

Cecal Goblet Cell Response on Intracellular Development of *Eimeria tenella* in Susceptible and Infected Chickens

Respons Sel Goblet Sekum terhadap Perkembangan Intraseluler *Eimeria tenella* pada Ayam Peka dan Terinfeksi

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Abstract

The purpose of this study is to know clearly illustration of response of cecal goblet cells on intracellular development of *E. tenella* in susceptible and infected chickens. Eighty males broiler, three weeks old were divided into two groups. Group one was consisted of at least 40 chickens and kept up to five weeks old. Then, they were infected with *E. tenella* and killed every two days from day 0 to day 12 post infection (pi) using goblet cell examined. The last five chickens were especially the daily oocyst production examined from day 7 to 12 pi. Group two was consisted of at least 40 chickens, three weeks old infected with *E. tenella*, when at 5 weeks old challenged with the same dose and they were reorganized procedure as group one. Response cecal goblet cells represented in ratio of mean number of active goblet cell per 10 crypt units of both groups compared with pre-infected chickens. Susceptible chickens assessed high number oocyst production, appeared coccidiosis clinical signs and contrary with infected chickens. Macroscopical and microscopical changes and goblet cells hypoplasia were seen in susceptible chickens, while to those changes of infected chickens were disappeared.

Key words: *E. tenella*, goblet cell, response

Introduction

The goblet cells are specialized epithelial cells that constitutively produce a protective mucus layer to protect the epithelium and also respond to pathologic challenge by increasing the rate of mucus production and by changing the constituents of the mucus (Meslin *et al.*, 1999; Sharma and Schumacher, 1995).

The eimerian parasites represent the principal pathogens of the intestinal tract in poultry and many other domestic animals, invading and destroying epithelial cells leading to the clinical disease, intestinal coccidiosis. Although each *Eimeria* spp. is highly host specific almost all vertebrate species (with the exception of humans and primates) can be infected with eimerian parasites and these interactions may be significant in terms of the evolution of the vertebrate enteric defense system.

Eimeria tenella (*E. tenella*) is one of pathogenic species of nine species of *Eimeria* gastrointestinal tract of poultry. It result in destroying cecal epithelial cells leading to the clinical disease involved blood diarrhea, decreasing production, high morbidity and

mortality. Clinical coccidiosis involves dramatic remodeling of the mucosa with extensive damage that leads to reduced absorption, hemorrhage and fluid leakage from damaged mucosa that is also associated with a dynamic immune and inflammatory response (Blagburn and Todd, 1984). Sub-clinical infection will result in localized damage to the epithelia offering the potential for invasion by gut dwelling microorganisms such as the opportunistic pathogen *Clostridium perfringens*. In facts, infection with eimerian parasites has been associated with increase in the incidence of clostridial necrotic enteritis in poultry (Williams *et al.*, 2003). The present study was designed to observe response of cecal goblet cells on intracellular development of *E. tenella* in susceptible and infected chickens.

Methods of The Research

Animals and parasites

Eighty males CP 707 broiler, Day Old Chick was purchased DOC supplier Co., housed in clean cages and fed with a standard diet without coccidiostat (Sabrani *et al.*, 1981) and tap water *ad libitum* in an air-

conditioned room ($23 \pm 1^\circ \text{C}$), under conventional condition with a 12:12 hr light: dark cycle. They were kept as outlined in the guide for the care and use of laboratory animals by The Faculty of Veterinary Medicine, University of Airlangga. The pathogenic agent used in this study, local isolated *E. tenella* from field and has been maintained in animals cage research of The Faculty of Veterinary Medicine by oral passage in CP 707 broiler.

Experimental procedures

Eighty males CP 707 broiler were randomly assigned into two groups and each group represented susceptible and infected chickens, respectively. All infective doses of *E. tenella* sporulated oocysts were orally given by throat tube, as 1×10^5 oocysts/chicken in 1 ml of distilled water. Faecal pellets were collected from the infected chickens between days 7 and 12 post infection (pi), and the total oocyst numbers/chicken calculated by McMaster chamber method (Yunus et al., 2005).

Group 1: This group observed the response of cecal goblet cell on intracellular development of *E. tenella* in susceptible chickens. Forty of five weeks old chickens were infected orally with 1×10^5 *E. tenella* and the day of infection was defined as day 0 (uninfected control). Five chickens were killed every two days from day 0 to day 12 pi using goblet cell examination. The last five chickens were used for the daily oocyst output examination.

Group 2: This group observed the same observation as the group 1 in infected chickens. Forty of three weeks old chickens were infected orally with 1×10^5 *E. tenella* and two weeks after the 1st infection challenged with the same dose of *E. tenella*. Post challenge infection was done the same procedure as the group 1. Five chickens were killed every two days from day 0 to day 12 pi using goblet cell examination. The last five chickens were used for the daily oocyst output examination.

Histological examination

Samples of cecum were taken from susceptible and infected chickens, fixed in 10% neutral buffered formalin overnight followed by startified dehydration in alcohol and embedding in the paraffin wax. Serial 4 μm thick sections were cut, deparaffinized, rehydrated and stained with hematoxylin and eosin for routine histology of intracellular development of *E. tenella*, or with Alcian Blue-Periodic Acid Shiff (AB-PAS) for clear determination of goblet cell. Intracellular development of *E. tenella* was expressed by total of oocyst output and histopathological examination. Cecal goblet cells were counted on the 10th day pi for each chicken on each group, which *E. tenella* infection at peak level of the daily oocyst

output. The number of cecal goblet cell was counted on at least 10 well-orientated crypt units. Effect of *E. tenella* infection was expressed by the mean of the number of goblet cells per 10 crypts (Chadee and Meerovitch, 1985; Seo et al., 2003).

Results were statistically analyzed using student's *t*-test and a *p* value below 0.05 was considered significant. All results were presented as mean $\pm$ standard deviation (SD).

Results and Discussion

The pattern and total of oocyst output of E. tenella infection: The temporal pattern of oocyst output per day confirms those previously reported (Stiff and Bafundo, 1993) with this isolate of *E. tenella*. Oocyst first appeared on the 7 days pi, then reached peak on the 10 days pi before numbers declined rapidly and the fewest oocysts were detected on 12 days pi. Basically, the same pattern of daily oocyst output was seen in both susceptible and infected chickens groups, but the infected chickens oocyst output per day as well as totally were significantly lower than susceptible chickens (Figs. 1 and 2).

The total numbers of oocysts produced of susceptible chickens in this study was $[6.6 \pm 0.5] \times 10^4$ /chicken and infected chickens $[1.8 \pm 0.5] \times 10^4$ /chicken, the period of patency was $[12.4 \pm 0.7]$ days (Fig. 2). The number of intracellular parasitic stages present within the cecal epithelia were in largest numbers at approximately 7 to 8 days pi especially in susceptible chickens. Infection was associated with various histopathological changes clearly in the cecum of susceptible chickens (Fig. 5).

Total of oocyst production in infected chickens were significantly decreased ($p < 0.01$) about 75 % compared with susceptible chickens and/or initial infected chickens (Fig. 2). Clinical signs (such as anemia, anorexia) of infected chickens were more slight than susceptible chickens. Consistency and colour of feces appeared normal in infected chickens compared susceptible chickens. Pathological changes and lesion score of cecum in infected chickens more slight compared than susceptible chickens (Unpublished data). Endogenous development of *E. tenella* (schizogony and gametogony) in infected chickens was suppressed and/or incompleated undergone. Several generations of schizont appeared degenerated consequently unbreak schizont, damaged cecal mucosa epithelial cells were not occurred and automatically there were no bleeding in cecum. Many abnormal endogenous development of parasites such as gametogony to result in disturbing syngamy of microgamete and macrogamete. Thus, oocysts formation were not perfectly continued. In contrast, endogenous development of parasites in susceptible chickens

occurred well and no inhibition. Infection with one species of *Eimeria* induces protective immunity in the host that is long lasting and exquisitely specific to that particular parasite (Yun *et al.*, 2000). While a large number of inoculating oocysts is generally required to generate an immune response against *Eimeria*, some exceptions have been noted, e.g. *E. maxima* is highly immunogenic and requires only a small number of oocysts to induce almost complete immunity. The early endogenous stages of the parasite life cycle are

considered to be more immunogenic than the later sexual stages (Yun *et al.*, 2000) although Wallach *et al.* (1990, 1995) showed that immunization with recombinant gamete associated antigen induced partial protection against challenge infection. Studies using oocysts irradiated to prevent intracellular development, but not invasion, demonstrated partial protection against challenge infection, thereby suggesting that sporozoites may also be immunogenic (Jenkins *et al.*, 1991).

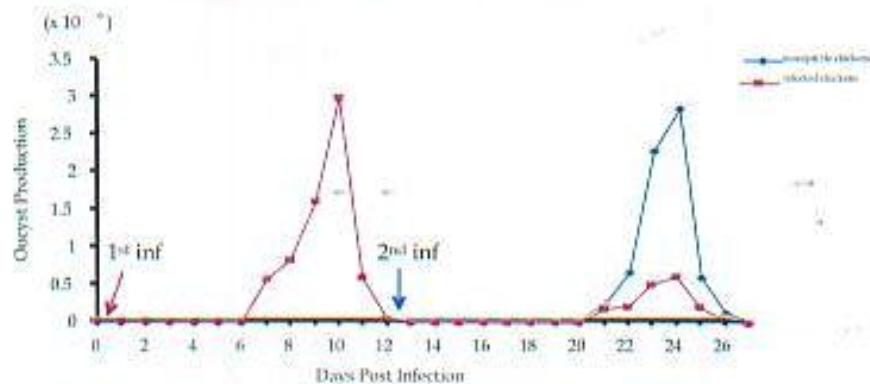


Figure 1. The pattern of oocyst production per day of susceptible and infected chickens on *E. tenella* infection. The initially oocyst output on the 7th day, then to peak level the 10th day and for limit around 12 days post infection. Each value of oocyst production per day represents mean of 5 chickens.

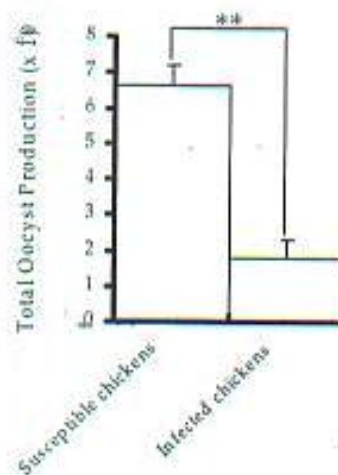


Figure 2. The effect of infection course on total of oocyst production from *E. tenella* infected chickens. From left to right, each column represents infection course status. Each value represents mean $\pm$ SD of 5 chickens. ** $p < 0.01$

The pattern of cecal goblet cells number during *E. tenella* infection: The numbers of cecal goblet cells of

susceptible chickens were declined from 2 days pi and continued to decline until 8 days pi (Fig. 3).

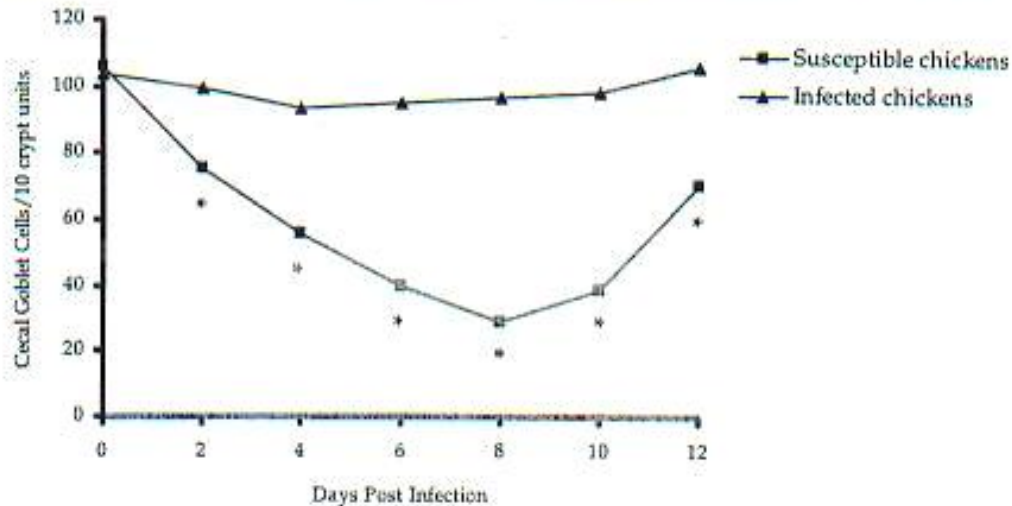


Figure 3. The pattern of cecal goblet cells number of susceptible and infected chickens during *E. tenella* infection course. Goblet cells are represented as the mean numbers per 10 crypt units with 5 chickens/group and * indicates significant difference compared with number of goblet cell in pre-infected chickens (day 0)

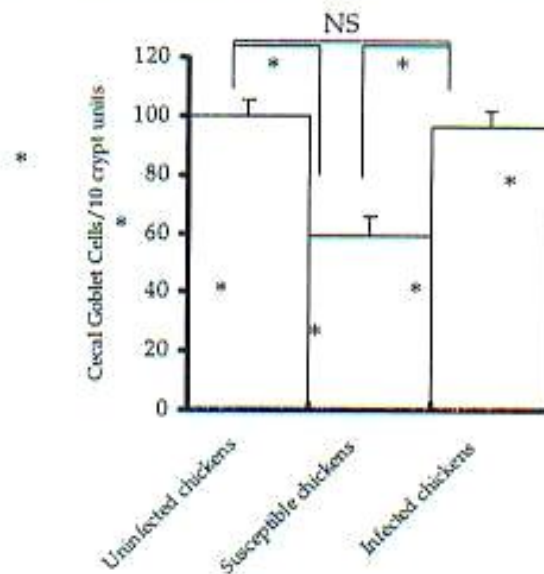


Figure 4. The effect of *E. tenella* infection course on goblet cell number of each chickens treatment group. From left to right, each column represents *E. tenella* infection course status. Each value represents mean $\pm$ SD of 5 chickens. * $p < 0.05$; NS, not significant

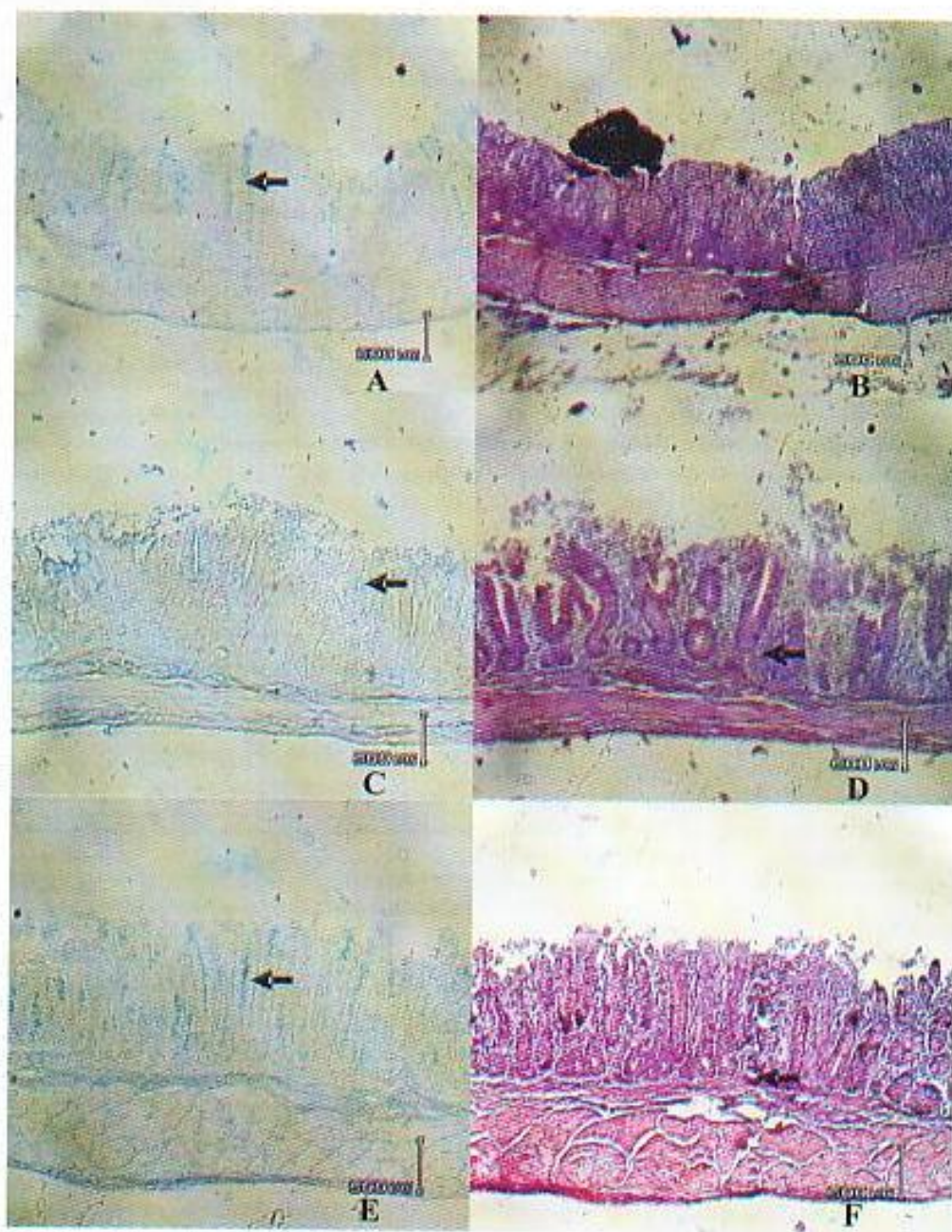


Figure 5. Histological assessment of cecal goblet cell response during infection with *E. tenella*. Panels A, B represent uninfected chickens; panels C, D represent susceptible *E. tenella* infected chickens at 8 days pi and panels E, F represent immune *E. tenella* infected chickens at 8 days pi. Cecal samples (ABCDEF) are depicted at magnification of $\times 100$. Tissue were fixed in 10% neutral buffered formalin and stained with Alcian Blue and Periodic Acid Schiff AB-PAS (panels A, C, E) or H & E (panels B, D, F). Representative goblet cells are indicated by an arrow (A, C, E) and parasites by an arrow (D, F)

At 8 days pi the numbers of cecal goblet cells were approximately four folds less numerous than with uninfected (day 0) chickens. Rapid recovery of goblet cell number in cecum occurred after 10 days pi. The numbers of cecal goblet cells of an infected chickens were relatively unchanged by infection of chickens with *E. tenella* (Figs. 3 and 4). Infection was associated with various histopathological changes in the infection sites (Fig. 5). The histopathological changes included substantial hyperplasia of crypt enterocytes, damage to the surface of mucosa and extensive infiltration in the lamina propria are most evident of the presence of infection in cecum. Numerous intracellular parasites were associated with those histopathological lesions (Fig. 5).

In *E. tenella* infection was predominantly parasite to invade cecal crypt and epithel, although mature gametocyte and/or zygote were present in surface of epithelial cells. This type of pathology is typical of those coccidial parasites that invade the large intestinal epithelia (Gregory and Catchpole, 1990) and the host inflammatory response appears to be responsible for at least some of these changes (Ruff and Allen, 1990). Indeed, with *E. nieschulzi* (a small intestinal tropic parasite), the damage to the epithelial surface was greatly reduced in T cell-deficient nude rats despite a dramatic increase in the numbers of parasites developing in the gut (Rose and Hesketh, 1982). A range of changes in the immune cell populations associated with coccidial lesion has been reported (Gregory and Catchpole, 1990; Ruff dan Allen, 1990), including intraepithelial lymphocyte populations (Vervelde et al., 1996), mast cells (Huntley et al., 1985; Rose and Hesketh, 1982) and polymorphonuclear cells (Vervelde et al., 1996).

Response of cecal goblet cells on E. tenella intracellular development in susceptible and infected chickens: Significantly, the decrease of cecal goblet cells number occurred in susceptible chickens compared with uninfected chickens ($p < 0.05$), but there were not differences between an infected and uninfected chickens (Figs. 3 and 4). The dramatic change in cecal goblet cells number were limited to the site of infection and not evident in an uninfected site, which another site to the developmental site of *E. tenella*. Since the *E. tenella* parasites develop in the crypt region that contains the multipotential stem cells (Cheng, 1974), the reduction in goblet cell may reflect damage to the stem cell population by development of parasites. Indeed, goblet cells arise by mitosis from multipotential stem cells at the base of the crypt (Cheng, 1974) and the depletion of goblet cell was most evident in infected crypts and much less in neighboring uninfected areas of the intestine. Changes in goblet cell numbers may affect the susceptibility of the parasite-infected host to limit the

capacity of opportunistic pathogens from interacting or penetrating the local epithelium. Indeed, subclinical coccidiosis is a predisposing factor in the development of necrotic enteritis under experimental or field conditions (Baba et al., 1992; Williams et al., 2003).

Conclusion

E. tenella intracellular development suppressed locally differentiation of cecal goblet cell in site infection. Susceptible chickens: *E. tenella* intracellular development suppressed the numbers of cecal goblet cells or site infection (hypoplasia) in association with development of endogenous stages of parasites life cycle. Infected chickens: Protective Immunity protects site infection of damage by *E. tenella* intracellular development so that the number of cecal goblet cells or site infection relatively unchanged.

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