

# Experimental Infection of Raccoon Dogs with *Sarcocystis cruzi* and *S. miescheriana*

Morihiro SAITO and Hiroshi ITAGAKI<sup>1)</sup>

Kumagaya Meat Inspection Center Saitama Prefecture, 179-1 Shimomasuda, Kumagaya, Saitama 310 and <sup>1)</sup>Department of Parasitology, Azabu University School of Veterinary Medicine, Fuchinobe, Sagamihara, Kanagawa 229, Japan

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**ABSTRACT.** Raccoon dogs were successfully infected with *Sarcocystis cruzi* and *S. miescheriana* by oral inoculation of infected cardiac muscle of cattle and pigs slaughtered in Saitama and Okinawa prefectures, respectively. Oocysts and sporocysts passed by raccoon dogs were similar in measurements and morphological features as reported of *S. cruzi* and *S. miescheriana* respectively. The prepatent and patent periods of both *S. cruzi* and *S. miescheriana* were 9 and 66–72 days respectively in raccoon dogs.—**KEY WORDS:** final host, life cycle, raccoon canine, *Sarcocystis cruzi*, *Sarcocystis miescheriana*.

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*Sarcocystis* species that infect cattle are *S. hominis*, *S. cruzi* and *S. hirsuta* and those which infect pigs are *S. suihominis*, *S. miescheriana* and *S. porcifelis*. Of these species of *Sarcocystis*, *S. cruzi* and *S. miescheriana*, and *S. hirsuta* and *S. porcifelis* utilize dogs and cats as the final host respectively, whereas the final host of *S. suihominis* and *S. hominis* is humans. In Japan three species of *Sarcocystis* that infect livestock have been known to occur: *S. cruzi*, *S. miescheriana* and *S. tenella*. Dogs have been experimentally confirmed to be the final host of *S. cruzi*, *S. miescheriana* and *S. tenella* [7], but some wild carnivores have been suspected of the final host because the prevalence of the former two species of *Sarcocystis* is high in cattle and pigs.

In this study raccoon dogs, *Nyctereutes procyonoides viverrinus*, were examined on the role as a final host of *S. cruzi* and *S. miescheriana*.

## MATERIALS AND METHODS

**Parasite:** Cardiac muscle infected with *S. cruzi* and *S. miescheriana* was obtained from cattle and pigs slaughtered in Saitama and Okinawa prefectures respectively.

**Animals:** Two male and 2 female raccoon dogs, *Nyctereutes procyonoides viverrinus*, 2 years old and bred and reared at a high school in Saitama Prefecture, were used and in addition 2 mongrel dogs, about 1 year old, were used as control.

**Fecal examination for *Sarcocystis* oocysts and sporocysts:** After fed with infected muscle, the animals were daily examined for oocysts and sporocysts in the feces and sporocyst per gram (SPG) was measured with a McMaster counting plate. When SPG value was less than 100 and during the prepatent period, all the feces excreted were examined for oocysts and sporocysts by the flotation method with saturated NaCl solution.

**Morphological observation of oocysts, sporocysts and sporozoites:** Oocysts and sporocysts, 500 each, were measured with a micrometer under a microscope. Fifty sporozoites were measured with a micrometer under a microscope after released from sporocysts by grinding

them in a mortar.

## RESULTS

One male and 1 female raccoon dogs were fed each 500 g cardiac muscle infected with *S. cruzi*, and 1 female animal was fed 500 g cardiac muscle infected with *S. miescheriana*. Another male raccoon dog was used as non-infected control.

Nine days after ingestion of infected muscle with *S. cruzi*, oocysts and sporocysts were passed in the feces of all the animals except for the control. About 10 days later oocysts disappeared from the feces, whereas sporocysts peaked in number from days 4 to 9 after the start of passage, and thereafter they decreased in number with time and disappeared from the feces days 68 to 72 after ingestion (Figs. 1, 2).

Raccoon dogs fed with *S. miescheriana*-infected muscle began to shed oocysts and sporocysts 9 days after ingestion. Oocysts disappeared from the feces 10 days later. Sporocysts were detected by the flotation method until day 66 after the start of shedding but could not be counted with a McMaster counting plate at any time of infection, because the number of sporocysts was very small. Neither oocysts nor sporocysts were shed by the control animals (Table 1).

Oocysts passed by raccoon dogs measured an average size of  $19.5 \times 15 \mu\text{m}$  and included two mature sporocysts but no polar cap, micropyle, polar granule nor external residual body (Fig. 3). Sporocysts were elongated ellipsoidal, measured an average size of  $16.5 \times 10.0 \mu\text{m}$ , and contained four sporozoites and one large internal residual body. No stieda body was seen (Fig. 4). Sporozoites with an average size of  $7.0 \times 3.0 \mu\text{m}$  were crescent in contour with one end more pointed than the other and included a nucleus.

Oocysts shed by a raccoon dog fed *S. miescheriana*-infected muscle were similar in structure to those passed by the animals fed *S. cruzi*-infected muscle, but *S. miescheriana* oocysts,  $16.5 \times 12.0 \mu\text{m}$  in average size, were smaller than those of *S. cruzi* (Fig. 5). Sporocysts

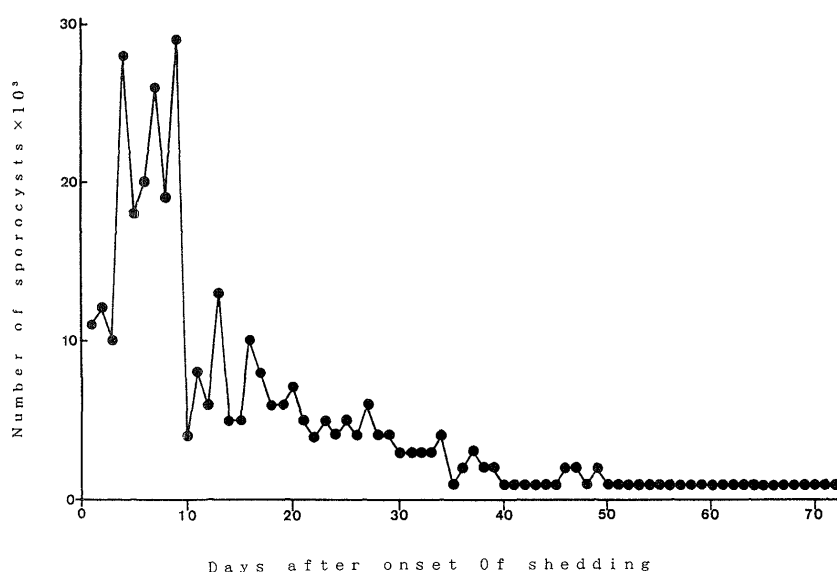


Fig. 1. Output pattern of sporocysts in a raccoon dog (No. 1) infected with *S. cruzi*.

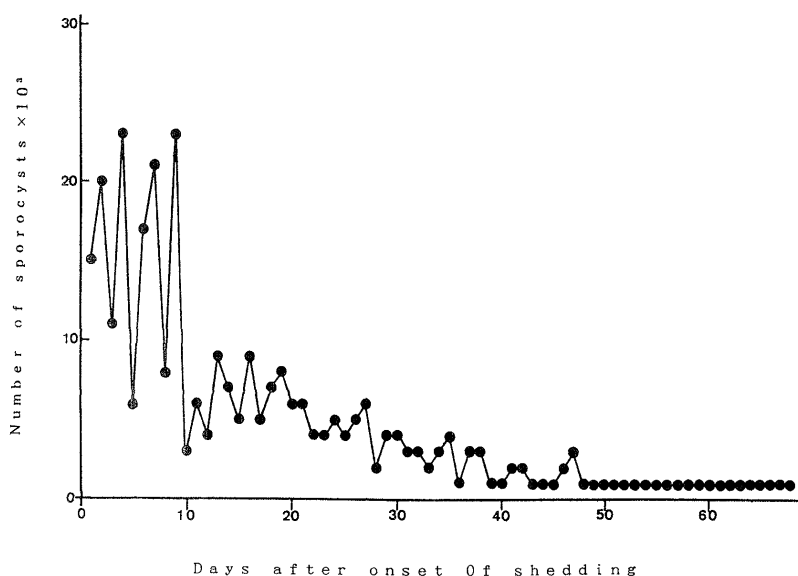


Fig. 2. Output pattern of sporocysts in a raccoon dog (No. 2) infected with *S. cruzi*.

Table 1. Prepatent and patent periods of *S. cruzi* and *S. miescheriana* in raccoon dogs fed infected muscle

Raccoon dog No.	Species	Sex	Age in years	Prepatent period in days	Patent period in days
1	<i>S. cruzi</i>	M	2	9	72
2	<i>S. cruzi</i>	F	2	9	68
3	<i>S. miescheriana</i>	F	2	9	66
4	control	M	2	—	—

shed by the animals infected with *S. miescheriana* were elliptical and slightly smaller than those passed by *S. cruzi*-inoculated animals but both sporocysts were the same in internal structure (Fig. 6). No morphological

differences were seen between sporozoites derived from the animals inoculated with *S. cruzi* and *S. miescheriana* respectively.

To confirm the infectivity of sarcocysts in the inoculated cardiac muscle of cattle and pig respectively, a part of the muscle was given to 2 dogs. Both animals passed the same oocysts and sporocysts as excreted by raccoon dogs.

#### DISCUSSION

The dog, fox, coyote, wolf and raccoon have been reported to be the final host of *S. cruzi* and *S. miescheriana* [3, 6, 8, 9], and *Sarcocystis* oocysts were detected from dogs [1, 2]. In Japan few reports have been published on the detection of *Sarcocystis* oocysts nor sporocysts from

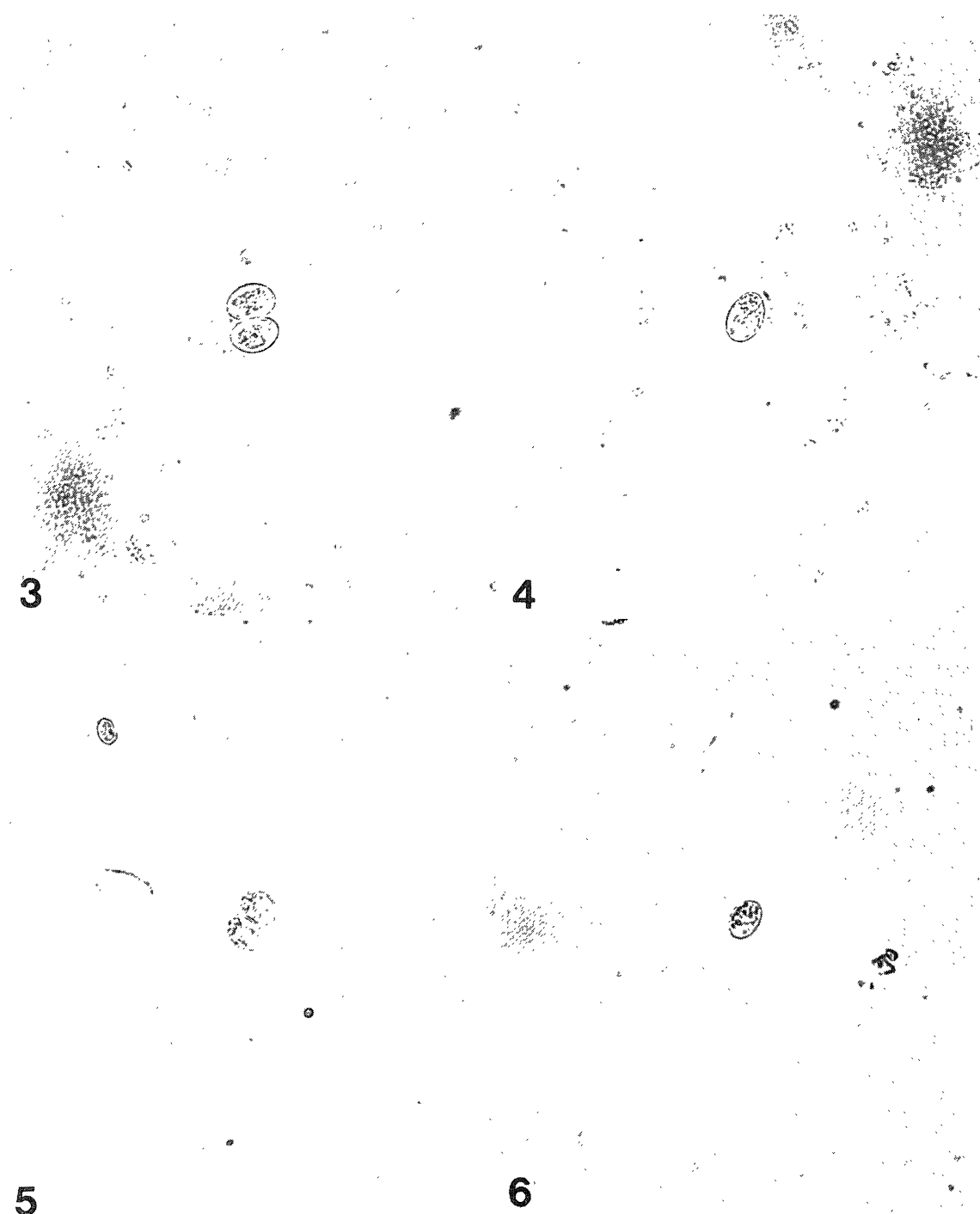


Fig. 3. Oocyst of *S. cruzi* passed by a raccoon dog.

Fig. 4. Sporocyst of *S. cruzi* passed by a raccoon dog.

Fig. 5. Oocyst of *S. miescheriana* passed by a raccoon dog.

Fig. 6. Sporocyst of *S. miescheriana* passed by a raccoon dog.

dogs and other carnivorous mammals, although the infection rates with *S. cruzi* and *S. miescheriana* in slaughtered cattle and pigs respectively are the same as reported in other countries [12, 13]. This suggests that some wild carnivorous mammals may fill the role of final host of both species of *Sarcocystis*. In recent years,

raccoon dogs frequently live near human residential quarters and hunt for food in breeding house of livestock. In this study raccoon dogs were experimentally confirmed to fill the role of final host for *S. cruzi* and *S. miescheriana* by feeding them on infected cardiac muscle of cattle and pigs slaughtered.

In the present experiments prepatent and patent periods of *S. cruzi* were 9 and 68–72 days (over 6 weeks) respectively. The measurements of oocysts, sporocysts and sporozoites excreted coincided with those reported [3, 8–11].

A raccoon dog fed *S. miescheriana*-infected muscle first passed oocysts and sporocysts in the feces on day 9, and sporocysts disappeared on day 66 after ingestion. The developmental features and measurements of oocysts, sporocysts and sporozoites were the same as reported [4, 5, 10, 14].

The same cardiac muscle infected with *S. cruzi* and *S. miescheriana* was also fed to dogs and the infection was successfully established.

All cattle, more than 5 years old, slaughtered in this country have been reported to be infected with *Sarcocystis cruzi* [11], but no reports have been published on the natural infections with *Sarcocystis* in dogs. We also could not detect the natural infections in stray dogs. These results suggest that some wild carnivorous mammals in addition to dogs may act as the final host of both *Sarcocystis* species in the field.

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