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SARCOCYSTIS AND RELATED ORGANISMS IN AUSTRALIAN WILDLIFE: IV. STUDIES ON *Sarcocystis cuniculi* IN EUROPEAN RABBITS *(Oryctolagus cuniculus)*

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Abstract: The role of the cat *(Felis domestica)* as a definitive host for *Sarcocystis cuniculi* of European rabbits *(Oryctolagus cuniculus)* was confirmed.

It was shown that after dosing with sporocysts from cats, rabbits developed sarcocysts and these became infective for cats at not less than 93 days post-infection (p.i.). The earliest infection detected was at 142 days p.i.

Infected muscle from an experimental rabbit did not transmit *Sarcocystis* when fed to other rabbits.

Microscopically, sarcocysts in European rabbits *(O. cuniculus)* were morphologically indistinguishable from those in cottontail rabbits *(Sylvilagus floridanus).*

INTRODUCTION

Although a number of papers have described successful transmission of *Sarcocystis* spp. of rabbits to cats and raccoons,[1,2,3,6] none have recorded transmission back to rabbits. In addition, these papers have been published in isolation, without any attempt being made to compare the organisms in the different hosts.

This paper confirms the transmission of *Sarcocystis cuniculi* from rabbits to cats, records the transmission from cats back to rabbits, and compares the morphology of sarcocysts of European *(Oryctolagus cuniculus)* and cottontail rabbits *(Sylvilagus floridanus).*

MATERIALS AND METHODS

Animals

The original "donor" rabbits were eight wild European rabbits *(Oryctolagus cuniculus)* collected at Bogan Gap in northern Tasmania in May, 1976 as part of the survey of *Sarcocystis* in mammals reported earlier.[5]

Experimental rabbits were crossbred domestic strains of the same species maintained at Mt. Pleasant Laboratories and the University of Kansas. These animals were four to nine months of age at the commencement of each experiment and both sexes were used. They were raised indoors, and during the period of experimentation were kept in cages and fed commercial rabbit cubes. Sarcocysts had not been found in these rabbit colonies over a period of years preceding the experiments.

Experimental cats *(Felis domestica)* were obtained as recently-weaned kittens and raised on commercial cat food at Mt. Pleasant Laboratories and the University of Kansas.

Experimental dogs *(Canis familiaris)* were born and raised at the University of Melbourne before transferral to Mt. Pleasant Laboratories. At no time prior to the commencement of the experiments did they receive raw meat.

Histologic Sections

Histologic sections of cottontail rabbit *(S. floridanus)* muscles containing sarcocysts were obtained from authors of previous papers.[2,3] Also, electronmicrographs of sarcocysts from European rabbits[5] and cottontail rabbits (W.J.

Hartley, University of Sydney, pers. comm.) were compared.

Methods

All faecal samples examined for *Sarcocystis* sporocysts were emulsified in 5-10 volumes of sucrose solution (sp.g. 1.15). This emulsion was then centrifuged at 1500 r.p.m. for 10-30 min. and the supernatant was diluted with four volumes of water. This diluted sample was then centrifuged for 10-20 min. and the resultant deposit was examined for the presence of sporocysts.

Faeces of the cats and dogs were examined on a number of occasions over several weeks prior to the commencement of each experiment, and for the seven days immediately preceding experimental feeding faeces were examined daily.

In the initial experiment, representing samples of striated and cardiac muscle were removed from the "donor" rabbits for histologic examination. Carcasses were then split longitudinally and each half fed to three kittens and two pups at Mt. Pleasant Laboratories over a period of five days. Cat and dog faeces were examined for coccidia for 18 days after feeding commenced. On the 18th day after feeding commenced the two cats remaining in the experiment were killed (the other cat was eliminated from the experiment because it began to pass *Toxoplasma* oocysts). Duodenal scrapings, jejunal and ileal contents, were pooled, suspended in saline, and given orally to rabbits 1-3 *(O. cuniculus).* Rabbit 4 was kept in a separate cage as an unfed control. The rabbits were exsanguinated 77 days post-inoculation (p.i.). Representative tissues, including myocardium, tongue, diaphragm and skeletal muscles were collected for histologic examination and the carcasses were then fed to another three kittens. Sera from the rabbits were examined for complement-fixing antibodies against *Sarcocystis (Sarcocystis* CFT using antigen prepared from macerated, boiled macrocysts of *S. tenella).*[4]

Some of the sporocysts collected from the first batch of kittens were sent to the University of Kansas where they were fed to seven laboratory rabbits (nos. 5-11) *(O. cuniculus).* These rabbits were killed on days 7, 14, 21, 30, 61, 93 and 142 p.i. and they all were examined histologically for sarcocysts. The last three (nos. 9-11) were fed to cats. The cat fed rabbit 11 was treated with 80 mg depomedrol and 400 mg cortisol intramuscularly on day 0. Faeces from these cats were continuously examined for sporocysts for 40-59 days p.i. In addition, muscle from rabbit 11 was dosed orally into another seven rabbits (nos. 12-18) which were killed at 173 days p.i. (n=5) and 418 and 902 days p.i., respectively. These last two rabbits (nos. 17 and 18) were each fed to a cat. It is pertinent to note that part of this experiment was contemporaneous with the next.

Sporocysts produced by the cat fed rabbit 11 were fed to five laboratory rabbits (nos. 19-23). Rabbits 19-21 were injected twice with 25 mg cortisone acetate at seven day intervals. Rabbit 19 died at 14 days p.i. and rabbits 20 and 21 were killed at 161 and 358 days p.i. respectively. The carcass of rabbit 22 was fed to two cats at 161 days p.i., and the carcass of rabbit 23 also was fed to two cats at 774 days p.i.

RESULTS

Six of the eight "donor" rabbits *(O. cuniculus)* were found to have compartmented, thick-walled sarcocysts in their skeletal muscles. The cysts measured up to 90 μm in diameter with a wall approximately 10 μm thick. Close examination revealed that the wall was composed of closely-packed villus-like projections. The sarcocysts of cottontail rabbits *(S. floridanus)* were practically identical except that they were larger, up to 300 μm in diameter. In particular, the cyst wall was of the same thickness and

structure as that of *S. cuniculi* in European rabbits. Ultramicroscopically, the projections forming the sarcocyst walls in both species of rabbits appeared identical in cross section. There was an outer limiting membrane surrounding a central core of about 100 closely-packed microfilaments. Unfortunately, only the projections in the walls of the sarcocysts of cottontails were cut in true longitudinal section and these were found to be villus-like containing microfilaments.

On day 12 p.i., two of the kittens in the first experiment which were fed *Sarcocystis*-positive rabbit carcasses began to pass sporocysts measuring 12.8 × 9.5 μm (n=30, range 11.6-14.5 × 8.7-10.0 μm). Each sporocyst contained four sporozoites and a residual body. Sporulated sporocysts were found in the lamina propria of the kittens' intestines, especially the jejunum. The third kitten began shedding *Toxoplasma gondii* oocysts on day 13 p.i. and was eliminated from the study. At no time were sporocysts detected in the faeces of the pups fed these carcasses.

Sarcocysts were not detected in the Mt. Pleasant rabbits 1-3 dosed with gut contents from the experimental kittens and examined at 77 days p.i., nor did feeding these rabbits to two more kittens lead to sporocyst production. Also, no sarcocysts were detected in the control rabbit 4 which, when fed to a kitten, did not result in sporocyst production. However, the dosed rabbits (nos. 1-3) had *Sarcocystis* CTF titres of $^{3}/_{320}$ - $^{3}/_{1280}$ compared with $^{3}/_{20}$ for the control animal (no. 4).

In contrast, rabbit 11 examined at 142 days p.i. at the University of Kansas had a few, thick-walled sarcocysts in skeletal muscles and diaphragm. These cysts were indistinguishable from those in naturally-infected rabbits and measured up to 75 μm in diameter. When rabbit 11 was fed to a cat, sporocysts averaging 12.9 × 9.5 μm (n=8, range 12.5-13.4 × 9.2-9.6 μm) were passed intermittently from days 20 to 29 p.i. However, sarcocysts were not found in rabbits 5-10 killed at 93 or less days p.i. and rabbits 9 and 10 killed at 61 and 93 days p.i. did not result in sporocyst production when fed to cats.

A few thick-walled sarcocysts were found in the diaphragm of rabbit 20 killed at day 161 p.i. When the carcasses of this rabbit and the non-cortisone treated rabbit 23 killed at 774 days p.i. were fed to two cats each, shedding of sporocysts occurred in two of two and one of two cats, respectively. Sarcocysts were not found in muscles of rabbit 19 which died at 14 days p.i., nor were developmental stages found in the liver or blood vessels.

Sarcocysts were not detected in rabbits 12-18 inoculated with muscle suspensions, nor did the feeding of carcasses of two of these rabbits to cats result in shedding of sporocysts. These rabbits also acted as controls for other rabbits (nos. 5-11, 19 and 20) at the University of Kansas.

DISCUSSION

These results confirm those of Tadros and Laarman[6] and Collins[1] that the cat is a definitive host for *S. cuniculi* of the European rabbit. In addition, we have shown that sporogony occurs in the lamina propria of the cat and that the period to infectivity in rabbits must be greater than 93 days and may be as great as 142 days. [1]

The only morphological difference we found between the sarcocysts found in the European rabbit *(O. cuniculus)* and the cottontail rabbit *(S. floridanus)* was that sarcocysts in the former host were smaller. This difference is probably

[1] In contrast to our findings, Cerna, Louckova and Nedvedova (1979, J. Protozool. 26: 40A) reported the presence of sarcocysts in experimental rabbits as early as 79 days p.i. Unfortunately, this reference consists only of an abstract and, therefore, the full details of materials and methods are not available for comparison.

related to age and, indeed, Tadros and Laarman[6] found cysts up to 500 μm in diameter in their European rabbits. Obviously, the relationship between these *Sarcocystis* spp. cannot be further elucidated without cross-infection trials. Fayer and Kradel[3] attempted to investigate this relationship when they dosed European rabbits *(O. cuniculus)* with sporocysts from cats fed cottontail rabbits. However, they kept their experimental rabbits for only 30 and 86 days which, from our results, would be too short a period to allow for the development of infective sarcocysts.

Until more definitive data is available, it is logical to relate the *Sarcocystis* spp. of rabbits to the species in which they occur i.e. *S. cuniculi* Brumpt, 1913 in the European rabbit *(O. cuniculus)* and *S. leporum* Crawley, 1914 in the cottontail rabbit *(S. floridanus)*.

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