Infectivity and Oocyst Excretion Patterns of *Cryptosporidium muris* in Slightly Infected Mice

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ABSTRACT. To determine the infectivity of *Cryptosporidium* to hosts in slight infections, we examined the infectivity and oocyst output patterns of *Cryptosporidium muris* in mice inoculated with small numbers of oocysts. One of the 25 ICR mice inoculated with 2.4×10^1 oocysts and 19 of the 25 mice inoculated with 2.4×10^2 oocysts shed oocysts in the feces after inoculation. Four of the 50 mice inoculated with 2.4×10^1 oocysts for 10 consecutive days also shed oocysts and their OPG values were similar to that of the mice which received 2.4×10^2 oocysts. Consequently, it is clear that less than 10% of the mice which received 2.4×10^1 *C. muris* oocysts for 10 consecutive days.

KEY WORDS: *Cryptosporidium muris*, light infection, mouse.

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Cryptosporidium has been recognized as a causative agent of diarrhea in calves and man [10], and a waterborne outbreak of *Cryptosporidium* infection is important in man [9]. The waterborne outbreak is considered to occur by repeated slight infection with cryptosporidian oocysts in drinking water. However, the details of infectivity of *Cryptosporidium* to hosts after slight infection have not yet been clarified. Previously, we reported that a positive correlation between the number of oocysts inoculated and the mean OPG values (the number of oocysts per gram of feces) was established in mice orally inoculated at doses varying from 10^3 to 10^6 *Cryptosporidium muris* oocysts [4] and in chickens which received 10^2 to 10^6 *Cryptosporidium* sp. oocysts [5] until 14 or 10 days after infection. Therefore, we examined the infectivity and oocyst excretion patterns of *Cryptosporidium muris* in mice by inoculation of small numbers of oocysts after repeated slight infection with *C. muris* oocysts.

The RN66 strain of *C. muris* used in this study was first isolated from a house rat by Iseki [3] and was supplied by his laboratory. The strain was subjected to passage in mice to multiply oocysts excreted. The oocysts were stored at 4°C in 2% potassium dichromate solution before use. The animals used in this study were 3-week-old ICR mice. They were obtained from a commercial source, confirmed to be free of natural coccidial infections by fecal examinations prior to experiments and raised under the coccidia-free environment. Two groups of 25 mice each were inoculated with 2.4×10^1 and 2.4×10^2 oocysts, respectively. Fifty mice were inoculated with 2.4×10^1 oocysts for 10 consecutive days. The sporulation of *Cryptosporidium* oocysts can not be confirmed morphologically, thus as the infected control 5 mice were inoculated with 2.4×10^6 oocysts. They were examined daily for oocyst discharge by sugar flotation method. When the oocysts were detected, OPG values was also examined as described in the previous paper [4].

The results were shown in Table 1. One of the 25 mice which received 2.4×10^1 oocysts shed oocysts in the feces from 21 days on after inoculation and 19 of 25 mice inoculated with 2.4×10^2 oocysts began to shed oocysts from 14–17 days. Four of the 50 mice received 2.4×10^1 for 10 consecutive days shed oocysts from 14–19 days on. The OPG values in the mouse which shed the maximum number of oocysts in each group of mice inoculated with the same dose of oocysts were showed in Fig. 1. The positive correlation were recognized among the number of oocysts inoculated, the OPG values and infectivity to mice in mice which received 2.4×10^1 or 2.4×10^2 oocysts. The OPG values of the mouse which received 2.4×10^1 oocysts for 10 consecutive days was similar to that of the mice inoculated with 2.4×10^2 oocysts, however, the repeatedly infected mice showed lower infectivity of the parasite than the mice given 2.4×10^2 oocysts. In the case of the repeated slight infection, the some inoculated oocysts was not considered to infect mice. All the infected control mice shed oocysts from 6–7 days on and the maximum OPG values were 10^6 (Fig. 1).

DuPont *et al.* [2] examined the intensity of infection in healthy volunteers given *C. parvum* oocysts and reported that one of the 5 and 10 of the 17 subjects who received 10^1 and 10^2 oocysts, respectively, became infected. From the results obtained in the present experiments, it is clear that less than 10% of the mice which received 2.4×10^1 *C. muris*

Table 1. Shedding of oocysts by mice after inoculation with small numbers of *Cryptosporidium muris* oocysts

Dose of oocysts given	No. of mice	No. of mice with oocyst shedding	Prepatent period
2.4×10^1	25	1	21
2.4×10^2	25	19	14–17
$(2.4 \times 10^1) \times 10$	50	4	14–19
2.4×10^6 (control)	5	5	6–7

Fig. 1. The *Cryptosporidium muris* OPG values of mouse with the maximum oocyst output in each experimental group inoculated with the same dose of oocysts. F: Oocyst positive by sugar flotation method. Dose of oocysts given: 2.4×10^1 , 2.4×10^2 , 2.4×10^1 for 10 consecutive days, 2.4×10^6 as infected control.

oocysts for 10 consecutive days became infected.

It is generally known that a single *Eimeria* and *Isoapora* oocyst can infect the appropriate host and produce oocysts [1, 6]. The flagella are absent in cryptosporidian microgametes [7], although they are present in *Eimeria* and *Isoapora* [8]. Therefore, the oocyst production was considered difficult in the light infection with *Cryptosporidium* because the microgametes were not sufficient in number for fertilization to be established.

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