

Infectivity to Mice of the Merozoites and Zoitites of *Isoospora rivolta* in Cats

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Abstract

Infectivity to mice of the merozoites and zoites of *Isoospora rivolta* in cats was examined. Cats given the ensheathed-zoites from mice that had been inoculated with 2.3×10^5 oocysts were necropsied on days 3, 4 and 5. The numerous merozoites were seen in the small intestinal mucosa and several zoites in the mesenteric lymph nodes of the cats. These merozoites or zoites were inoculated into mice. The mice were sacrificed between 14 and 41 days after inoculation and mince of the mesenteric lymph nodes and spleen were fed to cats, respectively. The cats shed oocysts from 5 or 6 days after feeding. The PAS (periodic-acid-Schiff stain) positive single zoites surrounded individually by a sheath were detected in the mesenteric lymph nodes of mice after inoculation with the zoites in the cats given the ensheathed-zoites. The single zoites were structurally similar to the ensheathed-zoites found in the intermediate host after oocyst inoculation. These results suggest that the merozoites and zoites of *I. rivolta* in cats are infectious for mice and grow into the ensheathed-zoites.

Key words: *Isoospora rivolta*, Infectivity to mice, merozoites, zoites

Introduction

Although sporozoites of the cyst-forming isosporoid coccidia were able to infect the intermediate hosts, only sporozoites of feline *Isoospora felis*, *I. rivolta* and canine *I. canis*, *I. ohioensis* grew into ensheathed-zoites in them (Frenkel, 1977; Dubey, 1979). The ensheathed-zoites did not divide in the intermediate hosts (Mehlhorn and Markus, 1976). Furthermore, Dubey (1977) described that the merozoites and zoites of these species in the definitive hosts did not infect the intermediate hosts. However, it is known that the merozoites and zoites of *Toxoplasma gondii* which is one of the cyst-forming isosporoid coccidia can infect the intermediate hosts. The present studies were undertaken to examine whether the merozoites in the intestine and

the zoites in the extra-intestinal organs of cats infected with *I. rivolta* are infectious for mice.

Materials and Methods

Animals

Mice and kittens served as experimental animals. The mice used were of the CFW strain weighing about 20g. The kittens weighing 320–600g were obtained from the Tama area, Tokyo, Japan. The feces of all animals were examined for coccidian oocysts by the sugar flotation method (specific gravity of sugar, 1.266) prior to experiments. Oocyst-free animals were raised in separate cages under the coccidia-free environment.

Oocysts and inoculation

A single oocyst of *I. rivolta* from the feces of a naturally infected kitten was inoculated into the other kitten and newly produced oocysts were transferred to mice and kittens to multiply oocysts used for further experiments. The oocysts obtained from the kitten were sporulated in 2.0% potassium dichromate solution in petri dishes at 25°C, and stored at 4°C.

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In order to avoid the contamination with the oocysts which passed through the alimentary tract of cats after oocyst inoculation (Matsui *et al.*, 1974), the donor cats were inoculated with the ensheathed-zoites from the infected mice. Five mice given 2.3×10^5 oocysts were necropsied on day 7. After assuring the presence of the ensheathed-zoites in their mesenteric lymph nodes, the mince of mesenteric lymph nodes and spleen were fed to 5 kittens.

Infectivity to mice

To determine the infectivity to mice of the merozoites and zoites, 4 of 5 kittens (donor cats) given the ensheathed-zoites from mice were necropsied on days 3, 4 and 5 after infection. Their mesenteric lymph nodes and small intestinal mucosae were examined to detect the parasites. The sporozoites with a clear large refractile globules were distinguished from the merozoites and zoites. After that, each of both organs from each of 4 donor cats was separately made into 10% suspension in physiological saline, respectively. Eight groups of 3 mice each were intraperitoneally or orally inoculated with 1.0 ml of these suspensions. The other 2 groups of 3 mice each, which were injected intraperitoneally with 0.25 mg/mouse of cortisone acetate 1 hr before, were inoculated with the suspensions prepared similarly from organs of the remaining donor cat (No. 497) killed on day 3. All mice were sacrificed between 15 and 24 days after inoculation, and their mesenteric lymph nodes and spleen were fed to 18 recipient kittens. The recipients were examined for oocyst discharge by sugar flotation method.

Morphology of the parasites in mice

Two kittens given the ensheathed-zoites were sacrificed on days 5 and 6, and all of their mesenteric lymph node suspensions were inoculated orally 2 to 3 times at 2-hr-intervals into 3 mice each. The mice were necropsied on days 14 and 70 after inoculation, and their mesenteric lymph nodes were microscopically examined as described in the previous paper (Matsui *et al.*, 1986).

Results

Infectivity to mice

At necropsy, numerous merozoites and schizonts were found in the small intestinal mucosa of donor cats fed the ensheathed-zoites. A few zoites were found in the mesenteric lymph nodes except cat no. 155. No sporozoites were detected in both organs. These merozoites and zoites infected mice by both the intraperitoneal and oral routes. All of 8 recipient cats, which were fed organs from mice inoculated with mesenteric lymph nodes of donor cats, shed oocysts from 5 or 6 days later. On the other hand, only 2 cats (No. 184 & 341) of 8 recipients, which were fed organs from mice inoculated with small intestinal mucosae of donor cats, shed a few oocysts (Table 1). Both 2 recipient cats (No. 506 & 507) fed organs from mice treated with cortisone acetate also shed oocysts, although the cat No. 507, ingested merozoites, shed fewer number of oocysts than those of the cat No. 506 ingested zoites.

Morphology of the parasites in mice

At necropsy, several zoites were found in the mesenteric lymph nodes of infected cats. After inoculation into mice with the zoites, a few single zoites surrounded by a sheath were detected in the mesenteric lymph nodes of mice. These single zoites had many PAS (periodic-acid-Schiff stain) positive granules (Fig. 1A). The single zoites detected on day 14 were $13.5\text{--}17.2 \times 4.0\text{--}7.5 \mu\text{m}$ in size and the sheath were $14.5\text{--}20.5 \times 5.8\text{--}9.2 \mu\text{m}$ in fresh preparations. The size of zoites detected on day 70 was similar ($15.5\text{--}18.8 \times 3.9\text{--}6.5 \mu\text{m}$) to that of the zoites observed on day 14, however, the sheaths were larger ($23.8\text{--}29.0 \times 11.3\text{--}15.0 \mu\text{m}$). These zoites were structurally similar to the ensheathed-zoites observed in mice inoculated with oocysts (Fig. 1B). No multiplication of the single zoites was observed.

Discussion

The ensheathed-zoites were first discovered in mice given *I. felis* or *I. rivolta* oocysts by Frenkel and Dubey (1972). Their typical morphological feature is monozoic (Frenkel, 1977). Mehlhorn and Markus (1976) reported that the ensheathed-zoites of *I. felis* had the typical appearance of coccidian sporozoites. However, the infectivity to mice of the other endogenous stages in cats has not yet been clarified.

Table 1 Shedding of oocysts by recipient cats after feeding of organs from mice inoculated with the merozoites or zoites of *Isospora rivolta* in cats

Donor cats				Mice		Recipient cats		
Cat No.	Necropsied (days)	Organs inoculated to mice	Detection of parasites	Route of inoculation	Organs fed to recipient cats	Cat No.	Oocyst discharge	Days of oocyst shedding
155	3	M	-	IP	M, S	178	+	5-15
				Oral	M, S	179	+	5-14
		I	+	IP	M, S	180	-	
				Oral	M, S	181	-	
245	4	M	+	IP	M, S	313	+	5-7
				Oral	M, S	314	+	5-9
		I	+	IP	M, S	315	death	
				Oral	M, S	316	-	
162	5	M	+	IP	M, S	182	+	6-8, 11-14
				Oral	M, S	183	+	6-7, 13-14
		I	+	IP	M, S	184	+	6
				Oral	M, S	185	-	
268	5	M	+	IP	M, S	309	+	5-12
				Oral	M, S	311	+	5-8
		I	+	IP	M, S	340	-	
				Oral	M, S	341	+	5, 12, 13
497	3	M	+	↓Oral	M, S	506	+	5-10
		I	+	↓Oral	M, S	507	+	6, 10-12

Infection: Each donor cat fed the mesenteric lymph nodes and spleen of the mouse which had been inoculated with 2.3×10^5 oocysts.

Organs inoculated or fed. I: Small intestinal mucous membrane, M: Mesenteric lymph nodes, S: Spleen

Route of inoculation. IP: Intraperitoneal

↓: Mice were injected intraperitoneally with 0.25 mg/mouse of cortisone acetate 1 hr before inoculation with the organs of an infected cat.

In the present experiments, when the organs of mice inoculated intraperitoneally or orally with the zoites or merozoites in the infected donor cats were fed to the cats, all of 8 cats fed zoites shed oocysts, and 2 of 8 cats fed merozoites shed a few oocysts. In case the mice were treated with cortisone acetate before inoculation with the merozoites, the recipient cats (No. 507) also shed a few oocysts after feeding of the organs of the mice. Therefore, it was confirmed that the merozoites and zoites were able to infect the mice by the intraperitoneally and orally routes. Although the number of merozoites inoculated into mice was greater than that of zoites, the merozoites showed low infectivity to mice. Matsui

et al. (1993) examined the possibility of invasion of the extra-intestinal organs of cats by the merozoites and zoites of *I. rivolta* and reported that a few merozoites may invade the extra-intestinal organs. Thus it was considered that a few merozoites which were able to invade the extra-intestinal organs of cats showed infectivity to mice.

The PAS positive single zoites surrounded by a sheath were found in the mesenteric lymph nodes of mice after inoculation with the zoites in the mesenteric lymph nodes of infected cats. These were structurally similar to the ensheathed-zoites found in mice after oocyst inoculation. Until now, the ensheathed-zoites were found in the intermedi-

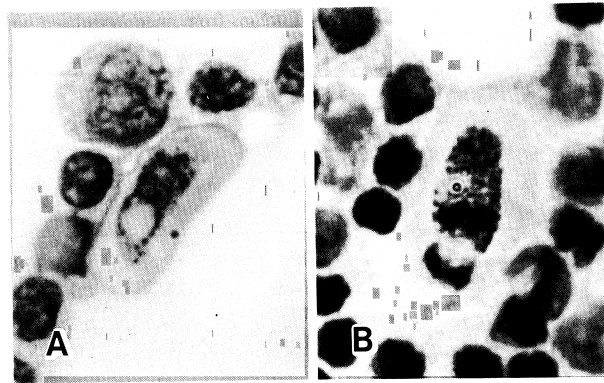


Fig. 1 Monozytic cysts of *I. rivolta* in the mesenteric lymph nodes of mice. Stained with PAS.

- A A PAS-positive zoite from a mouse on day 70 after inoculation with the mesenteric lymph nodes of infected cat. $\times 1177$.
- B An ensheathed-zoite from a mouse on day 70 after inoculation with oocysts. $\times 1177$.

ate host when oocysts were inoculated. It becomes clear that the zoites of *I. rivolta* in cats grow into the ensheathed-zoites after infection to mice.

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