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Effects of *Trichuris* Muris Infection On Mice (*Mus musculus*) Intestine

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ABSTRACT

Helminth infection can influence immunopathology in human body. Changes in the length of intestine and release an increased goblet cell is one of the defense mechanism against worm infections. In this study, we analyze effects of Trichuris muris infection towards goblet cells number and the length of villi and crypt intestine of mice This research was a true experimental with the posttest control group design. The subjects were 30 males mice types BALB/c which 2 months old with an average weight 20-30 grams. Samples were taken by simple random sampling. Mice divided into a control group, low-dose group and high-dose groups. All subject was observed for 30 days, and the intestine was taken for measured length of villi and crypt. Counting of goblet cells used preparation of intestine. Data analysis used one way Anova and continued by post hoc test. The result show that crypt and villi on large and small intestine will be longer in mice with *Trichuris muris* infection. 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 ($p =< 0.05$). *Trichuris muris* infection has influenced the length of villi and crypt intestine and the number of goblet cells in mice (*Mus musculus*).

Keywords: *Trichuris muris, length of intestinum, goblet cells number*

Introduction

Worm infection is still a neglected health problem in Indonesia because it is chronic and silent clinical symptoms. *Trichuris trichiura* is believed to infect about 800 million people worldwide, with the majority being children. Infected children show signs of malnutrition, stunted growth, intellectual retardation and educational deficits. *Trichuris muris*, a mouse model of *T.trichiura* infection in humans, have greatly contributed to improve knowledge on components of immune responses and how the immune systems induces parasite expulsion.[1]

The contraction of the smooth muscle that lines the gastrointestinal tract is one possible mechanism that aids expulsion of the *T.muris* parasite.[2] It has been demonstrated that the immune system can, in fact, mediate muscle contraction. Investigations into muscle hypercontractility in *T. muris* infection have also revealed an increase with infection.[3] Therefore, muscle hypercontractility may be an important effector mechanism for expelling *T. muris*.

Chronic intestinal nematode infections cause altered gut architecture, suggested that increased turnover of epithelial cells in the gut may prevent the parasite from remaining embedded within the host. Specifically, *T. muris* infection is associated with enhanced epithelial stem cell proliferation within the crypts of Lieberkühn in the large intestine, which is mediated by IFN-γ, and results in a massive crypt hyperplasia.[4]

The mucus layer forms the first barrier between the lumen and the small bowel epithelium, with mucin secretion. Changes in goblet cell proliferation and mucin mucus composition occurred in mice infected with *T. muris*. An increase in the amount of mucus makes Trichuris cyst more difficult in maintaining the acidity.[5] This study characterized goblet cell count and length of villi and intestinal crypt in mice with *T.muris* infection.

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Materials and Method

Mice: Male BALB/c mice with 2 month age and average weight 20–30 gram were obtained from Yogyakarta Veterinary Research and Investigation Center (BPPV). The animal studies were performed under the regulation Home Office Scientific Procedure Act (1986).

Parasite: *T.muris* was maintained as describe previously.[6] Mice were infected by oral gavage to give either 200 embryonated eggs (high level) and 40 eggs (low dose) in double distilled H2O. Worm burden are assessed in 30 day post infection.

Tissue Preparation: Large and small intestine was taken as long as 2 cm distal from the caecum and flushed out by NaCl 0.9%, then put into 10% NBF for fixation. Tissue were prepare by using gut bundle technique.[7] Tissue were then cut using microtome blades with a thicknes of 0.3–0.5 µm and arranged into a tissue cassette, and stain with hematoxylin eosin (HE).

Measurement of Length of Villi and Crypt Intestine: Histologic preparations have been made visually observed using a microscope with weak magnification (10x10). Full length longitudinal and transversal section were selected to analysis at 9-10 mice per group. Individual villi and crypt length were determined by selecting well orientated villi and crypt, and measuring from the base to the lumen.

Determine the Number of Goblet Cell: Calculating the number of goblet cell by looking on HE stained section at 9-10 mice per group. Goblet cells taken on the villi and crypt, viewed 10 cells that are bounded clearly, easily visible and uninterrupted.

Statistical Analysis: Statistical analysis was performed by using a one way analysis of varian (ANOVA) with post hoc. Analysis was performed by using SPSS program. *p-values* <0.05 were considered statistically significant.

Result and Discussion

A. The length of intestinal villi and crypta in Mice (*Mus musculus*) after infection with T*richuris muris*.

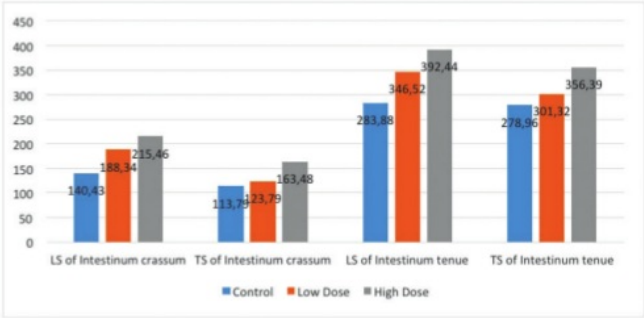
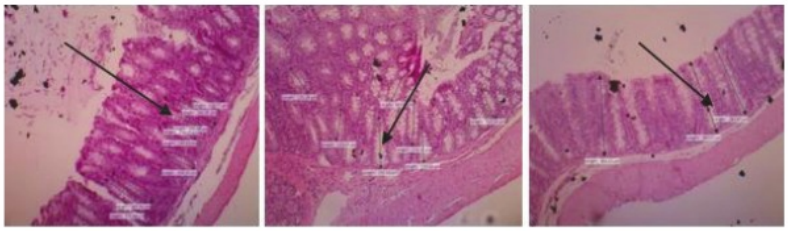


Figure 1: The length of intestinal villi and crypt in mice after *Trichuris muris* infection (in µm).

Figure 1 shows the differences in crypt and villi lengths in intestinal crassum and intestinal tenue in the treatment group. Based on statistical analysis with one way anova show that crypt and villi on intestinum crissum and intestinum tenue will be longer in mice with *Trichuris muris* infection. The measurements of length of villi and intestinal crypt are shown in Figure 2 below.



A B C

Figure 2: The measurements of length of villi and crypt in intestinal mice longitudinal section, stained with HE with magnification 10 x 10); A. Mice in control group; B. Mice given a low dose of *Trichuris muris* eggs, and C: Mice given ahigh dose of *Trichuris muris* egg

B. The number of Goblet cell in intestinum mice (*Mus musculus*) after *Trichuris muris* infection

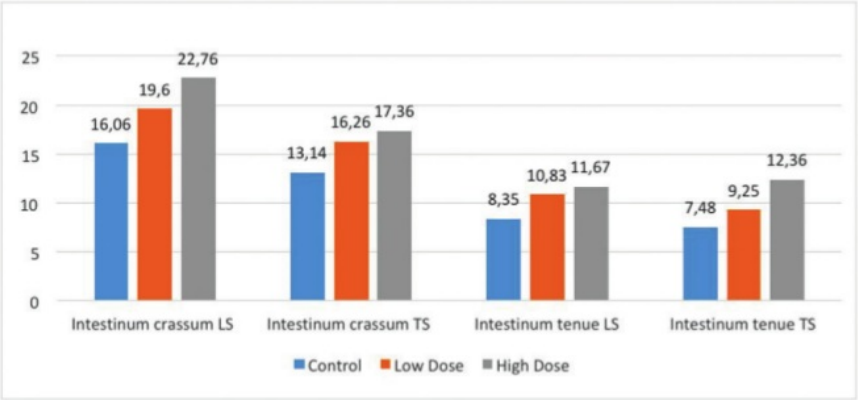


Figure 3: The average number of Goblet Cells in intestinum mice after Trichuris muris infection.

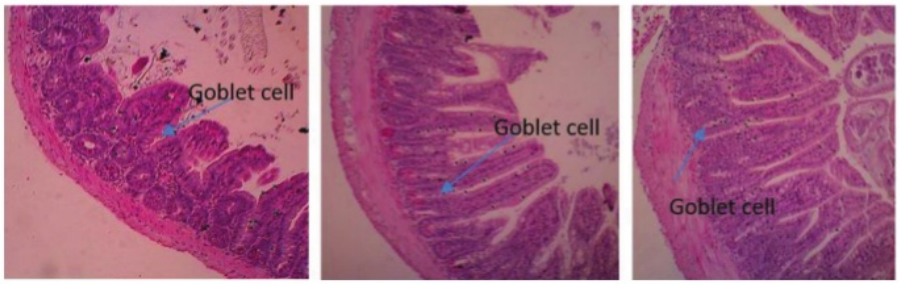


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Figure 4: Goblet cell in mice (Mus musculus) intestine after Trichuris muris infection with magnification 10 x 10, Transversal section, stained with HE (A: Intestinal mice in control groups, B: Intestinal mice in a low dose Trichuris muris infection, C: Intestinal mice in a high dose Trichuris muris infection)

Trichuris muris will invade epithelial cells within 24 hours after ingestion by mice. The intestinal mucosa forms a defense against the external environment in the form of intrinsic defenses consisting of epithelial cell layers and extrinsic defenses which are combination of goblet cells that produce mucous.[1]

Immune mediated expulsion of intestinal dwelling nematodes can involve the interplay between CD4+ T cell and gut epithelium. The immune response to *T.muris* has been well characterized in terms of cytokine production in the draining lymph node, with a type 2 dominated response an absolute requirement for worm expulsion.[1]

The development of a Th2-type of response is associated with fast parasite expulsion whereas a Th1 response is linked to establisment of chronic infection and increased immunopathology. The use of BALB/c strain in this research has been crucial in determining important cellular and molecular pathways of importance during *T muris* infection. In BALB/c mice, if they infected by high dose, immune response will develop Th2 type.[5]

T.muris are capable of modulating immune response of their host to prevent expulsion. Indeed, it has been shown that development of inappropriate Th1 response leads to chronic infection. It associated with high levels of IFN-γ, IL-12 and IL-18 in susceptible mouse. Resistance is also associated with decreased production of the Th1-inducing cytokine IL-18. Resistance are usually produced against high dose *T.muris* infection and lead to parasite expulsion by triggering expulsion mechanism such as increased epithelial cell turnover, mucin production and muscle hypercontractility. Two cytokines that play a major role in the resistance to infection are IL-4 and IL-13.[5]

Prolonged infection with gastrointestinal parasites can result in severe damage to surrounding tissues if not properly regulated. *T.muris* infection can cause

severe transmural inflammation in colon. Mucosal and submucosal inflammation result in destruction of normal crypt architecture during chronic infection.[2]

Chronic infection with *T.muris* has been associated with crypt hyperplasia accompanied by both increased epithelial cell proliferation and apoptosis. In this study a given low dose and high dose of *T. Muris* egg in 30 day, capable change the length of villi and crypt intestine of mice. These processes might be controlled by pro inflammatory cytokine IFN-γ and can also control the excessive extension of crypt in cronic infection. Resistant mice have accelerated epithelial cell turnover, a mechanism which is directly linked to faster parasite expulsion. These findings led to a model referred to as the 'epithelial escalator', where epithelial cells move from the bottom of the crypt (proliferation zone) to its top (shedding zone), moving the parasite embedded in the epithelial layer towards the lumen where the epithelium and parasite are shed. In this study, BALB/c mice were able to regulate epithelial turnover during infection. The difference in epithelial cell turnover between resistant and susceptible mice is due to differences in the immune response and their cytokine profile. Some studies have shown the role of IL-4 and IL-13 to the difference. On the other hand, IFN-γ and CXCL10 (IFN-γ-induced protein), both are associated with Th1 responses and susceptibility to *T. Muris* infections, responsible for down-regulatory epithelial cell transition.[8]

The second mechanism of expulsion parasite in gastrointestial tract is mucin production by goblet cells. Goblet cells are major producers of mucins (the major protein component of mucus) and form an important element of the innate defence in the gastrointestinal tract.[1] Goblet cell hyperplasia is observed during *T. muris* infection in both resistance and susceptible animals. This study showed that the number of goblet cells in the gastrointestinal tract would increase in mice infected with high dose compared with low-dose infected or uninfected ($p<0.05$).

Goblet cells are protective mucus cells that are protective to lubricate the gastrointestinal tract. The more inflammation that occurs the more mucus is produced by goblet cells as a cell defense. Goblet cell secretions contain glycoproteins (80% carbohydrates and 20% protein) released by the 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. 26 IL-4 and IL-13 is a regulation of the main source of improvement ITF (intestinal trefoil factor) that interacts with mucin to increase the viscosity of the mucous gel in goblet cells[2].

In this study, there was an increase in the number of goblet cells in *T.muris* infected mice compared with uninfected mice. After infection with *T.muris*, an increase of the number IEC that serves for expulsion of the parasite. IgE production increases in cases of worm infections. This is due to the activation of mastocyte degranulation of ECF-A (Eosinofil Chemotactic Factor) which can encourage the collection of eosinophilic cells that potentially kill the worms in tissues. 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.

As long as Trichuris infected the cysts occur hyperproliferation of cells in the intestine. It also shows that the mycotic trichuris persists in its host by eliciting a Th1 immune response and leads to enterocyte cell hyperplasia that occurs during chronic infection. While Th2 leads to an acute infection in the mechanism of expulsion of worms and resulted in goblet cell hyperplasia as activation of transcription factors involved in goblet cell differentiation.[8]

Acute infections, MUC4, Muc13 and Muc17 mucin cell surfaces when infected with Trichuris muris result in increased apical glycocalyx thickness along with increased glycoprotein synthesis in goblet cells involved in the process of expulsion of worms. The hypersecretion of glycoproteins is mediated by α3 (GABA-α3) gamma amino-butyric receptors under IL-13 control.[4]

Chronic infection leads to caecum morphological changes and no significant changes were observed in goblet cell numbers and an increase in epithelial cell count was associated with chronicity. During chronic infection, the barrier mucus decreases as the glycoprotein decreases resulting in commensal flora coming back into the epithelial cell lining, which can lead to exacerbation of epithelial inflammation. 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.

Conclusions

1. The average length of the villi and the intestinal crypt increased with the infection of *Trichuris muris*, the more infected eggs, the longer the size of villi and crypt (p values <0.05)
2. 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 values < 0.05).
3. *Trichuris muris* infection has influenced towards the length of villi and crypt and the goblet cells number in intestinum of mice (*Mus musculus*).

Ethical Clearance: This study approved and received ethical clearance from the Committee of Public Health Research Ethics of Medical Faculty, Lambung Mangkurat University, Indonesia. The informed consent included the research tittle, purpose, participants's right, confidentiality and signature.

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Conflict of Interest: The authors declare that they have no confict interest.

REFERENCES

1. Klementowiz JE, Travis MA, Grencis RK. Review: Trichuris muris: a model of gastrointestinal parasite infection, a review. Semin Immunopathol 2012;34: 8115-828.
2. Khan WI, Collins SM. Gut motor function: immunology cal control in enteric infection and inflammation. Clinical and Experimental Immunology 2006; 143: 389-397.
3. Khan WI, Richard MAH, Blennerhasset PA, dkk. Modulation of intestinal muscle contraction by interleukin-9 (IL-9) or IL-9 neutralization: correlate with worm expulsionin murine nematode infection. Infection and immunity 2003; 71: 2430-2438.
4. Potten CS, Booth C, Pritchard, OM. The intestinal epithelial stem cell: the mucosal barrier. International Journal of Experimental Pathology 1997; 78: 219-243.
5. Hurs RJM, Else KJ. Trichuris muris research revisited: a journey throught time. Parasitology 2013; 140: 1325-1339.
6. Wakelin D. Acquired immunity to Trichuris muris in the albino laboratory mouse. Parasitology 1967; 57:515-524.
7. Potten CS, Hendry JH. The microcolony assay in mouse small intestine, 1985; 155-159
8. Cliffe LC, Potten CS, Booth CE, dkk. An increase in epithelial cell apoptosis is associated with cronic intestinal nematode infection. Infection and Immunity 2007; 75 (4): 1556-1564

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