

## The Tissue Stages of *Iso spor a heydorni* in the Guinea Pig as an Intermediate Host

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### Abstract

The tissue stages of *Iso spor a heydorni* were studied in the guinea pig as an intermediate host. Guinea pigs, given  $3.6\sim 5.8 \times 10^7$  oocysts, were necropsied between 1 and 189 days after infection and the brain, mesenteric lymph nodes, small intestine and striated muscles were examined for parasites. Uninucleate parasites (zoites), which were morphologically similar to sporozoites, were seen in the small intestine and mesenteric lymph nodes between 1 and 14 days. They were banana-shaped and  $4.4\sim 7.2$  by  $0.9\sim 2.7 \mu\text{m}$  in size. Proliferative stages were not observed. The zoites were not infective to dogs. Between 28 and 77 days, similar uninucleate zoites were seen in the brain of guinea pigs and they were stained positive with periodic-acid-Schiff (PAS). These zoites were  $4.3\sim 5.5$  by  $1.3\sim 2.2 \mu\text{m}$  in size, and were not ensheathed. Each of cysts was found in the brain on days 77 and 189 after inoculation. The cysts were spherical, and  $10.6\sim 13.0 \mu\text{m}$  in diameter. They were surrounded by a thin wall, and contained 10 or more bradyzoites. The radial spine and septa were not observed in the cysts. These PAS-positive zoites and cysts were infective to dogs because oocysts were detected in the stool of the dogs approximately one week after feeding.

**Key words:** *Iso spor a heydorni*, *Hammondia heydorni*, Guinea pigs, Tissue stages, Intermediate host

### Introduction

*Iso spor a heydorni* is an intestinal coccidium of dogs, and the life cycle is obligatory hetero-oxenous. Cattle were first reported to be intermediate hosts of this parasite by Heydorn (1973) and Dubey and Fayer (1976). Soon after, other ruminants were reported as intermediate hosts of *I. heydorni* (Dissanaike and Kan, 1977; Dubey and Williams, 1980; Nassar *et al.*, 1983; Warrag and Hussein, 1983). However, the tissue stages of this parasite in the intermediate hosts have not yet been clarified, although the intestinal stages in dogs have been studied (Heydon *et al.*, 1975; Dubey and Fayer, 1976; Matsui *et al.*, 1986).

Previously, we isolated *I. heydorni* from a dog in Brazil and reported that the guinea pig was a suitable intermediate host of this parasite (Matsui *et al.*, 1981, 1987). The present study was performed to demonstrate morphology of the tissue stages of *I. heydorni*, using the guinea pig as an

intermediate host.

### Materials and Methods

#### *Oocysts and inoculation*

The oocysts of *I. heydorni* used in this study were obtained originally from feces of a naturally infected dog in Brazil and was maintained by alternate transfer to guinea pigs and dogs. The oocysts obtained from dogs were sporulated in 2.0% sulfuric acid solution in petri dishes at 25°C, and stored at 4°C. Sporulated oocysts were dilution-counted, and  $3.6\sim 5.8 \times 10^7$  oocysts were given to each guinea pig via stomach intubation.

#### *Animals*

The animals used were Hartley strain of guinea pigs weighing about 180g. Puppies, weighing 1120~2700g, were obtained from the Tama area, Tokyo, Japan. Feces of all the animals were examined by the sugar flotation method (specific gravity of sugar, 1.266) prior to experimentation. Oocyst-free animals were raised

in separate cages under a coccidium-free environment.

*Observation of sporozoites*

Sporozoites were obtained by excystation of oocysts from dog feces. Sporulated oocysts were collected by the sugar flotation method, and incubated at 37°C for 90 minutes in excysting solution consisting of 1% trypsin, 10% guinea pig's bile and saline. To observe morphological structures, suspensions containing the sporozoites were smeared on glass slides, air-dried, fixed in methyl alcohol, and stained with Giemsa's stain.

*Microscopic examination of the tissue stages*

Two infected guinea pigs were necropsied on days 1, 2, 3, 4, 5, 7, 9, 14, 21, 28, 35, 42, 57, 63, 77, 112, 119 and 189 after infection. The abdominal wall, brachial muscle, brain, diaphragm, gluteal muscle, heart, mesenteric lymph nodes and small intestine were microscopically examined by procedures previously described (Matsui *et al.*, 1986). Size of the parasites in smears and tissue sections were measured by a Nikon micro-meter.

*Infectivity test*

In order to determine the infectivity of tissue stages, the mesenteric lymph nodes and small

intestine of guinea pigs sacrificed on days 5, 9 and 14 after infection were fed to each puppy. Furthermore, distribution of parasites in the intermediate host was examined by administration of various organs of infected guinea pigs to puppies; brain, mesenteric lymph nodes, small intestine or striated muscles (abdominal wall, brachial muscle, diaphragm, gluteal muscle and heart) of the guinea pigs killed on day 21, 28, 57 or 77 were fed to each puppy. The recipients were examined daily for oocyst discharge by the sugar flotation method.

**Results**

*Pathogenicity*

No clinical symptoms were observed in the infected guinea pigs. Pathological changes were not observed when they were necropsied on the test days after oocyst inoculation.

*Sporozoites*

Artificially excysted sporozoites from oocysts were banana-shaped and 4.4~6.8 by 0.9~2.7 μm (5.8×1.5 μm on average) in size (Fig. 1).

*Tissue stages in the guinea pigs*

The results of detectable parasites were summarized in Table 1. At early time of infec-

Table 1 Detection of organisms of *Isospora heydorni* in the organs of infected guinea pigs.

Organs examined	Days after inoculation																	
	1	2	3	4	5	7	9	14	21	28	35	42	57	63	77	112	119	189
Jejunum, upper level	-	-	-	-	-	N	N	N	N	N	N	N	N	N	N	N	N	N
middle level	-	-	-	-	Z	-	-	-	N	N	N	N	N	N	N	N	N	N
lower level	Z	Z	Z	Z	Z	Z	Z	Z	-	-	-	-	-	-	-	-	-	-
Ileum	Z	Z	Z	Z	Z	Z	Z	Z	-	-	-	-	-	-	-	-	-	-
Mes. lymph nodes	-	Z	Z	Z	Z	Z	Z	-	-	N	N	N	N	N	N	N	N	N
Brain	N	N	N	N	N	N	N	-	-	P	-	P	P	-	PC	-	-	C
Abdominal wall	N	N	N	N	N	N	N	-	-	-	-	-	-	-	-	-	-	-
Brachial muscle	N	N	N	N	N	N	N	-	-	-	-	-	-	-	-	-	-	-
Diaphragm	N	N	N	N	N	N	N	-	-	-	-	-	-	-	-	-	-	-
Gluteal muscle	N	N	N	N	N	N	N	-	-	-	-	-	-	-	-	-	-	-
Heart	N	N	N	N	N	N	N	-	-	-	-	-	-	-	-	-	-	-

Mes. lymph nodes: Mesenteric lymph nodes

Z: Zoite detected    P: PAS-positive zoite detected    C: Cyst detected    N: Not examined

-: No parasites were detected

tion, uninucleate parasites(zoites), as shown in Figs. 2 and 3, were seen in the lower part of jejunum, ileum and mesenteric lymph nodes. The zoites detected from lower part of the jejunum on day 1 were  $5.1\sim 7.2$  by  $1.1\sim 2.0\ \mu\text{m}$  ( $6.1\times 1.7$

$\mu\text{m}$  on average) in size, and the other zoites from mesenteric lymph nodes on day 3 were  $4.4\sim 6.8$  by  $0.9\sim 2.7\ \mu\text{m}$  ( $5.8\times 1.5\ \mu\text{m}$  on average). They were banana-shaped and similar to excysted sporozoites. The detectable zoites decreased

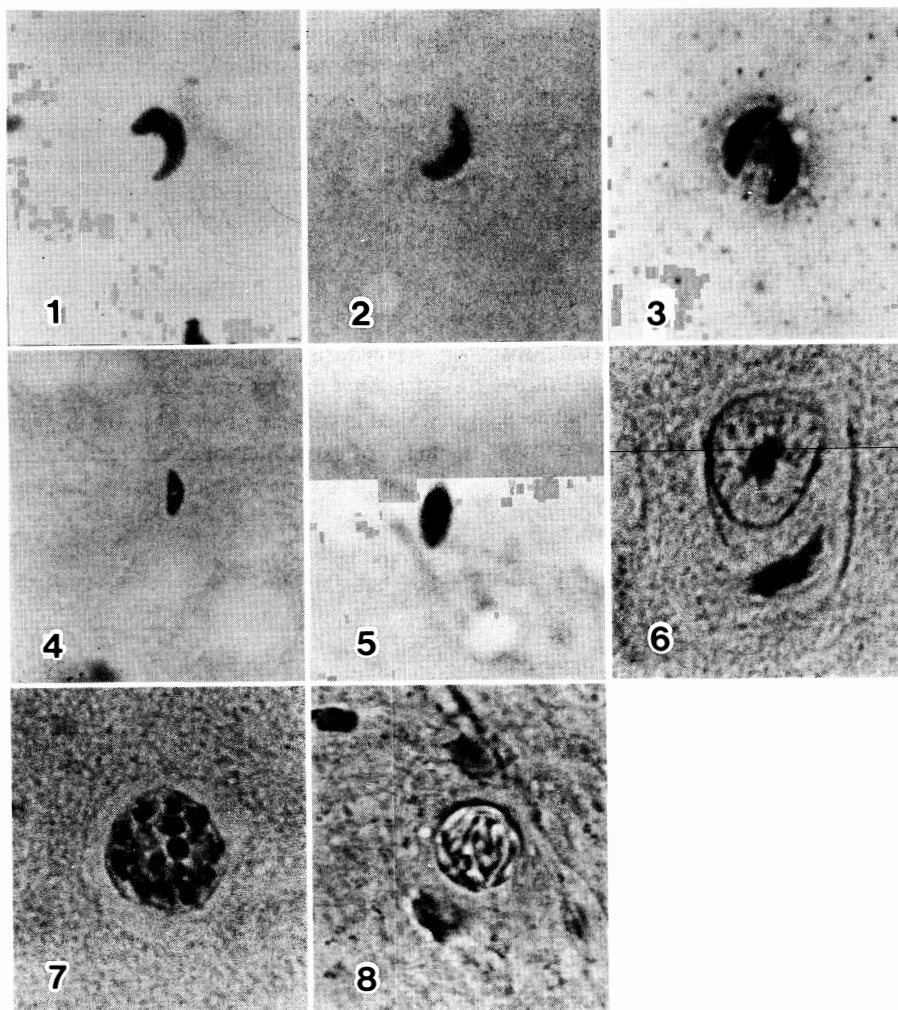


Fig. 1 A sporozoite of *I. heydorni* from excysted oocyst. Smears stained with Giemsa's stain.  $\times 1640$ .

Figs. 2-8 Tissue stages of *I. heydorni* in the organs of guinea pigs. Stained with Giemsa's stain (Figs. 2, 3 and 5), PAS (Figs. 4 and 7) and hematoxyline-eosin (Fig. 6). Fresh preparation (Fig. 8).

Fig. 2 A zoite in the lower part of jejunum on day 1.  $\times 1640$ .

Fig. 3 Zoites in the mesenteric lymph nodes on day 3.  $\times 1640$ .

Fig. 4 A PAS-positive zoite in the brain on day 28.  $\times 1640$ .

Fig. 5 A zoite in the brain on day 77.  $\times 1640$ .

Fig. 6 A zoite in a nerve cell of brain on day 77.  $\times 1650$ .

Fig. 7 A cyst in the brain on day 77.  $\times 1640$ .

Fig. 8 A cyst in the brain on day 189.  $\times 1640$ .

gradually in number. Neither binucleate zoites nor multinucleate schizonts were observed. The parasites were not detected in the duodenum, cecum and colon of these guinea pigs.

On day 28, 42, 57 and 77, a few uninucleate zoites were also seen in the brain. These zoites measuring  $4.3\sim 5.5$  by  $1.3\sim 2.2$   $\mu\text{m}$  on impression smears were stained positive with periodic-acid-Schiff (PAS), and did not have ensheathed (Figs. 4 and 5). Only one zoite measuring  $8.4$  by  $2.3$   $\mu\text{m}$  was found within a nerve cell of brain in the section on day 77 (Fig. 6).

Each of cysts was seen in the section of brain on day 77 (Fig. 7) and in the fresh squashed preparation of brain on day 189 (Fig. 8). The cysts were spherical and measured  $10.7$  by  $10.6$   $\mu\text{m}$  and  $13.0$  by  $12.1$   $\mu\text{m}$ . They were surrounded by a thin wall and contained 10 or more bradyzoites. The radial spine and septa were not seen in the cysts. Neither PAS-positive zoites nor cysts were detected in the muscle and small intestine.

The concept of the life cycle of *I. heydorni*,

including the results obtained from the present experiments and the endogenous stages in dogs reported previously (Matsui *et al.*, 1986), is represented diagrammatically in Fig. 9.

#### Infectivity test

The puppies did not shed any oocyst when they were fed with organs of guinea pig killed on day 5, 9 or 14 after oocyst inoculation. When puppies fed on the each organ from guinea pigs on day 21 and later, all the recipients shed oocysts except a puppy fed with mesenteric lymph nodes on day 77 (Table 2).

#### Discussion

Heydorn (1973) has reported the tissue stages of *I. heydorni* in the intermediate host; the thick-walled cysts were observed in the diaphragm and heart of experimentally infected cattle. Furthermore, the thin-walled cysts with septa were detected in the meat of naturally infected camel

Table 2 Shedding of oocysts by dogs after ingestion of various organs from guinea pigs inoculated with *Isospora heydorni* oocysts.

Days after inoculation	Organ ingested	Dog no.	Oocyst discharge	Prepatent period (days)	Maximum OPG
21	Brain	203	+	7	$1.6 \times 10^5$
	Mes. lymph nodes*	N			
	Small intestine	215	+	12	F
	Striated muscles†	216	+	7	$2.0 \times 10^6$
28	Brain	217	+	10	$1.3 \times 10^6$
	Mes. lymph nodes	220	+	7	$1.1 \times 10^6$
	Small intestine	218	+	7	$7.2 \times 10^6$
	Striated muscles	219	+	7	$8.8 \times 10^6$
57	Brain	206	+	6	$7.1 \times 10^6$
	Mes. lymph nodes	N			
	Small intestine	222	+	8	$6.8 \times 10^5$
	Striated muscles	221	+	9	$3.7 \times 10^5$
77	Brain	208	+	6	$6.9 \times 10^6$
	Mes. lymph nodes	228	-		
	Small intestine	222	+	8	$6.8 \times 10^5$
	Striated muscles	221	+	9	$3.7 \times 10^5$

\*: Mesenteric lymph nodes

†: Abdominal wall, Brachial muscle, Diaphragm, Gluteal muscle and Heart.

F: Oocyst detected only by sugar flotation method.

N: Not examined

by Warrag and Hussein (1983). However, these cysts were structurally similar to those of *Sarcocystis*, and dogs fed with the muscle organs containing these cysts shed both *Sarcocystis* sporocysts and *I. heydorni* oocysts. Thus, it is considered that the cattle and camel may have harboured natural infections of *Sarcocystis*. No other study on the tissue stages of this parasite have been reported.

In the present experiments, the tissue stages of *I. heydorni* in the guinea pig were clarified as follows; sporozoite-like organisms were seen in the small intestine and mesenteric lymph nodes at early times of infection but neither binucleate zoites nor multinucleate schizonts were observed. PAS-positive uninucleate zoites and a few cysts were detected in the brain on and after the 28th day. Although neither zoites nor cysts were detected in the mesenteric lymph nodes, small intestine and striated muscles of infected guinea pigs necropsied on day 21 and later, the dogs shed oocysts after feeding with these organs and brain.

The concept of the life cycle of *I. heydorni*, including the endogenous stages in dogs reported previously by Matsui *et al.*, 1986, is represented in Fig. 9. The tissue stages in the guinea pig are considered to develop as follows; the sporozoites invade the small intestine after excysting in the alimentary tract, move to the various organs and

muscles, develop into PAS-positive zoites, and a few of the PAS-positive zoites may develop into cysts. The PAS-positive zoites and cysts were infective to the final host, dogs. Therefore, it becomes clear that *I. heydorni* is one of the cyst-forming isosporan coccidia.

*I. heydorni* was named by Tadros and Laarman (1976). Dubey (1977) proposed that the name *Hammondia heydorni* was more suitable for it because the structure and life cycle were similar to those of *Hammondia hammondi*, whereas the tissue stages of *I. heydorni* have not been clarified until now. The structure and localization of cysts in the intermediate host are very important in the classification of cyst-forming isosporan coccidia (Frenkel *et al.*, 1979; Smith, 1981).

In the present experiments, the cyst of *I. heydorni* was demonstrated to be surrounded by a thin wall and to contain bradyzoites. The structure of *I. heydorni* cysts was similar to that of *H. hammondi*. However, this parasite was different from *H. hammondi* in some characteristics of tissue stages in the intermediate host. The cysts of *I. heydorni* were very few and smaller than those of *H. hammondi*. *I. heydorni* cysts were observed in the brain, however, *H. hammondi* cysts were found to be mainly located in the striated muscles (Frenkel *et al.*, 1979).

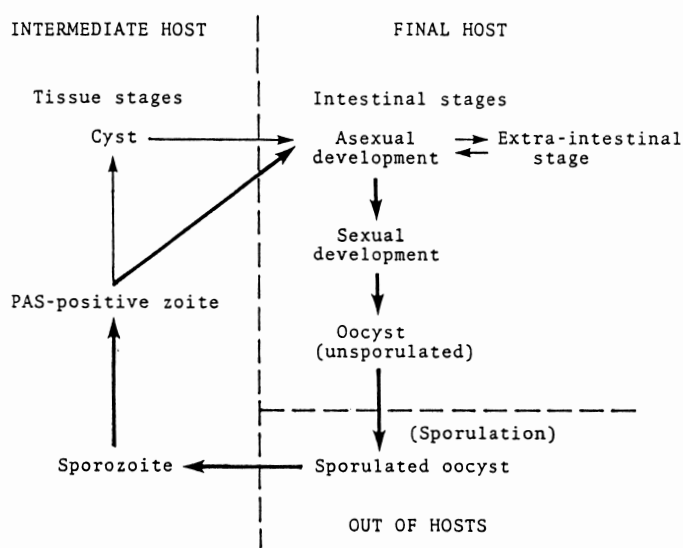


Fig. 9 Diagrammatic representation of the life cycle of *Isospora heydorni*.

Although *H. hammondi* tachyzoites were reported to multiply for at least 11 days in the small intestine and mesenteric lymph nodes (Frenkel and Dubey, 1975), no proliferative stages of *I. heydorni* were detected. Moreover, PAS-positive uninucleate zoites, which had not yet been observed in *H. hammondi*, were found in the tissue stages of *I. heydorni*. Therefore, the characteristics of tissue stage of *I. heydorni* are not consistent with that of "Genus *Hammondia*" in classification described by Frenkel *et al.* (1979) and Smith (1981).

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