

## THE EFFECT OF *Trichuris muris* EGG ON THE GOBLET CELLS IN INTESTINE OF MICE (*MUS MUCULUS*)

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**Abstract:** *Goblet cells are the immune response in the intestinal epithelium to produce mucus as a defense digestive tract mucosa caused by *Trichuris muris* infestation. The study purpose was to analyze the effect *Trichuris muris* towards goblet cells number in mice's intestine. This research was a true experimental with the posttest control group design. The subjects were 30 male mice types BALB / c which 2 months old with an average weight 20-30 gram. Samples were taken by simple random sampling. Mice divided into a control group, low-dose group (40 worm eggs) and high-dose groups (200 worm eggs) observed for 30 days and then counted the goblet cells number in each preparation. The data analysis was tested by shapiro wilk test and one-way ANOVA and continued by post hoc test. The results demonstrated the average value of the goblet cells number in the control group, low-dose and high dose increased by higher dose worm eggs and had the value ( $p = < 0.05$ ). This described there were *Trichuris muris*'s eggs influence towards the goblet cells number in mice (*Mus musculus*).*

**Keywords:** *Trichuris muris*, mice (*Mus musculus*), goblet cells in intestinum

## INTRODUCTION

Worms can be transmitted through worm-contaminated soil. Unhygienic residences, unclean lifestyles and dirty environments can be a risk factor. Intestinal worms can cause health problems were a group of "soil-transmitted helminths" or worms that are transmitted through the ground, such as *Ascaris lumbricoides*, *Trichuris trichiura* and *hookworm*.<sup>1</sup> According to the WHO in 2016, soil-transmitted helminths (STH) affects more than 1.5 billion people, or about 24% of the population, especially in the tropics and sub-tropics areas. In the infected humans shows no symptoms or signs of malnutrition, manifestations of diarrhea and abdominal pain, lethargy, and stunted growth and with the prevalence of more than 270 million pre-school children and over 600 million school-aged children.<sup>2</sup> The most common case is *Trichuris trichiura* infection for 57% case, followed by *Ascariasis lumbricoides* 23.8%, and 7.4% by *hookworm*. *Trichuris trichiura* infection, affects more than one billion people worldwide.<sup>3</sup> Cases in Indonesia is still in high prevalence of worm infection between 60-90%.<sup>1</sup>

Some people affected by parasites do not show symptoms primarily by worms or protozoa. This is because parasites always adapt as well as possible so that people who are infested do not know the existence of the parasite. The parasite that attacks the surface of the body (skin) of the host is called ectoparasites, and the one that attacks the internal organs to cause clinical symptoms called endoparasites. The process of entering the parasite into the host's body is called an infection while the attachment of ectoparasites is called infestation.<sup>4</sup>

The parasite stimulates more than one immunologic defense mechanism that is humoral and cellular immune response. The larger the size of the parasites that attack, so there will be the more types of antigens that evoke the body's immune response. IgE production increases in

cases of worm infections. It is because of the activation of mast cells degranulation ECF-A (Eosinophil chemotactic factor) to stimulate the collection of eosinophil cells that have the potential to kill worms in the tissue.<sup>5</sup> The immune response of the body against the worm will stimulate antigen presenting cells (APC) which can stimulate Th0 thus the immune response develops toward Th2. The release of intestinal worms depends on Th2-stimulated responses of cytokines such as IL-4 and IL-5. IL-4 stimulates B cells to produce IgE. IL-5 controls the increasing of the number of eosinophil cells.<sup>6</sup>

Cytokines are intercellular communication regulators to carry signals to target cells in the event of metabolic changes, cell division, protein synthesis and other cell secretions. Cytokines are released by leucocyte cells that affect immune cells by modulating an inflammatory response. The secretion of cytokines will carry immune cells to the site of infection, regulate mucus, increase muscle contractility and result in expulsion of the parasite from the epithelial lining.<sup>7</sup>

Immunity of worms can cause morphological changes as well as cell numbers depending on mucosal induction including mucus production mechanisms by goblet cells.<sup>8</sup> Goblet cells are critical elements of the immune response to *Trichuris muris*.<sup>9</sup> Goblet cell function in the intestinal epithelium produces mucus as an immune defense of the gastrointestinal tract that occurs due to inflammation.<sup>5</sup> It is alleged that the growing number of inflammatory cells, the more mucus produced by goblet cells as cell defense.<sup>10</sup>

*Trichuris* species is a group of parasitic intestinal worms which attacks the epithelium. *Trichuria Trichuris* species infecting humans, while *Trichuris muris* infects rats or mice.<sup>11</sup> *Trichuris muris* in rats serve as a useful model of *Trichuris trichiura* infection in humans and has proven to be a model in improving the understanding of the role of the immune

system either susceptibility or resistance to infection.<sup>9</sup> *Trichuris muris* that inhabit the intestinal lumen determines the mechanism against intestinal pathogens by attaching itself to the pathogens.<sup>12</sup> The *Trichuris* eggs invade epithelial cells in the first 24 hours after swallowing.<sup>13</sup> This research discusses the changes in the number of goblet cells and crypt section villi in the intestines of mice strain BALB/c given *Trichuris muris* eggs at a low dose and high dose. The reason for the BALB / c strain of mice is because it is resistant and can be observed in mucus regulation by MUC2 from goblet cell mucus secretion in the worm expulsion regulation.<sup>9</sup> Research on the numbers of goblet cells are still rare in Indonesia. There have been previous studies but with different methods.<sup>14</sup> So the researchers are interested in doing this research.

## RESEARCH METHODS

This research is experimental (True experimental) with posttest only control group design as its study design.

The subjects of this study were 30 male mice of BALB/c type that were 2 months old with an average weight of 20-30 grams. Samples were taken by simple random sampling using 30 mice based ON Federer formula.

Research instruments used in the form Neutral Buffer Formalin solution (BNF) 10% solution of absolute ethanol, xylol solution, a solution of paraffin, 99.5% glycerin solution, a solution of ewit (albumin), hematoxylin solution, lithium carbonate, a solution of eosin, DPX, Feed mice, eter and formaldehyde, 30 cages for the mice, handscoon, plastic, sonde, 60 pieces of round tubes, 60 pieces glass objects, 60 pieces cover slide, microscope, camera microscope (optical lab), laptops for picturize the objects, scapel knife, tweezers, filter, tissue casset, automatic processor machine, vacuum machine, blocking machine, freezer (-20 ° C), the engine microtome, microtome knife, water

bath 46 ° C, glass objects, glass covers, rack specifically for staining, oven 60 ° C.

The analysis was conducted to determine the effect of *Trichuris muris* on the number of goblet cells in the intestine of mice. Parametric test was conducted with more than two category groups and unpaired. Before it was started, there was also Shapiro-Wilk normality test and homogeneity test Levene-test which distributed data normally and its homogeneity  $p > 0.05$  or significance value  $> 0.05$ . One way ANOVA test and Post Hoc test was conducted to test two or more data population were the the same varians. Data was processed by computer statistics program.

This research was conducted from September 2<sup>nd</sup> to October 18<sup>th</sup>, 2016. This research was conducted at Veterinary Center Banjarbaru as a place of mice sampling and Pathology Clinic laboratory Lambung Mangkurat University Banjarmasin as a place of observation and analysis of data on the microscope.

## RESULTS AND DISCUSSION

Research on the effect of *Trichuris muris* eggs on the number of goblet cells in the mice intestine of (*Mus musculus*) was conducted on 28 mice out of 30 mice planned, because 2 mice died. The mice died on the fifth day after treatment that occurred at low doses. Cause of the death is unknown clearly.

The more doses given to the mice, the more number of goblet cells will be produced. The administration of 40 eggs had more goblet cell counts than the control group and 200 eggs had increased more goblet cells.

This shows that there is influence of *Trichuris muris* eggs on the number of goblet cells in the intestine of mice (*Mus musculus*). All groups have ( $p \Rightarrow 0.05$ ) so the hypothesis is accepted.

The digestive tract is responsible for the absorption of nutrients and water, but at the same time has an important role as a barrier to the external environment.

Intestinal homeostasis is the result of complex interactions between the environment, the cells of the intestinal epithelium (Epithelial Intestinal Cells), mesenchymal cells, vascular endothelial cells, and cells of the innate and adaptive immune systems.<sup>15</sup>

*Trichuris muris* especially invade epithelial cells in the first 24 hours of mice ingestion.<sup>13</sup> Intestinal mucosa forming a defense against the outside environment. The defense has two main components: intrinsic defenses consisting of epithelial cell layers and extrinsic defenses, which are a combination of goblet cell secretions that produce mucus.<sup>16</sup> Mucus in the mucosal surface will prevent pathogens to invade the epithelium.<sup>17</sup> Goblet cells are mucus-secreting cells (mucus) that are protective cells in lubricating the digestive tract. The more inflammation that occurs the more mucus is produced by goblet cells as a cell defense.<sup>10</sup> Secretion of goblet cells containing the glycoprotein (80% carbohydrates and 20% protein) released by exocytosis process. Increased goblet cells are under control of the Th2 cytokine although also an increase in IL-4 or IL-13 may also occur.<sup>16</sup> IL-4 and IL-13 are regulation from the major sources at the increasing the ITF (Intestinal Trefoil Factor), that interact with the mucin gel to increase the viscosity of mucus in goblet cells.<sup>18</sup>

During infection, the increased numbers of goblet cells more with *Trichuris muris* compared with uninfected mice.<sup>19</sup> Once the mice infected with *Trichuris muris*, they will have increasing in the IEC indicated for the expulsion of parasites.<sup>35</sup> The production of IgE is increased in the case of worm infections. It is because of the activation of mast cells degranulation ECF-A (Eosinophil chemotactic factor) to encourage the collection of eosinophil cells that have the

potential to kill worms in the network.<sup>5</sup> High dose infection (> 150 eggs) has a more dominant Th2 immune response whereas low-dose infection (<15 eggs) has a more dominant Th1 immune response.<sup>20</sup>

During infection with *Trichuris muris* occurs hyperproliferation of cells in the gut. It also shows that *Trichuris muris* survive in its host by generating the Th1 immune response and result in enterocytes cell hyperplasia that occurs during chronic infection. While Th2 leads to an acute infection in the mechanism of worms expulsion and results in the presence of goblet cell hyperplasia as activation of the transcription factors involved in goblet cell differentiation.<sup>16</sup>

In acute infection, the MUC4, Muc13 and Muc17 mucin cells surface, when infected with *Trichuris muris*, resulted an increasing on the apical glycocalyx thickness due to increasing synthesis of glycoproteins in a goblet cells involved in the process of expulsion of worms. The hypersecretion of glycoproteins is mediated by  $\alpha 3$  (GABA- $\alpha 3$ ) gamma amino-butyric receptors under IL-13 control.<sup>16</sup>

Chronic infection leads to caecum morphological changes and without significant changes were observed in goblet cell numbers and an increase in epithelial cell numbers that was associated with chronicity. During chronic infection, the barrier mucus, decreases as the decreasing glycoprotein and can result in commensal flora coming back into the epithelial cell lining, which can lead to exacerbation of epithelial inflammation.<sup>16</sup>

The role of macrophages during worm infections increases significantly as in the peritoneal cavity suggests that this occurs because the proliferation of IL-4 is more than the precursors in the blood.<sup>21</sup>

Table 1. Research result in effect of *Trichuris muris* and Hasil Penelitian Pengaruh Infestasi *Trichuris muris* the goblet cells in intestine of mice (*Mus musculus*) using LSD (Pos hoc)

Group	n	Average $\pm$ SD		Groups	n	Average $\pm$ SD		Anova Test
		Longitudinally				Crosswisely		
		<i>Intestinum crassum</i>	<i>Intestinum tenue</i>			<i>Intestinum crassum</i>	<i>Intestinum tenue</i>	
X <sub>0</sub>	10	16,06 $\pm$ 3,153	8,35 $\pm$ 1,692	X <sub>0</sub>	10	13,14 $\pm$ 3,001	7,48 $\pm$ 2,500	
X <sub>1</sub>	8	19,6 $\pm$ 2,663	10,83 $\pm$ 2,201	X <sub>1</sub>	8	16,26 $\pm$ 2,745	9,25 $\pm$ 2,706	<0.05
X <sub>2</sub>	7	22,76 $\pm$ 6,535 <sup>a</sup>	11,67 $\pm$ 3,926 <sup>a</sup>	X <sub>2</sub>	10	17,36 $\pm$ 4,556 <sup>a</sup>	12,36 $\pm$ 2,421 <sup>a,b</sup>	

## Remarks:

X<sub>0</sub> = control groupX<sub>1</sub> = low-dose group treated with 40 eggs of *Trichuris muris*X<sub>2</sub> = high dose group treated with 200 eggs of *Trichuris muris*

n = number

SD = Deviation Standard

<sup>a</sup> = There was a significant difference with the group X<sub>0</sub><sup>b</sup> = There was a significant difference with the group X<sub>1</sub>

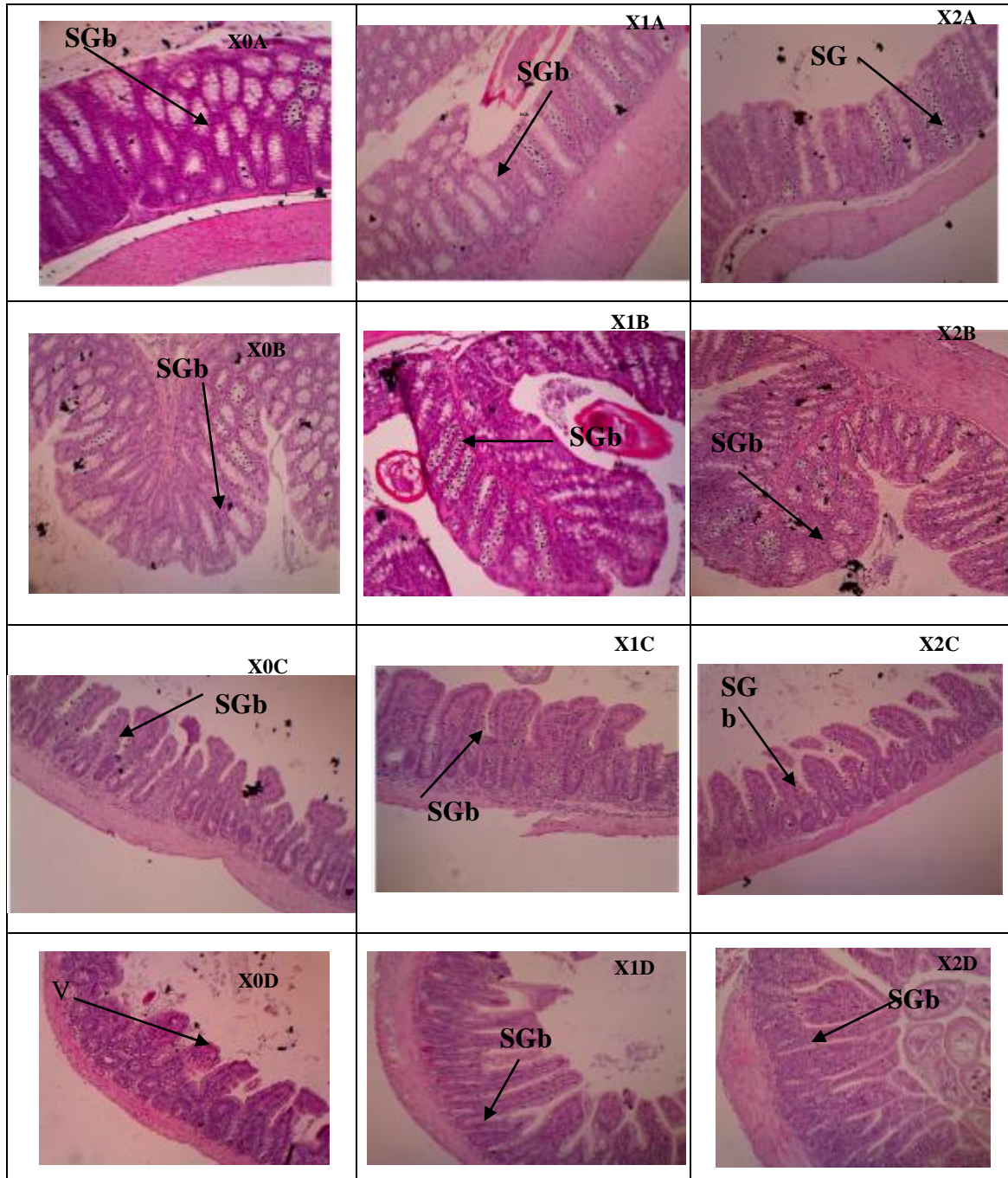


Figure Goblet Cells (SGB) in the mice (*Mus musculus*) intestine with 10x10 Magnification measured in control group (X0), Low dose group (X1) and High Dose group (X2). Samples were taken from *the intestine Crassum* longitudinal section (A), *intestinal Crassum* crosswise section (B), *intestinal Tenue* longitudinal section (C), and *intestinal Tenue* crosswise section (D).

## CONCLUSIONS

Based on the results obtained and the discussion of the *Trichuris muris* effect on goblet cells in the mice intestine can be concluded that: the average number of goblet cells in *the intestine crassum* longitudinal section was  $16.06 \pm 3.153$  in the control group (X0),  $19.6 \pm 2.663$  in low dose group (X1) and  $22,76 \pm 6,535$  in high dose group (X2) and there were statistically significant differences; the average number of goblet cells in *the intestine crassum* cross section is  $13.14 \pm 3.001$  in the control group (X0),  $16.26 \pm 2.745$  in the low dose group (X1) and  $17.36 \pm 4.556$  in the high dose group (X2) and there were statistically significant differences; the average number of goblet cells in *the intestine tenue* longitudinal section was  $8.35 \pm 1.692$  in the control group (X0),  $10.83 \pm 2.201$  in the low dose group (X1) and  $11.67 \pm 3.926$  in the high dose group (X2) and there were statistically significant differences; as well as the average number of goblet cells in *the intestine tenue* memlintang piece was  $7.48 \pm 2.5$  in the control group (X0),  $9.25 \pm 2.706$  at the low dose group (X1) and  $12.36 \pm 2.421$  in the high dose group (X2) and there are statistically significant differences.

Suggestions for this study is expected to do further research on the immunology mechanisms for number of goblet cells in the mucosa of the respiratory system, not only on the gastrointestinal course, but also more research on a strain of mice such as AKR, Swiss webster, and C57BL / 6 and other systems toward parasite worms which can be affected in the physiological function of mice and the presence of more doses between low doses and higher doses to know more about the effect of large doses.

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