EXPERIMENTAL INFECTIONS OF SARCOCYSTIS CRUZI, SARCOCYSTIS TENELLA, SARCOCYSTIS CAPRACANIS AND TOXOPLASMA GONDII IN RED FOXES (VULPES VULPES)

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EXPERIMENTAL INFECTIONS OF *SARCOCYSTIS CRUZI*, *SARCOCYSTIS TENELLA*, *SARCOCYSTIS CAPRACANIS* AND *TOXOPLASMA GONDII* IN RED FOXES (*VULPES VULPES*)

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ABSTRACT: Four littermate 6-wk-old red foxes (Nos. 1–4) were fed *Toxoplasma gondii*, *Sarcocystis cruzi*, *S. tenella* and *S. capracanis*. One littermate fox (No. 5) served as the control. Two foxes (Nos. 1, 2) were fed tissue cysts of *T. gondii* and two foxes (Nos. 3, 4) were fed oocysts of *T. gondii*. Twenty-one to 42 days later, the same five foxes were used to test the infectivity of meat of goat, sheep, and ox experimentally inoculated with *Sarcocystis*. Fox 2 was fed goat meat and shed *S. capracanis*-like sporocysts 10 days later. Foxes 3 and 4 were fed beef, and they shed *S. cruzi*-like sporocysts 9 days later. Fox 5 was fed sheep meat and shed *S. tenella*-like sporocysts 8 days later. Foxes were killed between 36 and 55 days of the experiment and their tissues were inoculated into mice to recover *T. gondii*. All foxes remained clinically normal and *T. gondii* was recovered from all inoculated foxes and not from the control. *Sarcocystis* sporocysts from foxes induced lethal infections in goats, sheep, and ox. The sporocysts, meronts, merozoites, and sarcocysts of fox-derived parasites were similar to those derived from coyotes or dogs. It was concluded that the red fox can act as a final host for the three pathogenic species of *Sarcocystis* in cattle, sheep, and goats.

INTRODUCTION

Red foxes shed sporocysts in feces after ingesting meat naturally infected with *Sarcocystis cruzi* of cattle, *S. tenella* of sheep, *S. miescheriana* of pigs, and *S. odocoileocanis* of white-tailed deer (*Odocoileus virginianus*) (Rommel et al., 1974; Fayer et al., 1976; Ashford, 1977; Erber and Boch, 1976; Crum and Prestwood, 1982). There is some uncertainty concerning the species of *Sarcocystis* of herbivores transmissible to foxes because no attempts were made to induce sarcocystosis in sheep and cattle through feces of foxes and because more than one species of *Sarcocystis* from a given intermediate host may cycle through a given final host (Beaver and Maleckar, 1981; Erber, 1982). The object of the present study was to determine whether the red fox can act as a final host for the pathogenic species of *Sarcocystis* in cattle, sheep, and goats. Opportunity was taken to study toxoplasmosis in the same foxes.

MATERIALS AND METHODS

A 3-wk-old litter of five red foxes was removed from a den near Roundup, Montana, and brought to the Veterinary Research Laboratory (VRL) 2 wk later. At VRL, the foxes were housed individually. They were fed only commercially available dog pellets and milk from the time they were removed from the den. All five foxes had no detectable serum antibody to *Toxoplasma gondii* in the Sabin-Feldman dye test.

Toxoplasma infection

The foxes were 6 wk old at the time of inoculation with *T. gondii*. Foxes 1 and 2 were each fed two mice infected with *T. gondii*; the mice had been inoculated with TC-1 strain of *T. gondii* (Dubey, 1979) 2 mo previously. The presence of cysts was ascertained by microscopic examination of the brains of the mice. Foxes 3 and 4 were each fed 350 infective oocysts of the GT-1 strain of *T. gondii* (Dubey, 1980a). The number of infective oocysts was determined by inoculation of the mice with ten-fold dilutions of oocysts as described (Dubey, 1980a) 3 wk prior to inoculation of the foxes. Fox 5 served as the uninoculated control.

Foxes were killed between 36 and 55 days postinoculation (DPI) (Table 1). At necropsy, portions (50 g or whole organ) of mesenteric lymph nodes, lungs, livers, brains, thigh muscles and hearts were removed and digested in acid-pepsin solution. After washing, the homogenate was inoculated subcutaneously into mice as described by Dubey (1980b); six mice were inoculated with each tissue. In addition to tissues collected for mouse inoculations, portions of spinal cords, spleens, urinary bladders, prescapular lymph nodes, and kidneys of foxes were fixed in 10% Millonig's buffered formalin. Paraffin-embedded tissues were cut in 5 μm sections, stained with hematoxylin and eosin, and examined microscopically.

The mice were examined for *Toxoplasma* infection. For this, impression smears of lungs and brains of mice that died were examined microscopically for *T. gondii* after staining with Giemsa's stain. Survivors were bled at 21 DPI and their serum samples were examined for antibody to *T. gondii*. Mice were

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TABLE 1. *Toxoplasma* recovered from red foxes which had been fed cysts or oocysts of *Toxoplasma gondii*.

Foxes				No. of mice positive for *Toxoplasma* six mice inoculated with following fox tissues					
No.	Sex	Inoculum	DPI[a]	Brain	Heart	Liver	Lungs	Thigh muscle	Mesenteric lymph nodes
1	M	cysts	36	1	4	0	4	2	ND[b]
2	F	cysts	36	0	3	1	4	4	1
3	M	oocysts	48	6	6	1	6	6	0
4	F	oocysts	55	6	6	3	6	6	1
5	F	none	36	0	0	0	0	0	0

[a] DPI = day postinoculation of fox.
[b] ND = not done.

killed 30 DPI and their brains were examined for *Toxoplasma* cysts.

Mice and fox sera were examined in the Sabin-Feldman dye test, and they were considered negative when antibody was not demonstrable in undiluted serum.

Transmission of *Sarcocystis* infections from cattle, sheep, and goats to foxes

All three species of *Sarcocystis* used in this study were isolated in Montana and their development and pathogenicity in the intermediate and final hosts had been reported in detail (Dubey et al., 1981, 1982a, b, 1983; Dubey, 1982, 1983a, b). Twenty-one days after *T. gondii* inoculation, fox 2 was fed meat from a goat experimentally infected with *S. capracanis*. The goat had been fed 6×10^5 sporocysts 76 days prior to necropsy.

Thirty-three days after *T. gondii* inoculation, fox 3 was fed meat from an ox experimentally infected with B1 isolate of *S. cruzi*. The calf had been fed 5×10^5 sporocysts 84 days prior to necropsy.

Forty-two days after *T. gondii* inoculation, fox 4 was fed meat from another ox experimentally infected with B1 isolate of *S. cruzi*. The calf had been fed 5×10^5 sporocysts 93 days prior to necropsy.

Fox 5 was fed meat from a sheep experimentally infected with *S. tenella*. The sheep had been inoculated with 5×10^4 sporocysts 276 days prior to necropsy.

Feces of the foxes were examined for coccidian oocysts (Dubey, 1976) twice weekly after inoculation with *T. gondii* and daily after inoculation with *Sarcocystis*. At necropsy, the sporocysts were collected from intestinal scrapings and stored in the balanced salt antibiotic solution at 4 C as described (Dubey, 1980, 1981).

Transmission of *Sarcocystis* infections from foxes to sheep, goats, and cattle

Six 2-mo-old goats, five 2–9-wk-old lambs, and a 4-day-old ox were used to test the infectivity of sporocysts from foxes. One goat was fed 10^7 sporocysts, one was fed 10^6 sporocysts, and one goat was fed 10^4 sporocysts from fox 2; the sporocysts had been stored for 1 day prior to feeding the goats. Three goats served as uninoculated controls. The inoculated goats were housed with the controls and were fed hay and grain.

One 15-day-old lamb (No. 1) and one 64-day-old lamb (No. 2) were each fed 5×10^5 sporocysts, and one 66-day old lamb (No. 3) was fed 10^4 sporocysts from the feces of fox 5; the sporocysts had been stored for 1 wk prior to feeding lambs. One 15-day-old lamb (No. 4) and one 66-day old lamb (No. 5) served as controls. Lambs 4 and 5 were siblings of lambs 1 and 3. The lambs were run with their dams.

The calf was fed 10^7 sporocysts from the intestinal scrapings of fox 4 which was killed on the same day the ox was inoculated. The calf was housed indoors and was fed milk until necropsy.

Hematological values (number of red- and white-blood cell counts, hematocrit, mean corpuscular volumes, differential leukocyte counts) of ill animals were obtained as described (Dubey, 1983b).

Inoculated sheep, goats, and ox were necropsied, and all their major internal organs listed by Dubey (1982) were examined histologically.

RESULTS

The foxes remained clinically normal throughout the study and *T. gondii* was recovered from the inoculated foxes (Table 1). All infected mice died of toxoplasmosis. Judging from the results of recovery of *T. gondii* in the mice, the oocysts were more infectious to the foxes than the tissue cysts. Neither *Toxoplasma* nor *Toxoplasma*-induced lesions were found in sections of any fox.

Fox 2 fed muscle tissue infected with *S. capracanis* shed sporocysts in feces 10 days later. The sporocysts (13–14 × 8–10 μm; $n = 20$) from the feces of fox 2 were structurally similar to those of *S. capracanis* in the feces of coyotes and dogs (Dubey et al., 1983). The goat fed 10^7 sporocysts suddenly became comatose, was in acute respiratory failure 13 DPI and was euthanatized; first-generation meronts were found in sections of arteries of the small intestine and

kidneys. The goat fed 10^6 sporocysts died of acute sarcocystosis 19 DPI and numerous second-generation meronts were seen in the kidneys and other tissues. The goat fed 10^4 sporocysts was mildly anemic but otherwise appeared normal; numerous cross-striated sarcocysts were found in muscles at necropsy 70 DPI. Control goats remained asymptomatic and were not necropsied. The clinical course, lesions, and the parasites in goats fed fox-derived sporocysts were similar to those in goats fed coyote-derived sporocysts (Dubey et al., 1981, 1983).

Fox 5 fed *S. tenella* infected muscles shed sporocysts 9 days later. The sporocysts (12.5–14 × 7.5–9; $n = 16$) from the feces of fox 5 were structurally similar to those of *S. tenella* in coyotes and dogs (Dubey et al., 1983). Both lambs fed 5×10^5 sporocysts became anemic on 25 DPI; one died 26 DPI and the other was euthanatized 27 DPI. The lamb fed 10^4 sporocysts and the control lambs remained asymptomatic and were examined at necropsy on 60 DPI. Numerous second-generation meronts were found in the tissues of the lambs necropsied 26 and 27 DPI. Numerous cross-striated sarcocysts and nonsuppurative myositis were found in the lamb necropsied 60 DPI. A few immature *S. tenella* sarcocysts (naturally acquired) were found in the tissues of lamb No. 3. Neither sarcocysts nor lesions were seen in the control lambs. Meronts, sarcocysts, clinical course, and lesions were similar to those of coyote-derived *S. tenella* (Dubey et al., 1982a, b; Dubey, 1983a).

Foxes 3 and 4 shed sporocysts (14–16 × 9–10.5 μm; $n = 20$) in their feces 9 days after ingesting infected beef; the sporocysts were structurally similar to those shed by coyotes (Dubey, 1982). The calf fed sporocysts was febrile (40–41 C) 15, 25, 26, 27 and 28 days after inoculation. On 27 DPI, the calf had mild watery diarrhea, appeared dull, and was euthanatized the next day because of weakness and inappetence. At necropsy, petechial to echhymotic hemorrhages were seen throughout muscles and viscera, and hemorrhages were particularly severe in the heart. The liver and visceral lymph nodes were icteric. Single and dividing merozoites were seen in mononuclear cells of the blood at 21 and 28 DPI. Second-generation meronts were found in kidneys and several other tissues. The parasites and lesions were similar to those in calves given coyote-derived sporocysts (Dubey, 1982; Dubey et al., 1982b).

DISCUSSION

The results of this study showed that red foxes can harbor mouse-virulent *Toxoplasma* without any ill effects. The GT-1 strain of *T. gondii* used to infect two foxes was pathogenic to mice, hamsters, squirrels, sheep, and goats (Dubey, 1980a, 1981, 1983c).

In the present study foxes were first infected with *Toxoplasma* and then *Sarcocystis*. However, it is unlikely that *Toxoplasma* modified the infections by *Sarcocystis* spp.

The results of this study show that red foxes are an efficient final host for the three species of *Sarcocystis* which are pathogenic for cattle, sheep, and goats. Although sporocysts from intestinal scrapings were not counted, microscopic examination showed that the number of sporocysts shed by foxes was probably comparable to those shed by dogs and coyotes. More importantly, sporocysts obtained from intestinal scrapings of foxes were much cleaner than those obtained from dogs. The intestines of foxes are considerably shorter than those of dogs and there is less mucous in the intestines of foxes than in dogs. These factors might have contributed to the recovery of clean sporocysts from the foxes, and may be of help to other researchers.

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