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Cyclic Transmission of the Small Type of *Isospora bigemina* of the Dog

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Wenyon (1926) reported first the existence of two sizes of *Isospora bigemina* oocysts (small and large types) from the dog, and those types of *Isospora bigemina* might be two different species (Heydorn, *et al.*, 1975a). However, the biological characteristics of two types of this parasite have not yet been elucidated completely. Heydorn (1973) reported that dogs did not shed fresh oocysts after oral inoculation with *Isospora bigemina* small type oocysts, but the dogs fed the muscle of dogs or cattle which were inoculated orally with oocysts shed fresh oocysts. Moreover, Dubey and Fayer (1976) reported that fresh oocysts were obtained from dogs by canine—canine cycle, but not by bovine—canine cycle. Some cyclic transmissions of the small type one were elucidated by Heydorn (1973) and Dubey and Fayer (1976). Each definitive host of *Isospora* type (*Toxoplasma, Isospora, Besnoitia, Hammondia, Sarcocystis,*) is restricted, and many kinds of animals have been known as intermediate hosts. However, the cyclic transmissions of *Isosphora bigemina* small type of the dog have not yet been clarified completely. Therefore, the present study was undertaken to demonstrate the infectivity of *Isospora bigemina* small type oocysts in various animals, especially, with the cyclic transmission between the guinea pig and the dog.

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Materials and Methods

Isospora bigemina small type oocyst: The original oocysts were isolated from the diarrheal feces of a naturally infected 3-month-old dog by W. M. Correa, one of the authors in Botucatu, Brazil. This fecal sample was cultured in 2% potassium dichromate solution. Oocysts which were morphologically distinguishable from *Isospora bigemina* small type were not detected in the sample.

Animals: Guinea pigs, mice, rats, rabbits, hamsters, dogs and cats were used as experimental animals. Conventional guinea pigs, rabbits and hamsters weighing about 300 g, 1,500 g, and 300 g were used, respectively. The mouse used was of the CFW strain weighing about 20 g, and the rat was of the Wistar strain weighing 150 g. Those mice and rats were raised and bred under the Coccidium—free condition in the laboratory. The puppies and kittens used

weighing about 750–1800 g, 450–470 g were obtained from the East Tama Area Branch, Tokyo Metropolitan Dog Retention Station. All the animals were subjected to the fecal examination for several times in order to confirm the oocysts-free before the experiments. Those oocyst-free animals were raised in separated cages, respectively, under the Coccidium-free condition.

Fecal examination: Fecal examination for all the animals was carried out every day after inoculation by the sugar floatation method (specific gravity of sugar, 1.266) until they were killed.

Experiment 1: A guinea pig, puppy, mouse, rat, and a rabbit were inoculated by oral route with average number of 5.0×10^4 of original oocysts. The puppies fed mince of the heart, diaphragm, abdominal wall, gluteal muscle, small intestine, liver, and mesenteric lymph nodes of donor animals which were killed between 60 to 107 days after oocyst inoculation were examined for oocysts discharge in their feces.

Experiment 2: Fresh unsporulated oocysts which were collected from the feces of dog fed the internal organs of guinea pig in experiment 1 were cultured by procedures previously described (Ito, *et al.*, 1974), and sporulated. In order to examine the infectivity of those oocysts, guinea pigs and dogs were inoculated by oral route with 6.0×10^6 of sporulated oocysts. Two of six guinea pigs were killed on the 10th and 20th day after oocyst inoculation, respectively, and the heart, diaphragm, abdominal wall, gluteal muscle, small intestine, liver, and mesenteric lymph nodes of each guinea pig were pooled, and those organs of guinea pig killed on the 10th day were fed to a puppy, and the similar organs of guinea pig killed on the 20th day were fed to another puppy. The four remaining guinea pigs were killed two by two on the 30th and 81st day, respectively, and their major internal organs and muscles were separated into four parts; the first part of them contained the heart, diaphragm, abdominal wall, and gluteal muscle, the 2nd part consisted of the small intestine, the third part contained the liver and mesenteric lymph nodes, and the fourth part consisted of the brain. The puppies fed each part of internal organs of guinea pigs were examined for the discharge of oocysts.

Three puppies inoculated orally with the oocysts were killed on the 10th, 20th and 31st day after inoculation, respectively. The heart, diaphragm, abdominal wall, gluteal muscle, small intestine, liver, and mesenteric lymph nodes of them were minced and fed to the other puppies. Those puppies were examined for the discharge of oocysts.

Experiment 3: Three guinea pigs were inoculated orally with 6.0×10^6 of oocysts. The heart, diaphragm, abdominal wall, gluteal muscle, small intestine, liver, and mesenteric lymph nodes of the two guinea pigs killed on the 63rd day after oocyst inoculation were pooled and divided into two parts, one part was fed a puppy, another part to a kitten. The remaining guinea pig was killed on the 83rd day after oocyst inoculation and the same organs as mentioned above were divided into two, and each part was fed to a puppy and a kitten. These recipient animals were examined daily for oocyst discharge.

Results

Description of *Isospora bigemina* small type oocysts used in the study: Oocysts are spherical or subspherical (Figs. 1, 2), and 10.5–13.5 μm×8.5–12.8 μm (12.4×11.2 μm on the average) in size. The length-width ratio was 1.00–1.33 (1.10 on the average). The oocyst wall was smooth and colorless. Sporocysts are subspherical or ellipsoidal (Fig. 3), and 7.5–11.3 μm×5.0–8.3 μm (9.3×7.0 μm) in size. The length-width ratio was 1.03–1.72 (av. 1.34). The

Table 1 Susceptibility of experimental animals to *Isospora bigemina* small type oocysts from the naturally infected dog

Donor animals		Recipient dog				
Animal	Days after inoculation	Dog no.	Oocyst discharge	Prepatent period (Days)	Patent period (Days)	Maximum of OPG
Guinea pig	70	16	+	8	11	3.6×10^7
Dog	78	24	+	8	53	3.7×10^7
Mouse	88	25	−			
Rat	106	26	−			
Rabbit	107	27	−			
Hamster*	60	49	−			

Inoculation with oocysts: Each donor animal was inoculated with original oocysts 5.0×10^4. (*: with oocysts 6.0×10^6 from dog no. 16) Subinoculated organs of animal: heart, diaphragm, abdominal wall, gluteal muscle, small intestine, liver, mesenteric lymph nodes.

Table 2 Shedding of oocysts by dogs after ingestion of organs from guinea pigs or dogs orally inoculated with *Isospora bigemina* small type oocysts

Donor animals				Recipient dog				
Animal	Number of animal	Days after inoculation	Organ ingested	Dog no.	Oocyst discharge	Prepatent period (Days)	Patent period (Days)	Maximum of OPG
Guinea pig	1	10	H.D.A.G.I.L.M.	37	−			
	1	20	H.D.A.G.I.L.M.	28	+	8	9	5.1×10^6
	2	30	H.D.A.G.	29	+	7	20	1.8×10^6
			I.	30	+	10	11	1.0×10^6
			L.M.	31	+	8	4	7.5×10^4
			B.	32	+	11	7	7.4×10^5
	2	81	H.D.A.G.	33	+	8	28	6.9×10^5
			I.	34	+	7	6	1.2×10^7
			L.M.	35	−			
			B.	36	+	7	12	2.7×10^7
Dog	1	10	H.D.A.G.I.L.M.	43	−			
	1	20	H.D.A.G.I.L.M.	46	+	9	6	5.8×10^3
	1	31	H.D.A.G.I.L.M.	50	+	9	4	$5,2\times10^4$

Inoculation with oocysts: Each donor animal was inoculated with oocysts 6.0×10^6.
Organ ingested ... H: heart, D: diaphragm, A: abdominal wall, G: gluteal muscle, I: small intestine L: liver, M: mesenteric lymph nodes, B: brain

micropyle, oocyst residuum and Stieda body were absent. The sporocyst residuum was present. Oocysts were shed in unsporulated state. When sporulated, the oocyst was *Isospora* type. Sporulation time was about 3 days at 25 C.

Experiment 1: No fresh oocyst was discharged from each animal inoculated with sporulated oocysts. Those animals were necropsied within a few months. However, no remarkable macroscopic pathological changes were observed. The two puppies fed several organs of a guinea pig or a puppy which were inoculated with oocysts began to shed fresh oocysts on the 8th day (Table 1). The number of oocysts per gram

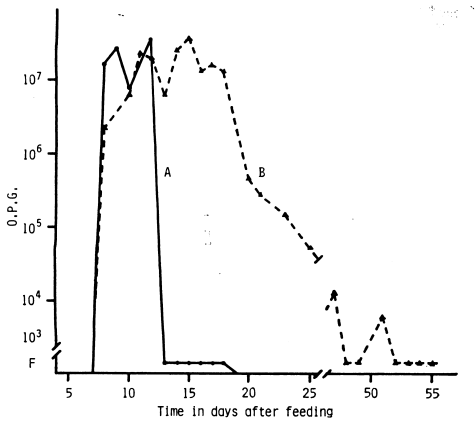

Fig. 4 OPG in dogs fed with the small type of *I. bigemina*.
A: Dog no. 16 (guinea pig—canine cycle)
B: Dog no. 24 (canine—canine cycle)
F: Oocyst positive by sugar floatation method

of feces (OPG) was counted through the patent period (Fig. 4). The two puppies were shedding numbers of oocysts for a few days, and showed the maximum of their OPG such as 3.6×10^7 or 3.7×10^7. Each puppy fed the organs of the mouse, rat, rabbit, and hamster, respectively, did not shed any oocysts.

Experiment 2.: The results are summarized in Table 2. The puppy fed the organs of the guinea pig killed on the 10th day after oocyst inoculation did not shed any fresh oocysts. However, the other puppy fed guinea pig killed on the 20th day shed oocysts, and showed the maximum of OPG such as 5.1×10^6. When the organs of the guinea pigs killed on the 30th day after inoculation were fed to four puppies in which one puppy (no. 29) fed the heart, diaphragm, abdominal wall, and gluteal muscle began to shed oocysts from 7 days after feeding, another puppy (no. 30) fed the small intestine shed oocysts from 10 days, and the third puppy (no. 31) fed the liver and mesenteric lymph nodes showed the discharge of oocysts from 8 days, and the other puppy (no. 32) fed the brain shed oocysts from 11 days. Likewise, those of killed on the 81st day were fed to four puppies, the puppy (no. 33) fed the heart, diaphragm, abdominal wall, and gluteal muscle began to shed oocysts from 8 days, either puppies (no. 34, no. 36) fed the small intestine or the brain showed the discharge of oocysts from 7 days, but the remaining puppy (no. 35) fed the liver and mesenteric lymph nodes did not shed.

The three puppies killed on the 10th, 20th, and 31st day after oocyst inoculation, respectively, did not shed fresh oocysts in their feces during the period of examination. The puppy fed organs of canine killed on the 10th day after oocyst inoculation did not shed oocysts, but the puppies fed organs of canine killed on the 20th and the 31st day, respectively, shed oocysts from 9 days after feeding. The number of oocysts was a few, and the maximum of OPG was 5.8×10^3 or 5.2×10^4.

Experiment 3.: Two puppies fed the internal organs of guinea pigs shed oocysts from 6 or 9 days after feeding, but neither of kittens shed oocysts.

Discussion

In the present experiments, no dog inoculated orally with oocysts discharged any fresh oocysts. When the internal organs of guinea pigs or dogs killed on the 20th day or more after oocyst inoculation were fed to dogs, the discharge of fresh oocysts was observed in the recipient dogs. The dogs discharged especially more oocysts in case fed guinea pig's organs than in case fed dog's organs. Therefore, the small type of *Isospora bigemina* isolated from a dog in Brazil may take guinea pig as a suitable intermediate host and their life cycle was confirmed to be obligatory heteroxenous. Since the infection was also established when the dog had been donor animal, it was considered that dogs served as a definitive and an intermediate host. When

several visceral organs of the infected guinea pigs were subinoculated into dogs separately, each dog discharged fresh oocysts, respectively. These results indicated that this organism may be parasitic to the various organs of guinea pigs. The prepatent period was 7–8 (rarely 6) days and the patent period was usually 9–20 days and sometimes more. The maximum number of OPG reached 10^6 to 10^7. On the other hand, each dog fed the organs of a mouse, rat, rabbit, or a hamster inoculated with oocysts did not shed oocysts. From the results, it was considered that those experimental small animals tested did not play a role as an intermediate host of this coccidia.

Some biological characters of the small type of *Isospora bigemina* of the dog were reported by Heydorn (1973), Fayer (1974), and Dubey and Fayer (1976). They found *Isospora bigemina* small type oocysts from the feces of dogs fed the muscle of cattle infected naturally. Heydorn (1973) inoculated orally those oocysts into the cattle, and found that the small type oocysts were discharged in feces of dogs fed beef of those infected cattle. The dogs inoculated orally with the oocysts did not shed any fresh oocysts. However, when they were killed 7 weeks later and their muscles were fed to the other dogs, those recipient dogs shed fresh oocysts (Heydorn, 1973). On the other hand, Dubey and Fayer (1976) reported that dogs fed beef of cattle inoculated orally with oocysts did not shed fresh oocysts. The dogs inoculated with oocysts did not shed fresh oocysts. However, when these dogs were killed 14 to 42 days after inoculation and their muscles were fed to the other dogs, those recipient dogs shed fresh oocysts (Dubey and Fayer, 1976). Those findings are compared with that of the present study. On the canine—canine cycle's case, the infection was established in both their experiments and the present one. On the bovine—canine cycle's case, the infection was confirmed by Heydorn (1973) but Dubey and Fayer (1976) failed in the establishment of the infection. We did not perform additional experiment of this cycle. On the other hand, they inoculated oocysts into the mice (Heydorn, 1973; Dubey and Fayer, 1976) and rabbits (Heydorn, 1973), and reported that those experimental animals did not serve as an intermediate host. Those results coincided with our results. But they did not investigate the level of infective activity of their organisms in guinea pigs. In the present experiments, the guinea pigs showed high susceptibility to this protozoa and high OPG value as the results of guinea pig —canine cycle. Therefore, the guinea pigs were considered to be a very suitable intermediate host. This finding is new knowledge which was obtained from the present experiments. It is of interest to note that guinea pigs which originated in South America may be a very suitable intermediate host of this protozoa isolated in Brazil in the present experiments. In either event, the biological difference between the protozoa observed in the present examination and the organisms studied by Heydorn (1973) and by Dubey and Fayer (1976) should be elucidated, the infectivity should be tested with their organisms in the guinea pig and our protozoa in the cattle. There is no report on the endogenous stage in the intermediate host, but Heydorn *et al.* (1975b) and Dubey and Fayer (1976) reported on the developmental stages in the intestine of dogs. We are also investigating on the developmental stages in the intestine of dogs compared with their results.

Summary

The cyclic transmission of the small type of *Isospora bigemina* of the dog were studied. The oocysts of *Isospora bigemina* used in the present experiment were iso-

lated from an infected dog in Brazil. The cyclic transmission of this coccidia was established succeedingly between dogs and guinea pigs. Guinea pigs and dogs did not shed fresh oocysts after inoculation by oral route with oocysts. They were killed on various days from 10 to 81 days after inoculation, and their visceral organs were fed to dogs. The recipient dogs began to discharge fresh unsporulated oocysts from 7 to 8 days after feeding with the visceral organs of guinea pigs or dogs which were killed on the 20th day or more. In experimental conditions, the maximum number of oocysts per gram of feces (OPG) reached 10^6 to 10^7. The number of oocysts discharged in the guinea pig—canine cycle were more than that obtained from canine —canine cycle. In the same way, a mouse, rat, rabbit, and a hamster were inoculated with oocysts and killed on various days from 60 to 107 days, and the viseral organs were fed to the dogs, respectively. Nevertheless, each dog did not shed any oocysts.

The oocysts were 12.4×11.2 μm in size. Neither micropyle, oocyst residuum nor Stieda body was formed, but a sporocyst residuum was present. Sporulation time was about 3 days at 25 C. From the results of the present experiments, it seems clear that the small type of *Isospora bigemina* of the dog takes guinea pigs as a suitable intermediate host. Therefore, the life cycle of this parasite was esteemed as obligatory heteroxenous.

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イヌ寄生 *Isospora bigemina* 小型種の生活環

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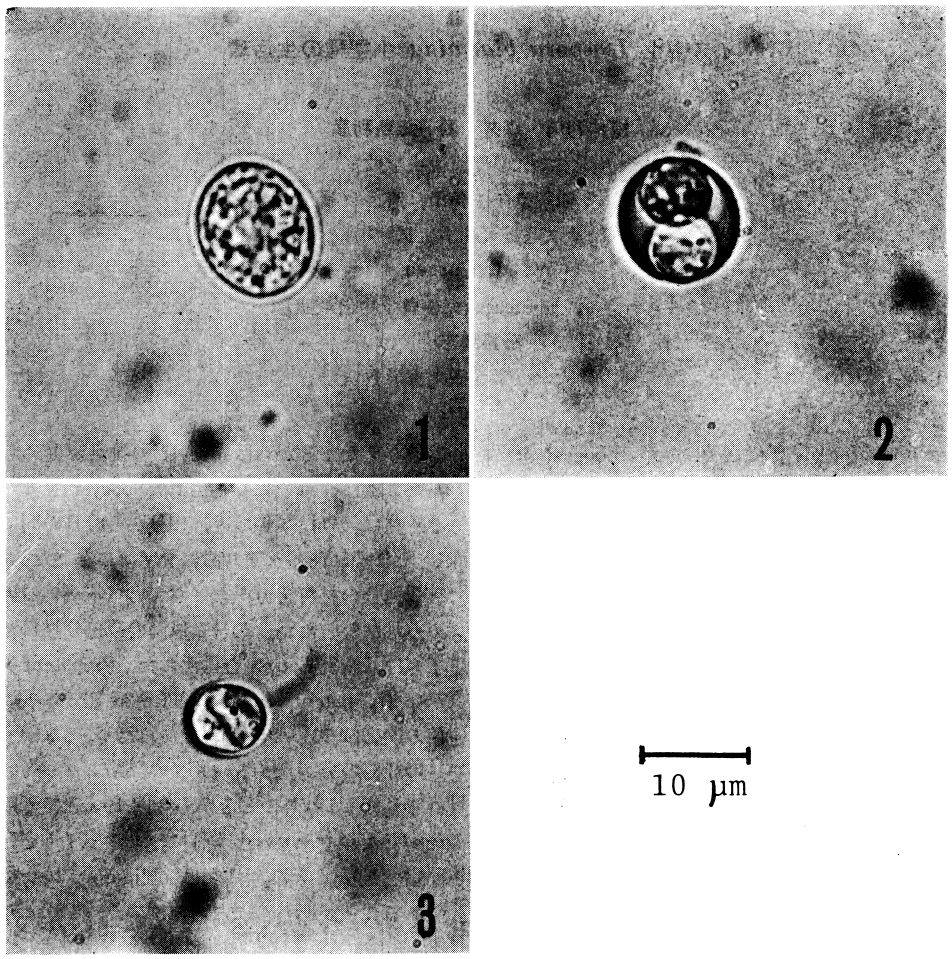
Isospora bigemina 小型種に相当する oocyst を Brazil の Botucatu でイヌより分離し，その生活環について検討した．Oocyst の形は類円形で，その大きさは 10.5～13.5 μm×8.5～12.8 μm（平均 12.4×11.2 μm），内部残体は存在するが，外部残体，micropyle，Stieda body は認められない．Sporulation time は約3日であった．Original oocyst をモルモット，イヌ，マウス，ラット，ウサギにそれぞれ経口投与したが，いずれも oocyst の排泄は認められなかった．これらの動物を 2～3 カ月後に殺処分し，それぞれその心臓，横隔膜，腹膜，臀筋，小腸，肝臓，腸間膜リンパ節をプールしてイヌに食べさせたところ，モルモットまたはイヌの臓器を与えたイヌが，ともに8日目から新たな oocyst を排泄した．しかしその他の動物の臓器を与えたイヌからは oocyst の排泄が認められなかった．次に，新たに排泄された oocyst をモルモットとイヌに経口投与した後，10日から81日の間に殺処分し，それぞれ前述と同じ臓器をイヌに与えた結果，10日目殺処分の材料を与えたイヌでは oocyst の排泄が認められなかったが，20日目以降の殺処分材料を与えたイヌは，全例 oocyst を排泄した．Prepatent periodは7～8日，patent period は約9～20日であった．OPG 値の最高は 10^6～10^7 個台であったが，モルモット～イヌの経路の方が，イヌ～イヌの経路よりも高い傾向がみられた．これらのことから今回 Brazil で分離された *Isospora bigemina* 小型種は，モルモットを好適な中間宿主とし，その生活環は obligatory heteroxenous であることが明らかになった．



Explanation of Figures

Fig. 1 An unsporulated oocyst of *Isospora bigemina* small type.
Fig. 2 A sporulated oocyst of *Isospora bigemina* small type.
Fig. 3 A sporocyst of *Isospora bigemina* small type.