Oral Presentation (MP-6)

Coccidiosis Intestinal Dysmotility in C57BL/6 Mice

Riki Siswandi[1], Ayako Yoshida[1], Hiroyuki Satoh[2], Nariaki Nonaka[1]*

[1]Laboratory of Veterinary Parasitic Diseases, [2]Laboratory of Veterinary Clinical Radiology, Graduate School of Medicine and Veterinary Medicine, University of Miyazaki, Japan
*corresponding author: nnonaka@cc.miyazaki-u.ac.jp

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INTRODUCTION

Recently, anecdotal report was arisen that coccidiosis in cattle resulted in delayed intestinal motility. The occurrence of delayed intestinal motility has been never briefly reported. However, some study indicated a delay in intestinal motility. A study in cattle coccidiosis [1] reported a transient increase of nutrients apparent digestibility, especially of crude fiber index, during clinical coccidiosis and that anorexia and intestinal leakage impaired the nitrogen balance, causing weight depression. The observation may have reflected intestinal hypomotility, however, this work did not observe the intestinal motility directly. Another study [2] indicated that *Eimeria* infection in rabbit could induce intestinal motility disturbance. The disturbance, however, did not indicate as general delay in intestinal transit, because some part of the intestine experienced faster motility and another part indicated slower motility.

As one of the cosmopolitan diseases of production animal, the occurrence of intestinal dysmotility by coccidiosis need to be clarified. And a control measure against intestinal dysmotility of coccidiosis need to be proposed soon after the clarification of its clinical existence. Therefore, we designed a study with murine *Eimeria* as model to provide evidence for better approach toward this emerging issue.

MATERIALS AND METHODS

Animals used in this study were C57BL/6 male mice. All mice were purchased at six weeks of age from SLC Inc. Japan. All mice were kept as outlined in the "Guide for the Care and Use of Laboratory Animals" by University of Miyazaki.

After one week of adaptation, mice were assigned into different group of treatments. To investigate the ability for two species of murine *Eimeria* to induce intestinal dysmotility, we conducted a comparative study on groups of *E. pragensis* (Ep) and *E. vermiformis* (Ev) infected mice and control group. Each of the infected groups was inoculated with 1000 sporulated oocysts of corresponding species. In the control group, sham inoculation with distilled water (DW) was administered instead of oocysts. Each group in this study consist of five mice.

In addition of those groups, we designed a feed apprehension study group for evaluating the effect of low appetite (named as stress group). Five mice were assigned to this group and received sham inoculation with DW and limited feed intake, as much as 3 grams per day, instead of ad libitum feed intake. The intestinal motility was evaluated and compared with infected groups and control group.

The infectivity of *Eimeria* was measured by counting the number of oocysts per gram of feces (OPG) with modified McMaster's method, using a saturated salt solution with a specific gravity of 1.2. Feces were collected daily from each mouse started from 7 days post-infection (dpi) until the end of study. The severity of coccidiosis also analyzed by observation of clinical symptom.

Observation of intestinal motility were performed at 7 dpi by contrast gastrography of barium sulfate (Ba_2SO_4). The observation was performed at 24 hours post Ba_2SO_4 administration. Ba_2SO_4 (4 gr/ml) were administered in 0,3 ml to each mouse by oral gavage. The intestinal content transition was analyzed by the methods described by Giron, et al. [3].

In addition to the x-ray evaluation, the first time transit of barium was also observed by periodical observation of barium stained fecal pellet discharge after barium administration. The observation was conducted every 30 minutes until the white fecal pellet stained with barium was observed.

Statistical associations between variables were analyzed using the Spearman's rank correlation or Wilcoxon rank-sum test. All analyses were performed using the statistical program R (www.r-project.org).

RESULTS AND DISCUSSION

OPD (number of oocysts excreted per day) evaluation of Ep and Ev infected groups revealed a successful infection (Figure 1). Both groups showed similar OPD production on 9 dpi. The patency of oocyst production in this study is parallel with previous report, in which oocyst excretion was started from 7 dpi for Ep and 8 dpi

for Ev.

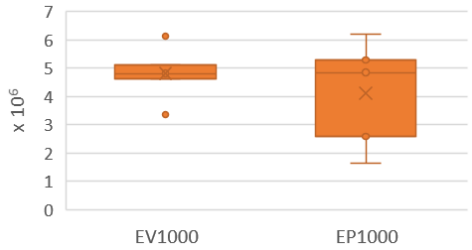


Figure 1 Oocyst excretion per day of Eimeria pragensis (Ep) and Eimeria vermiformis (Ev) infected group at 9 dpi.

Clinical symptoms were notably observed in the Ep infected group especially on the peak of oocyst excretion at 9 dpi. The Ep infected group showed symptoms such as pili-erection (100% of the animal of the groups), lethargy (100%), anorexia (100 %), lower fecal output (100 %), bloody stool (60 %), diarrhea (60%), and death (20 %). Meanwhile the Ev infected group showed lower feed intake and slightly reduced body weight gain during the infection study. There was no clinical sign observed in control group which received sham inoculation of distilled water. The occurrences of clinical sign in Ep group suggested to evaluate the effect of Ep in lower dose of infection. By lowering the infection dose of Ep, we can avoid severe clinical symptoms described above which potentially affect the outcome of the study.

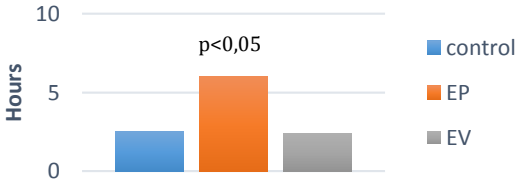
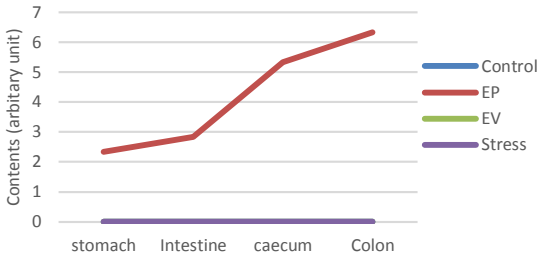


Figure 2. First time transit (hours) of barium sulfate after oral administration in infected groups and control group

The observation of first time transit of barium at 7 dpi revealed that Ep infected mice showed significant delay in barium discharge from the body when compared to control and Ev infected groups (Figure 2). While control and Ev group need approximately 3 hours to discharge the ingested meal, the time is doubled in Ep group which was around 6 hours.

With x-ray observation at 24 hours post-barium administration, no trace of barium was observed in the control, Ev infected, and stress groups. Meanwhile in the Ep infected group, the barium was still observed in the small intestine, caecum, and colon (Figure 3). This retention of barium in all parts of gastrointestinal tract could be defined as total hypomotility of intestine or pan-enteric ileus.

Interestingly, the stress group with experienced feed apprehension during observation did not indicate any disturbance in intestinal motility. We designed the stress group with limited feed intake, as low as half amount of daily feed intake of the control group. The apprehension of feed intake was started on 6 dpi, 24 hours before barium administration, and continued daily until all barium was discharged from the body. By designing the stress group, we wanted to compare the intestinal motility of infected group with fasting mice in the stress group. In the Ep infected group, the reduction in feed intake was started after oocyst shedding (7 dpi) which was parallel to the decrease in intestinal motility (Figure 3).



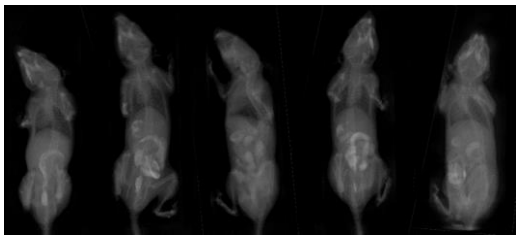


Figure 3. Radiographic index of Ba_2SO_4 in GI tract at 24 hours after administration (top). Ev, control and stress groups had no trace of barium at observation time.; Distribution of Ba_2SO_4 in GI tract of Ep infected group at 24 hours after administration (bottom).

The delayed intestinal motility in the Ep infected group could be induced by another factors like restraint stress or fasting [4]. With similar restraint stress applied in all group of this study, we eliminated the possibility of restraint stress, limited feed intake and anesthesia related intestinal motility disturbance. Therefore, we concluded that the decreased intestinal motility in the Ep infected group was induced by the infection of Ep in the intestinal tissue.

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