Mucosal Mast Cells Contribution in Intestinal Defense of Chickens (*Gallus domesticus*) Infected Naturally by *Ascaridia galli*

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Abstract
This study was aimed at finding out the investigation of mucosal mast cells in intestines of chicken that were naturally infected by *Ascaridia galli*. Amount of ten intestine of freshly slaughtered chickens (*Gallus domesticus*) found from local abatoir in Banda Aceh were divided into two groups containing five intestines of each. Mucosal mast cells count were done of which histologic slides were made in stained serial histological sections with Alcian blue (pH 0,3) and Safranin-O (pH 0,1) of the intestines. The result showed that the mucosal mast cells increased significantly ($P < 0.05$) in intestines of chickens infected naturally by survival *A. galli* adult worms. It was concluded that the intestinal defense of chickens against parasite infection is associated with the mucosal mast cells contribution by creating an environment hostile to the establishment and survival of intestinal nematodes, *A. galli*.

Keywords: mast cell, *Ascaridia galli*, chicken, intestine

Introduction

Ascaridia galli is an important intestinal nematode of poultry in many parts of the word and among the most pathogenic of parasites to localize in intestine of laying hens. Chronic infection develops after continuous ingestion of infective stages larvae and usually result in anemia, edema, wieght-loss, and diarrhea, which in severe cases, can result in death.

Mast cell responses have been suggested in protection against *A. galli* infections. Infections with 1000 dosis *A. galli* embryonated egg in laying hens have shown that mast cells accumulate in the small intestine following infection (Darmawi *et al*., 2013). Previously, De-yuan *et al*. (2003) described that mast cells in the thymic medulla and jejunal mucosa of chickens infected experimentally with *A. galli,* suggesting that mast cells responses may be involved in controlling these infection.

According to our previously investigation known that the intestines infected by *A. galli* showed histopathological changes of villi the cover of desquamation, hyperplasia and fusion. Demage level of the small intestine chicken was largely determined by the number of infecting by *A. galli*. On the other hand, the more number of *A galli* infected to chickens, the higher level of damage would be occured in the small intestine of chicken (Balqis *et al*., 2013). Hambal *et al*. (2013) explained that duodenum, jejunum and ileum infected by *A. galli* showed hyperemia, and inflammatory cell infiltration in part of *A. galli* infection. In addition, Darmawi *et al*. (2013) observed that mucosal mast cells involved in intestinal defense mechanism. The chickens infected orally with 1,000 embryonated eggs of *A. galli* were able to increase significantly mast cells response on days 14 post infection. The *A. galli* infection could trigger the involment of mucosal mast cells response in jejunal defense of laying hens against parasitic diseases caused by *A. galli*. In this study, we asseyed the mucosal mast cells made a contribution to intestinal defense of chickens against parasite infection caused by *A. galli*.

Materials and Methods

Amount of ten intestine of freshly slaughtered chickens (*Gallus domesticus*) found from local abatoir in Banda Aceh were divided into two groups containing five intestines of each. The first group determined by intestines were not find *A. galli* adult worm infection as controls. The second group determined by intestines contained amount of *A. galli* adult worms infected naturally.

Histological Procedure

Intestine's segment was dissected, flushed with cold sterile saline solution, opened longitudinally, and placed, mucosa side up, onto small pieces of blotting paper. The segments were then fixed in 10% buffered normal formalin. This process was performed for each laying hen using sterile instruments for each dissection. Fixed samples were dehydrated in the ascending concentrations of ethanol (50%, 60%, 70%, 80%, 96% (1), 96% (2) and 100%). The samples were cleared in xylol and were embedded in paraffin wax. Three of each histological sections (3-5 µm of thickness) were stained with Alcian blue (pH 0,3) and Safranin-O (pH 0,1) (Sigma). After washing, sections were counterstained with eosin and mounted as described by Darmawi *et al.* (2013) with certain modifications.

Mast Cell Staining

The number of mast cells per 10 villus crypt units (VCUs) was counted on each section. Mast cell counts were performed under light microscopy using an eyepiece square graticule (eyepiece × 10, objective × 40), and data expressed as mean number of mucosal mast cells (MMCs) per VCU as described by previous authors (McDermott *et al.*, 2003; Noviana *et al.*, 2004; Li *et al.*, 2004; Königová *et al.*, 2008; Darmawi *et al.*, 2013) with certain modifications.

Statistical analysis

Data were analyzed by the Student t test, where t tests were used for comparisons of mast cell numbers. *P* values of < 0.05 were taken to indicate a significant difference.

Results and Discussion

In this study we found that the number of mucosal mast cells in healthy chickens is stable, but their numbers increase in *A. galli* infection (Table 1).

Table 1. Mucosal mast cell number/10 villus crypt unit (mean ± SD) in the intestines from uninfected, and infected naturally by *A. galli* in chickens

Groups	Mucosal mast cell number in intestines
Uninfected	321.32 ± 57.42*
Infected	391.41 ± 89.73*

* Significantly different from uninfected and infected chickens ($P < 0.05$).
Results shown are representative of two independent experiments. MMC/10VCU ± SD

In this study, mucosal mast cells significantly increased in intestines of chickens infected naturally by *A. galli* in comparison with uninfected chickens as shown in Table 1. Our results in this study agree with and support those of Onah & Nawa, (2004), Li *et al.*, 2004), (Vukman *et al.*, 2013), Ball *et al.*, (2013), De-yuan *et al.* (2003), Suzuki *et al.* (2008), and Königová *et al.* (2008) who showed mast cells are contributed in intestinal defense against worm infection. Our previous study explained that large numbers of mast cells were observed in the jejunum of infected laying hens. Laying hens infected with embryonated eggs of *A. galli* accumulated mast cells in the jejunum (Darmawi *et al.*, 2013).

In the chickens, *A. galli* adult worms establish in the lumen of intestine known "lumen phase". However, *A. galli* parasite was not only able to survive in the lumen but could also penetrate in the barrier intestinal mucosal defense with migration to the tissue "histotrophic phase". Previously investigator described that *A. galli* infective larvae invaded in the epithelium and located in the lamina propia after hatching in lumen (Luna-Olivares *et al.*, 2012). There are some histopathological changes in intestine of *Gallus domesticus* caused by *A. galli* infection. In confirmation of our previous study, we found that the chicken (*G. domesticus*) infected naturally by *A. galli* caused the infiltration of inflamatory cell and hemorrhagy in the intestine (Hambal *et al.*, 2013). In addition, the more small intestine of the chickens suffered histopathological changes namely desquamation, hiperplasia, fusion that occurs in the jejunum (Balqis *et al.*, 2013).

Various authors described that increased numbers of mucosal mast cells are often observed in affected tissues during helminth infections (Onah & Nawa, 2004; Li *et al.*, 2004; Vukman *et al.*, 2013; Ball *et al.*, 2013; De-yuan *et al.*, 2003; Suzuki *et al.*, 2008; Königová *et al.*, 2008). This phenomenon supported by Darmawi *et al.* (2013) who observed that mastocytosis occurred in jejunal sections of laying hens challenged with embryonated of *A. galli.* Infection induces mucosal mast cells degranulation in the intestinal that is considered to be a host defense mechanism against the parasite. In support of this hypothesis, various authors described that mast cells involved in mucosal defense mechanism. Various authors described that mucosal mast cells play an important role as effector cell activated in response to most helminth infections, contribute to expulsion of a number of

gastrointestinal nematode parasites. Migration of mast cells induced by tegumental coat antigen of *Fasciola hepatica* (Vukman *et al*., 2013). Mastocytosis was activated in jejunal of mice challenged with *Strongyloides venezuelensis* (Onah & Nawa, 2004). Mucosal mast cells are an important effector for *Trichinella spiralis* expulsion in rats and mice (Suzuki *et al*., 2008). The larger numbers of mucosal mast cells in abomasal mucosa of lambs infected with *Haemonchus contortus* (Shakya *et al*., 2009), followed by *H. contortus* expulsion from abomasums of sheep (Ortolani *et al*., 2013).

Conclusion

The mucosal mast cells contribute to intestinal defense in chickens by creating an environment hostile to the establishment and survival of intestinal nematodes, *A. galli*.

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