 min and distilled water for 2 x 5 minutes. Then it was incubated with DAB (diaminobenzidine) for 20 min and washed again with sterile PBS for 3 x 5 min and distilled water for 2 x 5 min. Afterward HE (hematoxylin meyer) was poured directly for 10 min and the process ended by directly dripping the sample with water faucet over the 10 minutes. Once dried, the samples was ready to be examined under a microscope [9].

III. THE RESULTS

Pili Protein Identification S. dysenteriae

The results of pili protein collection was consistent to our previous study as much as four times separation by SDS-PAGE method of Laemmli (1970) to determine the MW (Fig 1).

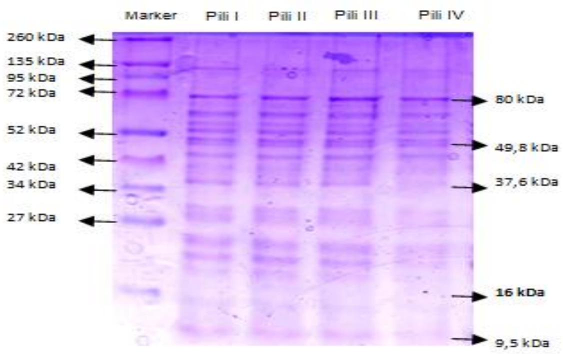

Figure 1. Profile of protein sub unit pili mw from *s.dysenteriae*

Profile of protein sub unit pili MW from *S.dysenteriae* uses pili bacteria cutter four times and Pili I, Pili II, Pili III and Pili IV are identical. The method of the shearing pili bacteria by using bacterial pili cutter is same and carried out for 30 seconds at a speed of one piece to 5000 rpm. The result is different if it is compared with previous study. Based on the results of SDS PAGE analysis 16 protein bands with molecular weight of 9.5 kDa to 105.9 kDa were obtained.

Hemagglutinin test

HA test used a protein subunit pili with MW 80 kDa, 49.8 kDa, 37.3 kDa, 16.7 kDa and 9.5 kDa (Fig 2)

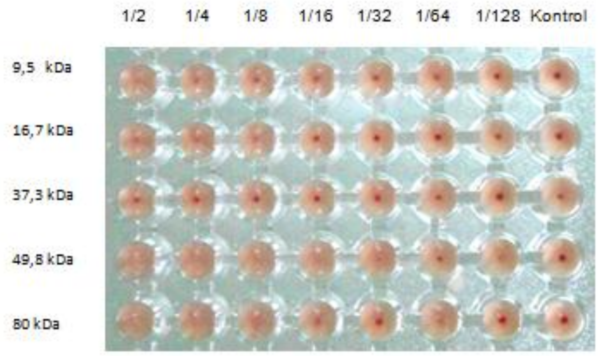

Figure 2. Profile of five protein hemagglutination sub unit pili with MW (9.8 kDa, 16.7 kDa, 37.3 kDa, 49.8 kDa **and** 80 kDa) *S. dysenteriae*.

It appears that the protein sub unit pili with MW 49.8 kDa showed highest HA titer (1/32) h. Based on the results of it protein was selected to produce polyclonal antibody.

Dose response method

Dose response trials used various doses of dilutions of antibody protein sub unit pili with MW 49.8 kDa from 1/400, 1/800, 1/1600, 1/3200, 1/6400, 1/12800 and 0 (control) ($P < 0.05$) (Fig 3).

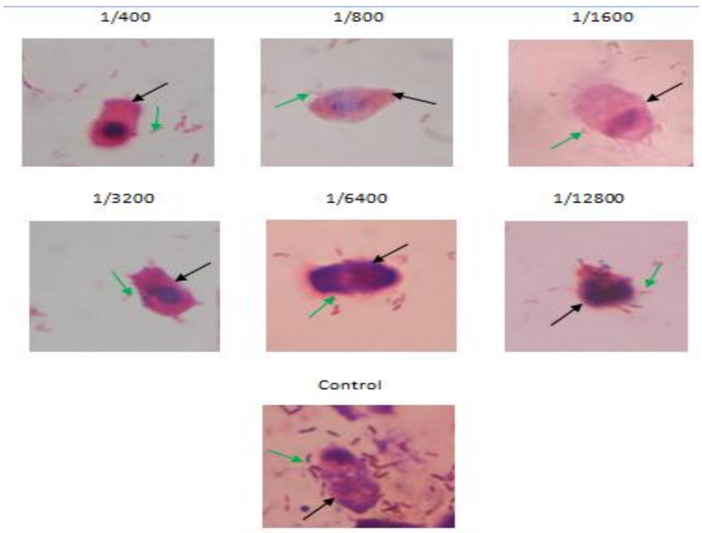

Figure 3: The result atachment of *S. dysentriae* into enterocyte after coated with various concentration of antibody protein sub unit pili with MW 49.8 kDa into enterocyte.

Based on the above result the amount of bacteria which attach into enterocyte can be calculated. The adhesion index (AI) is calculated by counting the number of bacteria attach to 100 enterocytes (Table 1). Statistical analysis will be done based on the observation of the AI. The result is depicted in table 1.

Table 1: The result of statistical analysis of the effect various concentration of antibody protein sub unit pili with MW 49.8 kDa to the AI

Group	n	Mean ± Stan.Dev.	p-value
Titer 1/400	4	79,50 ± 30,36[abc]	
Titer 1/800	4	104,00 ± 19,41[abc]	
Titer 1/1600	4	154,50 ± 15,76[abcd]	
Titer 1/3200	4	226,00 ± 27,43[cde]	0,000
Titer 1/6400	4	302,75 ± 40,31[de]	
Titer 1/12800	4	447,25 ± 23,16[f]	
Control	4	614,00 ± 69,72[g]	

Description: The same notation denote no significant difference and in the contrary with on is significant difference.

Immunocytochemistry test

The effect of dilution with the use of various doses of concentration of antibody protein HA sub unit pili with MW 49.8 kDa. *S. dysenteriae* in immunocytochemistry test (IT) will produce color in enterocyte. The result shows the brown and blue color (Fig 4).

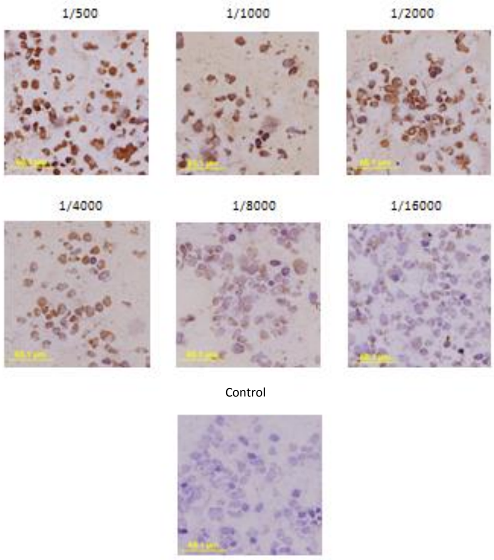

Figure 4: The result of IT to the appearance color of enterocyte with the use of various doses of concentration antibody protein sub unit pili with MW 49.8 kDa *S. dysenteriae*

Description: The same notation denotes no statistically significant difference and on the contrary to the difference notation it is statistically significant.

Protein HA sub unit pili with MW 49.8 kDa *S. dysenteriae* coated to the enterocyte, and subsequently after it is subjected with anti body protein in accordance of the procedure of IT brown or blue color will appear into enterocyte. The brown color means positif result due to the attachment of protein and perfect anti body. If it is not perfect it will become blue. Statistic analysis of the positive cell positive through evaluation of the color in every sample treatment will produce the result as depicted in table 2.

Table 2. The relationship between various doses of concentration of antibody protein HA sub unit pili with MW 49.8 kDa. *S. dysenteriae* and positive result by using IT.

Group	n	Mean ± Stan.Dev.	p-value
Titer 1/500	4	94,75 ± 0,96[a]	
Titer 1/1000	4	88,75 ± 4,27[b]	
Titer 1/2000	4	88,00 ± 6,65[b]	
Titer 1/4000	4	76,25 ± 27,43[c]	0,000
Titer 1/8000	4	73,25 ± 5,74[c]	
Titer 1/16000	4	37,75 ± 11,53[e]	
Control	4	0,00 ± 0,00[f]	

Description: The different notation means no significant difference (p value <0.05) and when it Contains the same notation it means no significant difference (p> 0.05).

Results of Kruskal-Wallis analysis indicated that there are significant differences in the treatment of the results IT with the use of varying dose (p = 0.000) (95% confidence level). The results of comparative analysis of each treatment through post hoc test (Mann-Whitney), showed that almost all the dose dilution provided results that differed significantly (95% confidence level), unless the titer of 1/1000 was not different from 1/2000 and the titer of 1 / 4000 did not differ significantly with titers of 1/800

IV. DISCUSSION

The initial step in the development of colonization by *S. dysenteriae* is their ability to adhere to the mucosal surface of host cell. Avanita (2011) found that *S. dysenteriae* has a protein adhesion sub unit pili with MW 49.8 kDa that is able to inhibit the adhesion of *S. dysenteriae* into enterocytes of mice [14].

The initial step is to confirm the adhesion protein subunit pili with MW 49.8 kDa *S. dysentriae* by using of SDS PAGE. Fig 1 shows that profile of MW of subunit pili *S. dysentriae* in every cutting is consistent and not different. Previous studies have the different result of profile MW of subunit pili *Salmonella Thypi* and *Vibrio cholerae* O1 (Sumarno *et al.*, 2011 and Sumarno *et al.,* 2012) The profile of protein sub unit pili of these two bacteria by using SDS-PAGE is consistent but the protein of molecule adhesion gradually decreases. Once of the reason is the different procedure to collect the protein subunit pili bacteria which used pili bacteria cutter.

In this study the method for isolation protein subunit pili *S. dysentriae* was perform first until forth cutting of pili bacteria carried out for 30 seconds and at a speed 5000 rpm. While in the isolation protein sub unit pili *V. cholerae* and *S. Thypi* show difference in the duration of time and acceleration of speed [7,9].

As it is known that in some intestinal pathogens including *Vibrio mimicus*, there is a positive correlation between the capability of properties of HA with the ability of adhesion to the intestinal mucosa [15]. Many research have shown that some bacterial adhesion could agglutinate red blood cellsl (played as HA) such as the bacterium *Campylobacter pylori* [16,17]. Recently it has been clarified that protein HA sub unit pili with MW 37.8 kDa *V. cholerae* O1 is an adhesion molecule and might be one of the candidate cholera vaccines [7].

According to Todar the protein is an molecule adhesion if antibody of protein will inhibit the attachment of the specific receptor [18]. To confirm that protein sub unit pili with MW 49.8 kDa *S. dysentriae* is an molecule adhesion the antibody of protein sub unit pili with MW 49.8 kDa S. *dysenteriae* should inhibit the attachment S. dysentriae into enterocyte.

The first method used to confirm that the antibody of protein HA sub unit pili with BM 49.8 kDa *S. dysenteriae* is antibody of adhesion molecule due to the response of protein HA sub unit pili with BM 49.8 kDa S. dysenteriae is the dose response method attachment to confirm inhibition. This method uses antibodies HA protein subunit pili with MW 49.8 kDa *S. dysenteriae*. These antibodies are used to inhibit the attachment of *S. dysenteriae* in enterocytes of mice with multiple doses of dilution.

Figure 2 shows that the highest HA titer (1/32) belongs to protein sub unit pili with MW 49.8 kDa *S. dysentriae*. Its protein was picked out as an antigen to produce antibody in the mice. Before using this antibody to corfirm attachment inhibition of *S. dysenteriae* into enterocyte, the antibody should be chosen with the highest dilution attached with antigen and the result is maximum. The examination can use Elisa or dot blod method. Using dot blod method, the result of the lowest antibody titers is able to maximize the respond to antigen as much as 1/1600 (data not show).

Anova results of the protein antibody subunit pili with MW 49.8 kDa *S. dysenteriae* showed that there were significant differences in relation to the treatment dose in the adhesion index (p = 0.000), which shows the increasing antibody doses decreased HA protein subunit pili with MW 49.8 kDa *S. dysenteriae* and then increased adhesion index. In addition there are a close relationship and a significant effect of treatment dose of

HA protein antibody sub-unit of 49.8 kDa pili *S. dysenteriae* of adhesion index values as indicated by the Pearson correlation coefficient of 0.724, p = 0.000, R square 0.52, p = 0.000. These results indicate that HA protein antibody subunit pili with MW 49.8 kDa S. dycsentriae can inhibit the adhesion of *S. dysenteriae* in enterocytes of mice. It can be concluded that the polyclonal antibodies used in the test dose response is an antibody of molecule adhesion.

The second method is the presentation of adhesion molecule which attach with molecule receptor as a part of the cell as detected by IT. Adhesion molecule can attach to the molecule receptor and receptor is a constituent as apart of the cell. The Fig 4 and table 2 show the result of the examination of the protein sub unit pili with MW 49.8 kDa *S. dysenteriae* that can attaches into enterocyte. The IT is an indirect experiment to know that the protein sub unit pili with MW 49.8 kDa S. dysentriae attaches into enterocyte. We only see the attachment of molecule adhesion on enterocyte from a kind of color on enterocyte. In this phenomenon the function of the molecule adhesion on enterocyte is as an antigen. Blue color can be interpreted that there is no matching antigen antibody reaction. If the molecule adhesion as an antigen does not join with the antibody protein sub unit pili with MW 49.8 kDa *S. dysenteriae* , a methylene blue replaces antibody and attaches to the antigen. Attachment of methylen blue to antigen will develop blue color. On the contrary if protein antibody sub unit pili with MW 49.8 kDa *S. dysenteriae* attaches to the antigen on enterocyte, the methylen green can't replace it and the antigen green color is not visualized and the color becomes brown.

The visualization of bacteria attachment to a receptor on a cell host usually uses a light microscope. If a result of bacteria attachment is not clear, maybe electron microscopic examination can replace the visualization.

From the results of IT (Fig 4) in the treatment group it appears that the enterocytes coated with the HA protein sub unit pili with MW 49.8 kDa pili *S. dysenteriae* in the dilution 1/500 showed positive results (brown color) almost for the entire cell enterocytes, and with the increasing dilution of cells it also showed the increasing positive results. On the sample control cell enterocytes showed negative results (blue color). The above results indicate that the HA protein sub-unit of 49.8 kDa pili *S. dysenteriae* is an adhesion protein that has the ability to attach to enterocytes and the dilution of 1/500 showed positive results of IT for almost the entire cell enterocytes because all enterocytes coated by HA protein sub unit pili with MW 49.8 kDa *S. dysenteriae* are further recognized by the antibody HA protein sub unit pili with MW 49.8 kDa *S. dysenteriae* (primary antibody) and visualized by chromogen DAB as brown. The reduction in the protein levels of enterocytes means the less HA is coated by a protein subunit of 49.8 kDa pili *S. dysenteriae* enterocytes, the less it is recognized by HA antibody protein sub unit pili with Mw 49.8 kDa *S. dysenteriae* thus it increases the cell entrecotes with negative results as shown by the the expression of blue color. IT has also been used to prove that the protein adhesion subunit pili with MW 48 kDa *Salmonella Typhi* 16 is adhesive protein. It can be concluded that the polyclonal antibodies used in the IT molecule is an antibody of HA protein sub unit pili with MW 49.8 kDa *S. dysenteriae* . This conclusion is reinforced by the results of statistical calculations on a 100 cell enterocytes. The Kruskal-Wallis analysis showed that there were significant differences in the treatment of the results immunocytochemistry dose (p = 0.000), which shows that the more doses of HA protein subunit pili with MW 49,8 kDa *S. dysenteriae* increases , the more IT of enterocytes with positive results will be. In addition there is a close and substantial effect of the treatment dose of HA protein subunit pili MW 49.8 kDa *S. dysenteriae* of IT positive results shown by the Pearson correlation coefficient of 0.627, p = 0.000, R square 0.393, p = 0.000.

Several other studies Shin (2002) also showed the same phenomenon. IgY could reduce the adhesion of the bacteria *Helicobacter pylori* in receptor cells. In getting the results of this, polyclonal antibody can inhibit the adhesion of bacterial cells *V. cholerae 01* in intestinal epithelial cells by using a barrier method of agglutination with the highest titer of 1/256. Western-blot results still showed positive results up to dilution 1/1000 [19]. In addition Langermann (2001) conduct preclinical vaccine studies with FimH adhesion (derived from uropathogenic *Escherichia coli*) that have shown that antibodies generated against adhesion molecule can impede colonization, block infection, and prevent disease [20].

In addition to determine whether HA antibody protein subunit pili with MW 49.8 kDa *S. dysenteriae* is an antibody molecules adhesion, IT method can also be used to determine the antigenicity of HA protein subunit pili with MW 49.8 kDa *S. dysenteriae* in inducing humoral immune responses in vivo. Observation results show IT of IgG in the serum of mice gave a positive reaction to enterocytes that had been coated with HA protein subunit pili with MW 49.8 kDa *S. dysenteriae* (Figure 4), it seems that the HA protein subunit pili with MW 49.8 kDa *S. dysenteriae* coated enterocytes surrounding the cell membrane.

An antigen may not be antigenic, and antigen must be immunogenic antigens. IT results (Figure 4) show that the HA protein subunit pili with MW 49.8 kDa *S. dysenteriae* can be detected with polyclonal antibody anti HA protein subunit pili with MW 49.8 kDa *S. dysenteriae*, so it can be said that these antigens are immunogenic, because it managed to induce humoral immunity of mice which were immunized with these antigens. In addition Sumarno (2011) has shown that the HA protein subunit pili with MW 37.8 kDa *V. cholerae* was able to induce an immune response and could increase the sIgA. The demonstration that antigen HA protein subunits pili with MW 49.8 kDa *S. dysenteriae* is immunogenic can be used in subsequent studies as the basis in creating vaccine component to prevent infection of *S. dysenteriae* in relation to its ability to inhibit the attachment of *S. dysenteriae* in enterocytes.

Based on the results and discussion of research it can be concluded that: Antibodies of HA protein subunit pili with MW 49.8 kDa BM *S. dysenteriae* as an adhesion molecule is an antibody which is evidenced through the method of dose response and IT.Antibody of HA protein subunit pili with MW 49.8 kDa *S. dysentriae* can inhibit the adhesion of *S. dysenteriae* dysenteriae to the enterocytes of mice. The need to do further research lies on: characterization of molecular subunits in pili adhesion protein found in *S. flexneri*, *S. boydii*, and *S. sonnei*.

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