Invasion of the Extra-intestinal Organs of Cats by the Merozoites and Zoites of *Isospora rivolta*

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**Summary**

The possibility of invasion of the extra-intestinal organs of cats by the merozoites and zoites of *Isospora rivolta* were examined. Four cats (donor) given the organs of mice that had been inoculated with $2.3 \times 10^4$ oocysts were necropsied 3 days thereafter. Numerous merozoites in their small intestines and a few zoites in the mesenteric lymph nodes were found. Their intestinal mucosa and extra-intestinal organs (liver, mesenteric lymph nodes and spleen) were separated and fed to 2 recipient cats (the 1st recipient), respectively. The 1st recipient cats were sacrificed on day 9 and the extra-intestinal organs of each group were fed to other cats (the 2nd recipient). Both of 2nd recipients began to shed oocysts from 7 or 9 days after feeding. A greater number of merozoites in donor cats were inoculated into 1st recipients, whereas the 2nd recipient shed few oocysts. This result indicate that a few merozoites and zoites may invade the extra-intestinal organs of the 1st recipient cats.

**Introduction**

The re-shedding of *Isospora belli* oocysts by chronically infected patients is often observed. The same phenomenon has been recognized in the cats infected with feline *Isospora felis*, *Isospora rivolta* and canine *Isospora canis*, *Isospora ohiensis*. These coccidia and *I. belli* are closely related coccidians. The asexual and sexual development stages, namely merozoites, schizonts, gametocytes, zygotes and unsporulated oocysts, of such coccidia are observed in the small intestine. The zoites are also detected in the extra-intestinal organs (mainly mesenteric lymph nodes) of appropriate host but do not undergo division. Furthermore, Matsui et al. reported that no organisms were detected in the small intestine of cats infected with *I. rivolta* after cessation of oocyst shedding, though the zoites remained infective for a long time in the extra-intestinal organs. Thus it has been considered that the zoites are waiting or dormant stages as hypnozoites of *Plasmodium* and the cause of re-shedding of oocysts. However, the question of whether endogenous forms invade the extra-intestinal

Key words: *Isospora rivolta*, Extra-intestinal organs, Merozoites, Zoites, Cats.
organs of definitive host has not yet been clarified. *I. rivolta* is a common coccidium of cats with monoxenous and heteroxenous life cycles*8*. Unsporulated oocysts are shed in feces of infected cats. After sporulation, the sporulated oocysts, containing sporozoites, are infectious to cats. The sporozoites in oocysts are also infectious to the intermediate hosts and form the ensheathed-zoites*9*. Moreover, both of the merozoites and zoites in cats are infectious to the other cats. After inoculation with the merozoites, zoites or ensheathed-zoites to cats, all of them infect the cats intestine, multiply continuously, and form oocysts*5,10*. The zoites in the extra-intestinal organs of such cats are detected after the inoculation with the oocysts or ensheathed-zoites*5*, but not examined after the inoculation with the merozoites and zoites. In order to determine whether the merozoites and zoites of *I. rivolta* invade the extra-intestinal organs of cats, the extra-intestinal organs of cats given merozoites or zoites were fed to other cats and examined for oocyst discharge.

Materials and Methods

1. Animals
Mice and kittens served as experimental animals. The mice used were of the CFW strain weighing about 20 g. The kittens weighing 330~560 g were obtained from the Tama area, Tokyo, Japan. The feces of all kittens were examined for coccidian oocysts by the sugar floatation method (specific gravity of sugar, 1.266) prior to experimentation. Oocyst-free animals were raised in separate cages under a coccidium-free condition.

2. Oocysts and inoculation
A single oocyst of *I. rivolta* from the feces of a naturally infected kitten was subjected to mice and kittens to multiply oocysts. The oocysts obtained from kittens were sporulated in 2.0 % potassium dichromate solution in petri dishes at 25℃, and stored at 4℃. The mice were inoculated with $2.3 \times 10^6$ oocysts each. They were necropsied on day 7 and were examined to detect the ensheathed-zoites in the mesenteric lymph nodes. After that, the mince of mesenteric lymph nodes and spleen were fed to kittens.

3. Experiment
Four kittens (donor cat) given organs of mice as mentioned above were necropsied on day 3 and their lower part of jejunum and mesenteric lymph nodes were examined to detect the parasites. Mince of the extra-intestinal organs (liver, mesenteric lymph nodes and spleen) and mucosa of the lower part of jejunum and ileum were separated (extra-intestinal and intestinal groups). The same organs from each of two kittens were pooled and fed to the 4 recipient kittens (1st recipient). The 1st recipients were confirmed to discharge oocyst, and sacrificed on day 9 after feeding. The mixture of extra-intestinal organs from each of two 1st recipients were fed to two other kittens (2nd recipient). The 2nd recipients were examined daily for oocyst discharge by sugar floatation method. The possibility of invasion of the extra-intestinal organs of cats by the merozoites and zoites was judged by oocyst shedding from the 2nd recipients.

Results
The results were summarized in Table 1. When the donor cats were necropsied, numerous merozoites and schizonts were found in their low part of jejunum and ileum but sporozoites were not detected. A small number of zoites were found in their mesenteric lymph nodes. All of the 1st recipients given organs of the donor cats shed oocysts from 3 to 9 days.
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Table 1 Shedding of oocysts by recipient cats after ingestion of the extra-intestinal organs from cats given the merozoites and zoites of Isospora rivolta in cats.

<table>
<thead>
<tr>
<th>Donor cats</th>
<th>1st recipient cats</th>
<th>2nd recipient cats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat No.</td>
<td>Organs ingested</td>
<td>Oocyst discharge</td>
</tr>
<tr>
<td>440</td>
<td>3</td>
<td>L,M,S…</td>
</tr>
<tr>
<td>443</td>
<td>3</td>
<td>L,M,S…</td>
</tr>
<tr>
<td>444</td>
<td>3</td>
<td>I</td>
</tr>
<tr>
<td>445</td>
<td>3</td>
<td>I</td>
</tr>
</tbody>
</table>

Infection: Each donor cat was fed with the mesenteric lymph nodes and spleen of mouse which had been inoculated with $2.3 \times 10^4$ oocysts.

Organ ingested. I: Small intestinal mucous membrane L: Liver M: Mesenteric lymph nodes S: Spleen

After feeding. In case the extra-intestinal organs of these 1st recipients were fed to the 2nd recipients, they began to shed oocysts from 7 or 9 days after feeding. The number of shedding oocysts from the 2nd recipient in intestinal group was smaller than that from cat in extra-intestinal group. The patent period of former was shorter.

Discussion

Dubey and Frenkel$^{15}$ reported the existence of the extra-intestinal stages of I. felis and I. rivolta in cats. Dubey$^{4}$ found the zoites in the mesenteric lymph nodes of cats 12 hr after inoculation with I. rivolta oocysts. Therefore, it has been considered that the sporozoites may invade directly the extra-intestinal organs of cats. However, the possibility of invasion by the merozoites and zoites has not been clarified.

In order to avoid the contamination with the oocysts which passed through the alimentary tract of cats after oocyst inoculation$^{9}$, the donor cats were inoculated with the ensheathed zoites from the infected mice in the present experiment. In case the extra-intestinal organs of the 1st recipient cats received the merozoites or zoites were fed to the 2nd recipient cats, both of cats shed oocysts. Therefore, it became clear that the organisms existed in the extra-intestinal organs of the 1st recipient cats. A greater number of merozoites than the zoites in donor cats were inoculated into 1st recipients, whereas the 2nd recipient shed a few oocysts. This result suggests that a few merozoites and zoites may invade the extra-intestinal organs of the 1st recipient cats.

Spontaneous oocyst re-shedding was also observed in the definitive hosts infected with Hammondia hammondii$^{11}$, I. heymori$^{11}$ or Toxoplasma gondii$^{9}$. It is well considered that the sporozoites and merozoites of these coccidia and I. belli may also invade the extra-intestinal organs of appropriate host.

Conclusion

The possibility of invasion of the extra-intestinal organs of cats by the merozoites and zoites of Isospora rivolta in cats were examined. The result suggests that a few merozoites and zoites may invade the extra-intestinal organs of cats.
References