INFLUENCE OF INTESTINAL GOBLET CELL BY *Eimeria vermiformis* INFECTION ON THE EXPULSION OF *Nippostrongylus brasiliensis* IN CO-INFECTED HOST

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ABSTRACT

Goblet cell plays an important role on expulsion of *Nippostrongylus brasiliensis* by increase in the number in intestine. In contrast, infection with *Eimeria vermiformis* lead to decrease goblet cell number. On the other hand, *Eimeria vemiformis* results in the development of small intestinal Th1-type and infection of *Nippostrongylus brasiliensis* results on development of Th2-type response. This paper discussed the influence of goblet cell in co-infected host on elimination of *Nippostrongylus brasiliensis*. For this purpose, *Nippostrongylus brasiliensis* and *Eimeria vermiformis* were infected to ICR mice, expulsion of the worm were delayed in co-infected group compare to single infected group and associated with the changes of goblet cell number. The results clearly showed that *Eimeria vermiformis* infection influenced to the delay of elimination of *Nippostrongylus brasiliensis* in immune group. Thus, the influence may effect through immune system was suggested.

Keywords: Goblet cell, *Nippostrongylus brasiliensis, Eimeria vermiformis.*

Ảnh hưởng của tế bào hình chén trong việc thải trừ giun *Nippostrongylus brasiliensis* ở vật chủ đồng nhiễm với *Eimeria vermiformis*

TÓM TẮT

Tế bào hình chén đóng vai trò quan trọng trong việc thải trừ giun *Nippostrongylus brasiliensis (N. brasiliensis)* thông qua việc tăng số lượng tế bào và tăng tiết chất nhầy ở ruột. Trong khi đó, cầu trùng *Eimeria vermiformis* lại làm giảm số lượng tế bào hình chén ở ruột và hình thành miễn dịch tế bào Th1 ở ruột thì *N. brasiliensis* lại sản sinh đáp ứng miễn dịch Th2. Bài báo này sẽ thảo luận những ảnh hưởng gây ra do tế bào hình chén ở chuột đồng nhiễm trong việc thải trừ *N. brasiliensis*. *Nippostrongylus brasiliensis* và *Eimeria vermiformis* được gây nhiễm cho chuột ICR. Kết quả cho thấy, việc thải trừ giun bị giảm ở chuột đồng nhiễm so với chuột đơn nhiễm và có liên quan đến số lượng của tế bào hình chén. Kết quả này cho thấy, *Eimeria vermiformis* có ảnh hưởng đến việc thải trừ *Nippostrongylus brasiliensis* và có thể thông qua hệ đáp ứng miễn dịch ở chuột đồng nhiễm.

Từ khóa: tế bào hình chén, *Nippostrongylus brasiliensis, Eimeria vermiformis.*

1. INTRODUCTION

Co-infection is widely found in infected hosts. The interaction between parasites and host response in co-infected host was rarely studied. *Nippostrongylus brasiliensis* are well known as a helminth of intestinal tract (Oliver et al., 2004; Ogilvie and Hockley, 1968; Urban et al., 1998). In the gut lumen, the worm was expelled by mucin affection (Nawa et al., 1994) through goblet cell hyperplasia mechanism (Wells, 1963; Miller and Nawa, 1979). While goblet cell plays a role in expulsion of nematoda, in *Eimeria vermiformis* infection, the depletion

of goblet cell number was detected (Linh et al., unpulished data). On the other hand, *E. vermiformis* results in a Th1 type response characterized by production of gamma interferon (IFN-γ) while *Nippostrongylus brasiliensis* is a Th2 type response characterized by CD4+ T cell mediated (Kopf et al., 1993; McKenzie et al., 1999). Th2 cell-mediated mucus production acts positively in the protection of mucosal surfaces from pathogens. The control of goblet cell production in type 2 inflammatory processes was attributed to IL-4, IL-9, and IL-13 (Temann et al., 1997; Longphre and Gallup., 1999). IL-4 and IL-13 were considered the major mediators of goblet cell production in the type 2 cytokine-driven inflammatory process evoked by intestine helminth infection.

Recently, Oliver et al. (2004) reported that *N. brasiliensis* did not alter immune response of *Toxoplasma gondii* and did not alleviate gut pathology or prevent death of mice. Marshall et al. (1999) reported that Th2 response mounted after infection with *Schistosoma mansoni* was ameliorated the intestinal Th1 type immunopathology in *T. gondii* infected mice. However, the change of goblet cell in co-infected host was not mentioned.

The present study focused on the change of goblet cell number and its influence on the expulsion of *N.brasiliensis* in co-infected host.

2. MATERIALS AND METHODS

2.1. Experimental animals

Males of ICR mice at 9 weeks of age were housed in clean cage and given standard diet and clean water ad libitum in an air conditioned room (23±3°C), under conventional condition with a 14:10hr light:dark cycle. All protocols were approved by the Institutional Review Board for animal experiment of the University of Miyazaki.

2.2. Parasite infection

E. vermiformis was passaged in mice, and oocysts were purified and sporulated (Rose et al., 1984). After microscopical scoring of stocks for sporulation, mice were given 100 or 500 sporulated oocysts in 100 µl of water by oral gavage. During infection, feces were collected until 13 days after infection. Oocysts were counted on McMaster chambers after salt flotation.

E. pragensis was routinely maintained in our laboratory by oral passage through C57BL/6mice (Mesfin and Bellamy, 1979). All infective doses of *E. pragensis* sporulated oocysts were orally given by a gastric tube at 100 oocysts/mouse in 0.1ml of DW. During infection, feces were collected until day 13 after infection and oocysts were counted on McMaster chambers after salt flotation.

The strain of *N. brasiliensis* used in this study was maintained in our laboratory by serial passage in Wistar rats using subcutaneous inoculation of 3,000–4,000 third-stage larvae (L3) prepared using the charcoal culture method (Ishikawa et al., 1994). The rats were infected with L3 of *N. brasiliensis* by subcutaneous inoculation into the flank region. Infection was confirmed by counting fecal egg output as eggs per day (EPD).

2.3. Experiment design

Experiment 1

45 mice were used for experiment and 15 mice were used for each group. 5 mice were killed at day 7, day 10 and day 13 for autopsy samples.

The infection was divided into 3 groups:

Group 1 infected with *N. brasiliensis* only.

Group 2 infected with *N. brasiliensis and E. vermiformis*

Group 3 infected with *N. brasiliensis and E. pragensis.*

During infection, feces were collected from day 4 to day 12. Oocysts and eggs were counted on McMaster chambers after salt flotation. Worm number was measured every three days from day 7 to day 13 after infection. The experiments were designed as follow.

Experiment 2

The experiment was divided into 3 groups. 45 mice were used for experiment and 15 mice were used for each group. 5 mice were killed at day 2, day 4 and day 6 after immunization for autopsy samples.

During infection, feces were collected from day 4 to day 12. Oocysts and eggs were counted on McMaster chambers after salt flotation. Worm number and eggs were counted at day 2, day 4 and day 6 after worm transfer.

Group 1: Control group, direct transfer of the worm to intestine of mice at day 28

Group 2: *N. brasiliensis* was infected at day 20 before worm transfer.

Group 3: 20 days before worm transfer, *N. brasiliensis* was infected and 8 days later, *E. vermiformis* was infected.

The experiments were designed as follow.

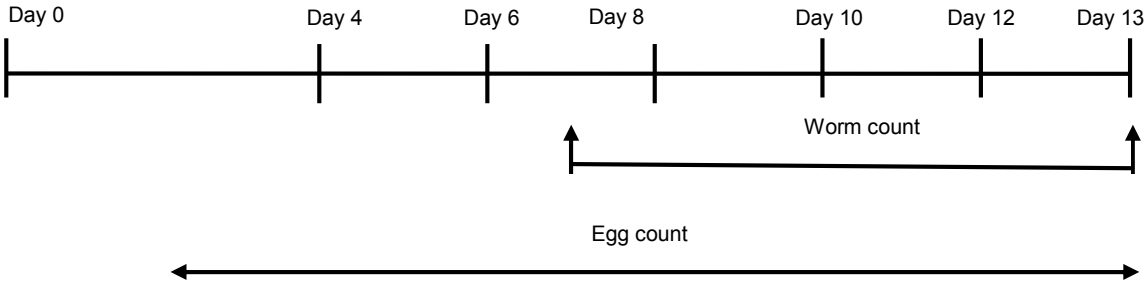
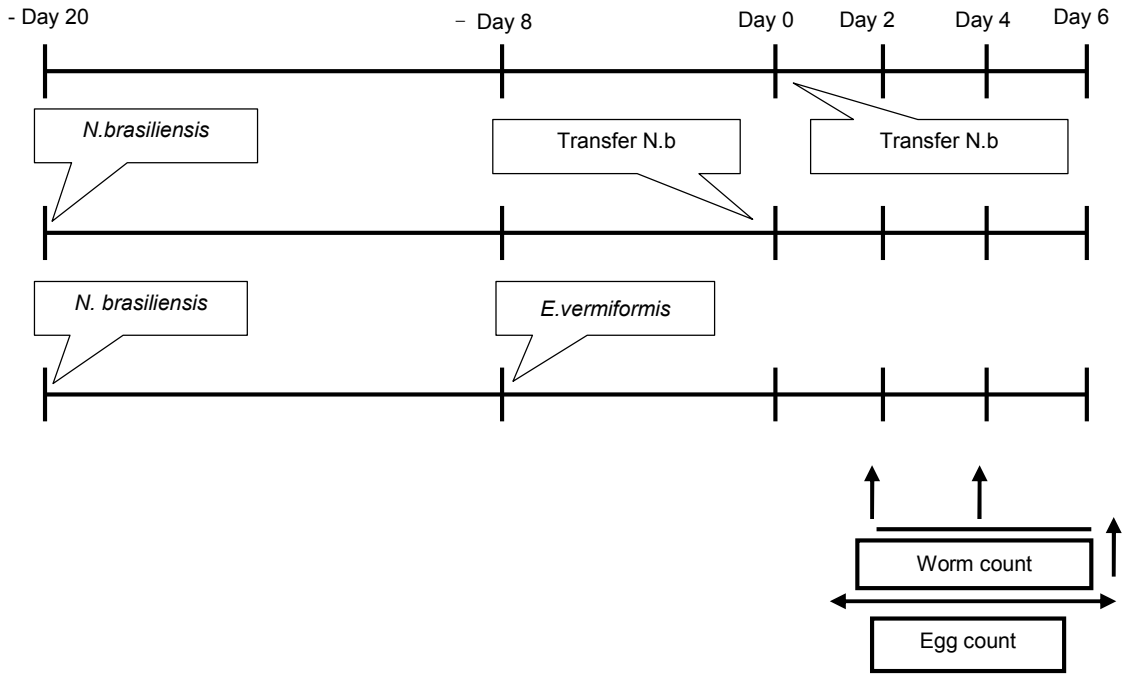


Fig. 1.



2.4. Histopathological examination

The intestine was cut open longitudinally and fixed in 10% neutral buffered formalin pH 7.4, processed in paraffin, cut in 4-µm thick serial sections and stained with Alcian Blue for determination of GC. The number of GC in small intestine was counted on at least 10 well-orientated crypt-villus units and 3 well-orientated crypt-villus units in colon for each animal and expressed as the mean numbers of GC.

2.5. Statistical Analysis

The mean and the standard errors (SE) were calculated by the Student T test. $P < 0.05$ was considered as significant.

3. RESULTS

Since goblet cells were known as an important factor on expulsion of *Nippostrongylus brasiliensis*, in contrast, infection of *Eimeria vermiformis* and *Eimeria pragensis* resulted in depletion of goblet cells, we examined whether *Eimeria* infections influence the elimination of *Nippostrongylus brasiliensis* in co-infected host.

Results from experiment 1 showed that fecal egg counts decreased from day 6 in group 1 and group 2 compared to group 3 (Fig. 3). Number of EPG in group 3 was stable from day 5 to day 9 and decreased until day 12. Number of worm burden was high in day 10 in group 2 and 3 compared to group 1. Worm elimination happened clearly in group 1 from day 10 to day 13. At day 7 of infection, worm burden was not significantly different between three groups and between group 3 compared to single infected group (group 1) (Fig. 3, Fig. 4).

Thus, *Eimeria pragensis* slightly influenced the elimination of *Nippostrongylus brasiliensis*. In group 2, number of worm burden was high at day 10 and significant difference in number of worm burden was found on day 13 compared to group 1 and group 3 (Fig. 4). Therefore, *Eimeria vermiformis* infection may play a big role on delay of elimination of *Nippostrongylus brasiliensis*.

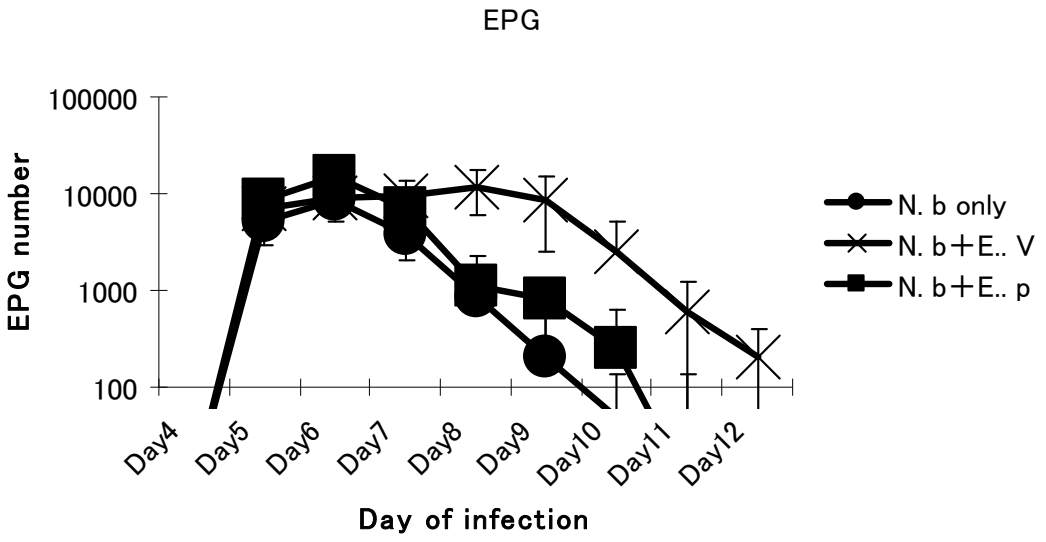


Fig 3. EPG number counts in mice. Mice were orally infected as designed and the EPG was determined from day 4 to day 12 post infection

*Note: Values represent the mean standard deviation of experiments (*P < 0.05, **P < 0.01 compared with control values).*

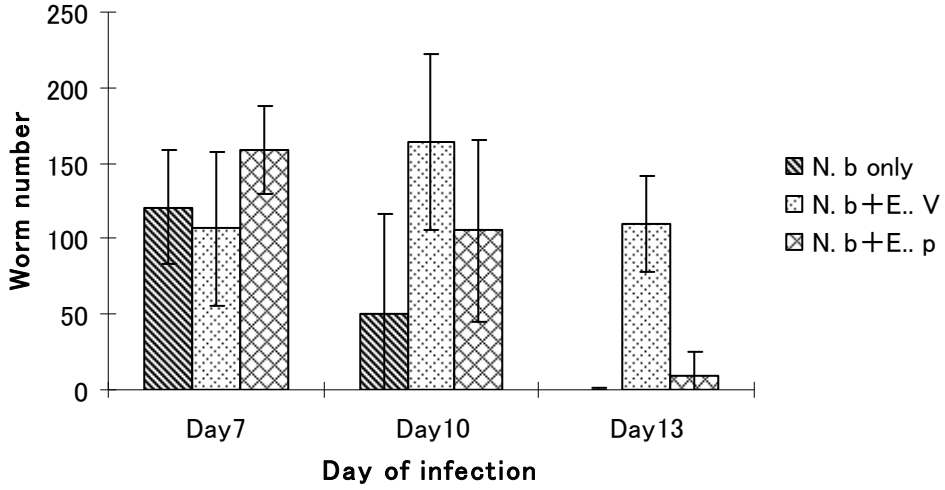


Fig. 4. Number of worm in infected mice.
Animals were infected with 4,000 L3 of *N. brasiliensis*

*Note: Samples were examined on days 7, 10, and day 13 p.i. *P < 0.05, **P < 0.01 compared with control values. Bars represent means ± SE (N =5).*

Goblet cell number was significantly lowered in co-infected group with *Nippostrongylus brasiliensis* and *Eimeria vermiformis* compared to single infected group with *Nippostrongylus brasiliensis* (Fig. 5), suggeting that depletion of goblet cells caused by *Eimeria vermiformis* infection may delay the elimination of *Nippostrongylus brasiliensis*. In co-infected group with *Nippostrongylus brasiliensis* and *Eimeria pragensis,* number of goblet cells in small intestine was not change compared to control group (Fig. 5) but the depletion of goblet cells occurred in large intestine (Fig. 6). However, there existed no significant difference between EPG and worm burden number in group co-infected with *Nippostrongylus brasiliensis* and *Eimeria pragensis* compared to single infected group with *Nippostrongylus brasiliensis* (Fig. 3 and Fig. 4). This suggests that *Eimeria* pragensis may not influence the expulsion of *Nippostrongylus brasiliensis*.

Primary infection of *Nippostrongylus brasiliensis* caused rapidly expulsion of the worm regarding T-cell function of the host. The effect of secondary infection of *N. brasiliensis* by transfering the worm to intestine of mice on worm expulsion and goblet cell changes were examined.

Results from Fig. 7 and Fig. 8 showed that on day 2, EPG and worm burden number were not significantly different between three groups but the significant differences were found as early as day 4 after transfering the worm in group B compared to control group and completely decreased on day 6. In group C, the number of EPG and worm burden decreased from day 4 and continued to decrease on day 6 compared to control group but higher than group B.

In addition, impairment of goblet cell hyperplasia was observed in group C from day 2 to day 6 after transfer the worm (Fig. 9). By this time, depletion of goblet cells caused by *Eimeria vermiformis* was observed.

The results suggested that the secondary infection of *Nippostrongylus brasiliensis* may activate Th2 response resulting in increased goblet cell number and lead to elimination of *Nippostrongylus brasiliensis*. Thus, the delay of elimination of *Nippostrongylus brasiliensis* showed in group C (Fig. 7) was influenced by the infection of *Eimeria vermiformis*.

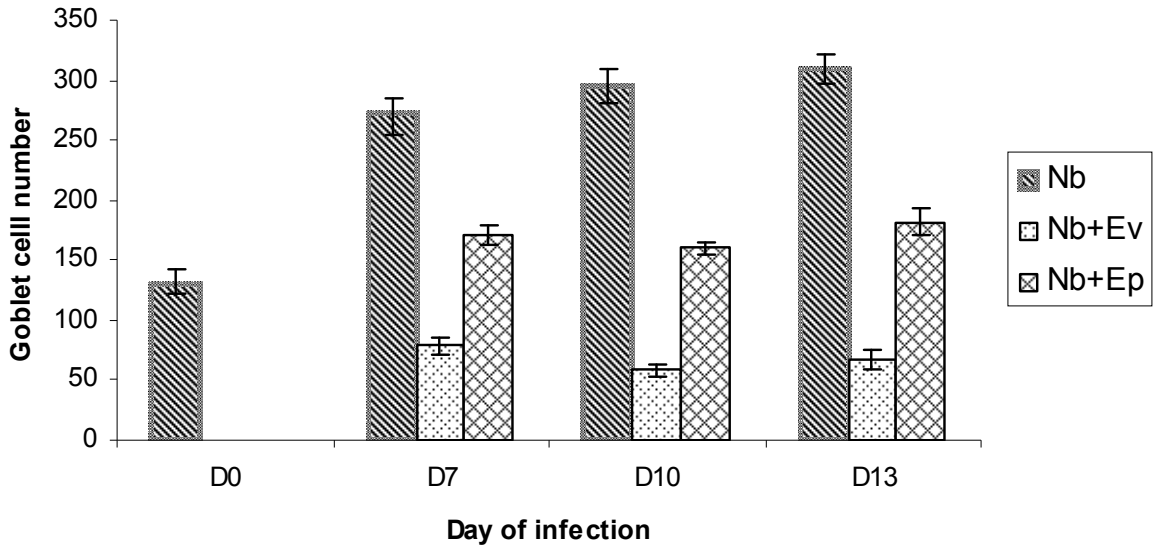


Fig. 5. Goblet cell (GC) number in small intestine in different groups at day 0, 7, 10 and 13 post infection

*Note: The number of GC was counted on at least 10 well-orientated crypt-villus units for each animal and expressed as the mean numbers of GC. *P < 0.05, **P < 0.01 compared with control values. Bars represent means ± SE (N =5).*

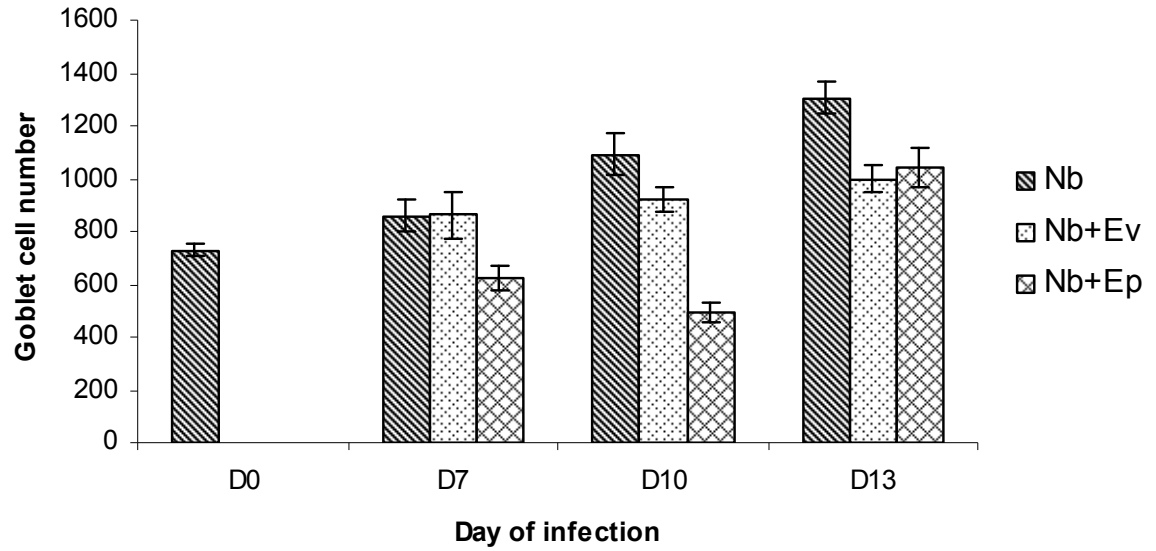


Fig. 6. Goblet cell (GC) number in large intestine in different groups at day 0, 7, 10 and 13 post infection

*Note: The number of GC was counted on at least 3 well-orientated crypt-villus units in colon for each animal and expressed as the mean numbers of GC. *P < 0.05, **P < 0.01 compared with control values. Bars represent means ± SE (N =5)*

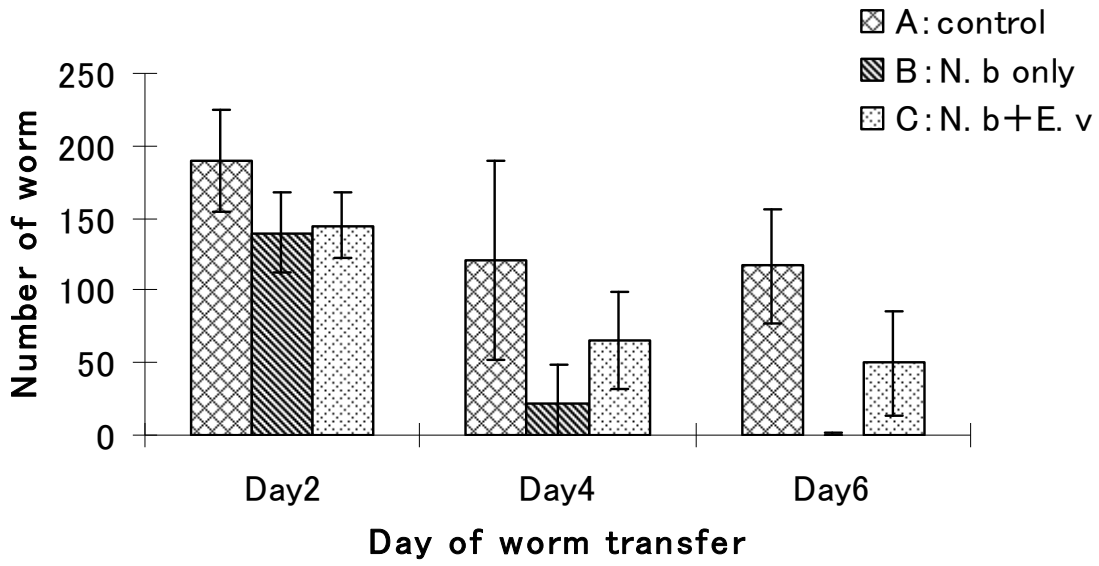


Fig. 7. Worm number was counted every at day 2, day 4 and day 6 after worm transfer

*Note: *P < 0.05, **P < 0.01, ***P< 0.001 compared with controlvalues. Bars represent means ± SE (N =5)*

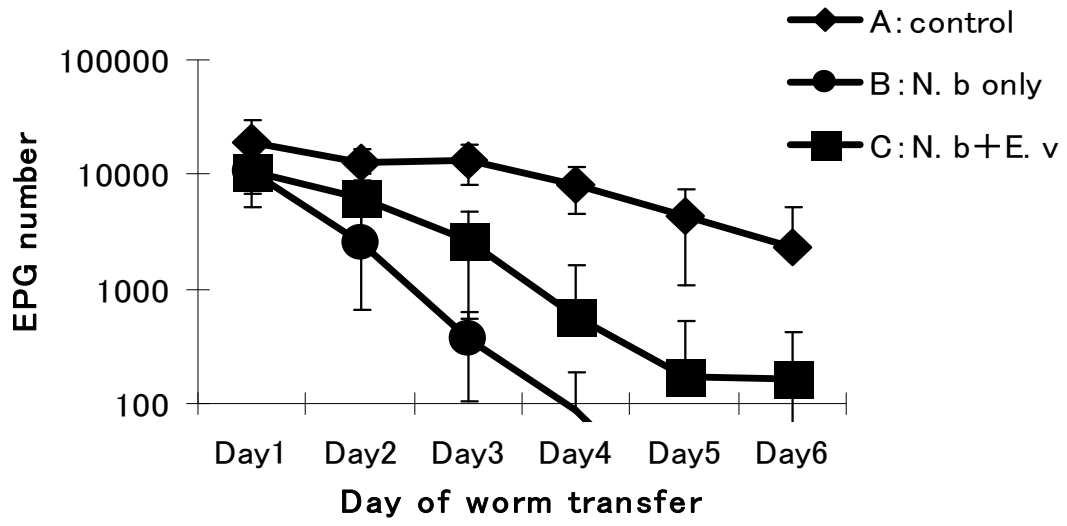


Fig. 8. EPG number counts at day 2, day 4 and day 6 after worm transfer

*Note: *P < 0.05, **P < 0.01, ***P< 0.001 compared with control values. Bars represent means ± SE (N =5).*

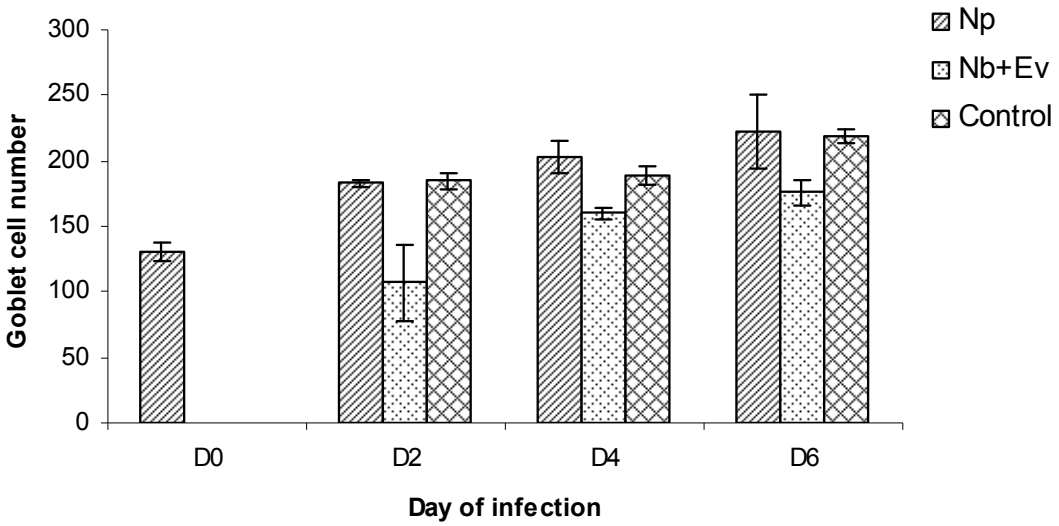


Fig. 9. Goblet cell (GC) number in small intestine in different groups at day 0, 7, 10 and 13 post infection

Note: The number of GC was counted on at least 10 well-orientated crypt-villus units for each animal and expressed as the mean numbers of GC. Bars represent means ± SE (N =5).

4. DICUSSION

Expulsion of *N. brasiliensis* has long been discussed (Miller and Nawa, 1979; Nawa et al, 1994; Urban et al., 1998; William et al., 2007). Our work provides evidences of the influence on expulsion of *N. brasiliensis* by *Eimeria* infections. Expulsion of *N. brasiliensis* is associated with intestinal golet cell hyperplasia (Tomita et al.,1995; Deplancke and Gaskins, 2001). Our data also indicated that number of goblet cells in both small intestine and large intestine increased in group infected with *N. brasiliensis* only compared to uninfected group. On the other hand, *Eimeria* infection caused depletion of goblet cells in intestinal tract (Rose et al., 1992; Yunus et al., 2005). In this experiment, we observed the depletion of goblet cell in co-infected group compared to group infected with *N. brasiliensis* only. However, the depletion of goblet cells was associated with the worm remain in co-infected host on day 10 and day 13 of infection in co-infected group with *N. brasiliensis* and *E. vermiformis* but not in group co-infected with *E. pragensis*. So far, *E. pragensis* was known as a pathogen that invades the large intestine epithelia (Yunus et al., 2005). In addition, no changing of goblet cell response in small intestine in co-infected host with *N. brasiliensis* and *E. pragensis* compared to uninfected group was confirmed.

Taken together, *E. pagensis* may not influence the delay of worm expulsion in co-infected host.

Changing in goblet cell number may effect the susceptibility of the parasite infected host to limit the capacity of opportunistic pathogens from interacting or penetrating the local epithelium (Yunus et al., 2005) or alter the host immune response to eliminate pathogens in intestinal tract (Deplancke and Gaskins, 2001).

In general, Th1 type response to infection with *Eimeria* led to imunopathology (Rose et al., 1992; Smith and Hayday, 2000). In contrast, Th2 cytokine driven expulsion of *Nippostrongylus brasiliensis* associated with intestinal goblet cell hyperplasia and mucus production (William et al., 2007).

To investigate the capicity of Th2 microoganisms to modulate *T. gondii* induced Th1 response, Marshall et al. (1999) co-infected mice with *S. mansoni* and *T. gondii*. The Th2 response induced by infection of *S.mansoni* blocked IFN-γ and NO production.

Since *N. brasiliensis* induced Th2 type responses were down-regulated by subsequent infection with *T. gondii* (Oliver et al., 2004), we investigated how immune regulation during the infection with *N. brasiliensis* followed infection with *E. vermiformis*. The elimination of *N.brasiliensis* occurred as soon as day 4 after transfering the worm compared to the control group. During primary infection, *N. brasiliensis* did not secrete significant amounts of degranulator until day 5-6 after infection (Miller et al., 1983). The increased intestinal worm or egg may due to the disruption of eosinophilopoiesis in primary infection (Michell et al., 2007).

Although in *N. brasiliensis* infection, worm expulsion was completed by day 7 of secondary infection (Michell et al., 2007), similar to the results shown in group B. On the other hand, delay of elimination of the worm wá observed in co-infected mice with *E.vermiformis*. Significant differences were observed from day 4 to day 6 after transfering the worm. During this time, depletion of goblet cells was induced by *E. vermiformis*. This seemed that Th1 type response by *E. vermiformis* somehow supressed Th2 response induced by *N. brasiliensis* in co-infected host.

The present study indicated that *E. vermiformis* influences to delay the elimination of *N. brasiliensis* in co-infected host. It is not clear how *E. vermiformis* influences the expulsion of *N.brasiliensis*, but this may be via to goblet cell response under immune mechanism. Further study is needed to clarify this point.

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REFERENCES

Deplancke, B and Gaskins, H.R. (2001). Microbial modulation of innate defence: goblet cells and the intestinal mucus layer. Am J Clin Nutr.,73: 1131S-1141S.

Ishikawa N, Horii Y, Oinuma T, Suganuma T, Nawa Y. (1994). Goblet cellmucins as the selective barrier for the intestinal helminths: T-cell-independentalteration of goblet cell mucins by immunologically 'damaged' *Nippostrongylus brasiliensis* worm and its significance on the challenge with homologous and heterelogous parasite. Immunology, 81: 480-486.

Kopf, M., Le Gros, G., Bachmann, M., Lamers, M.C., Bluethmann, H., and Kohler, G. (1993). Disruption of the murine IL-4 gene blocks Th2 cytokine responses. Nature, 362: 245-248.

Longphre, M., Li, D., Gallup, M. (1999). Allergen induced IL-9 directly stimulates mucin transcription in respiratory epithelial cells. J Clin Invest., 104: 1375-1382.

Marshall, A.J., Brunet, L.R., van Gessel, Y., Alcaraz, A., Bliss, S.K., Pearce, E.J., and Denkers, E.Y. (1999). *Toxoplasma gondii* and *Schistosoma mansoni* synergize to promote hepatocyte dysfunction associated with high levels of plasma TNF-alpha and early death in C57BL/6 mice. J. Immunol., 163: 2089-2097.

McKenzie, G.J., Fallon, P.G., Emson, C.L., Grencis, R.K., and McKenzie, A.N. (1999). Simuntaneous disruption of interleukine IL-4 and IL-13 defines individual roles in T helper cell type 2-mediate response. J. Exp. Med., 189: 1565-1572.

Mesfin, G.M and Bellamy, J.E.C. (1979).Effect of acquired resistance on infection with *E. falciformis* var. *pragensis* in mice. Infect. Immun., 23: 108-114.

Miller, H.R.P. and Nawa, Y. (1979). *Nipostrongylus brasiliensis*: intestinal goblet cell response in adoptively immunized rats. Exp. Parasitol., 47: 81.

Miller, H.R.P., Woodbury, R.G., Huntley, J.F., Newlands, G. (1983). Systemic release of mucosal mast cell protease in primed rats challenged with *Nippostrongylus brasiliensis*. Immun., 49: 471-479.

Nawa, Y., Ishikawa, N., Tsuchiya, K., Horii, Y., Abe, T., Khan, A.I., Shi, B., Itoh, H., Ide, H., and Uchiyama, F. (1994). Selective effector mechanism for expulsion of intestinal helminths. Parasite Immunol., 16: 333-338.

Ogilvie, B.M., and Hockley, D.J. (1968). Effect of immunity of *Nippostrongylus brasiliensis* aldult worms: reversible and irreversible change in

effectivity, reproduction and morphology. J. Parasitol., 54: 1073-1084.

Oliver, L., Ildiko, R.D., and Klaus, J.E. (2004). Infection with *Toxoplasma gondii* reduce established and developing Th2 responses induced by *Nippostrongylus brasiliensis* infection. Infection and immunity, 72(7): 3812-3822.

Rose, M. E., D. G. Owen, and P. Hesketh (1984). Susceptibility to coccidiosis: Effect of strain of mouse on reproduction of *Eimeria vermiformis* Parasitology, 88: 45-54.

Rose, M.E., Millard, B.J and Hesketh, P. (1992). Intestinal changes associated with expression of immunity to challange with Eimeria vermiformis. Infect. Immun., 60(12): 5283-5290.

Smith, A.L and Hayday, A.C. (2000).Genetic dissection of primary and secondary responses to a widespread natural pathogen of the gut, Eimeria vermiformis. Infect. Immun., 68(11): 6273-6280.

Temann, U.A., Prasad, B., Gallup, M., W. (1997). A novel role for murine IL-4 in vitro: induction of MUC5AC gene expression and mucin hypersecretion. Am J Respir Cell Mol Biol., 16: 471-478.

Tomita, M., Itoh, H., Ishikawa, N., Higa, A., Ide, H., Marukumo, Y., Maruyama, H., Koga, Y and Nawa, Y. (1995). Molecular cloning of mouse intestinal trefoil factor and its expression during goblet cell changes. Biochem., 311: 293-297.

Wells, P.D. (1963). Mucin secrecting cells in rats infected with *Nipostrongylus brasiliensis*. Experimental Parasitology, 14(1): 15-22.

William, G.C.H., Antony, J.C., Claire, J.H., Helen, M., Elmarie, M., Berenice, A., Fred, D.F., Gary, K.O., Dave, E., Frank, B. (2007). Delayed goblet cell hyperplasia, acetylcholine receptor expression, and worm expusion in SMC-specific IL-4Rα- deficient mice. Plos Pathog., 3(1): 0046-0053.

Urban, J.F., Noben-Trauth, N., Donaldson, D.D., Madden, K.B., Morris, S.C., Collins, M., and Finkelman, F.D. (1998). IL-13, IL-4α, and Stat6 are required for the expulsion of the gastrointestinal nematode parasite *Niposstrongylus brasiliensis.* Immunity, 8: 255-264.

Yunus, M., Horii, Y., Makimura, S and Smith, A. (2005). Murine goblet cell hyperplasia during *Eimeria pragensis* infection is ameliorated by clindamycin treatment. J. Vet. Med. Sci., 67(3): 311-315.