

SPONTANEOUS AND EXPERIMENTAL INFECTION OF DOMESTIC RABBITS BY *SARCOCYSTIS CUNICULI* BRUMPT, 1913

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Abstract. In 43 animals, representing 36% of a sample of 117 domestic rabbits, antibodies to *Sarcocystis* were detected using indirect immunofluorescent antibody test (IFAT). Experimental transmission of the parasite from rabbit to cat and back to rabbit proved that the organism involved was *Sarcocystis cuniculi* Brumpt, 1913. The antibody response in experimental transmission was revealed by IFAT: the level of antibodies was generally low and the antibodies disappeared in less than 100 days p.i., while the parasites still remained viable in host's muscle tissue. The diagnosis of rabbit sarcosporidiosis in the definitive and intermediate hosts is discussed.

Sarcosporidia parasitic in domestic rabbit *Oryctolagus cuniculus* (Linné, 1758) were first noticed by Mauz 1867 (in Kalyakin and Zasukhin 1975). Brumpt (1913) described the species *Sarcocystis cuniculi* having muscle zoites $13-16 \times 5-6 \mu\text{m}$. A related species, *Sarcocystis leporum* was reported by Crawley (1914) from the Eastern cottontail rabbit *Sylvilagus floridanus*. Although both species were synonymized by some authors, new evidence shows that both species are independent (Fayer and Kradel 1977).

The developmental cycle of *S. cuniculi* was first described by Tadros and Laarman (1977) who successfully infected cats with cystozoites (muscle zoites) from wild *Oryctolagus cuniculus*.

Our aim was to obtain an idea on the occurrence of rabbit sarcosporidiosis in this country, to confirm the species identity of the parasite and to study its life cycle, employing experimental transmission. Further, the possibility of using the indirect immunofluorescent antibody test (IFAT) for the serological diagnosis of rabbit sarcosporidiosis was tested.

MATERIAL AND METHODS

Part of the examined sera were obtained from an experimental rabbit farm of the Agriculture University, Faculty of Agronomy, Prague-Suchbát. Other sera were obtained from rabbits grown by amateur breeders in two localities: one near Prague, one in SW Bohemia.

The sera were examined by IFAT using *S. dispersa* cystozoites as antigen. The use of a heterologous antigen was based on previous findings that members of the genus *Sarcocystis* share common antigen(s) demonstrable by IFAT (Černá and Kolářová 1978). Experimentally infected animals (see below) were serologically examined by the same method.

In some rabbits direct examination for the presence of *Sarcocystis* was performed by microscopic examination of muscle tissue. Pieces of oesophagus and diaphragm were homogenized in physiological saline for one minute, filtered through three layers of gauze and centrifuged. Cystozoites liberated from muscle cysts were found in the sediment.

Oesophagi of experimentally infected rabbits were examined histologically using Harris and Mayer hematoxyline staining for the demonstration of the cyst wall structure.

For experimental infection the meat of a spontaneously infected (serologically and microscopically positive) rabbit was fed to kittens. The kittens were infection-free; they were held before the

experiment in the household of one of us (M. L.) and after weaning they were fed with boiled chicken meat.

Sporocysts of the parasite were isolated from the gut contents of the kittens. The sporocysts were administered perorally to five two-month-old rabbits ($K_I - K_V$) which were serologically negative before the experiment. The infection doses used were as follows: $K_I - 1 \times 10^6$ sporocysts, $K_{II} - 5 \times 10^5$ sporocysts, $K_{III} - K_V - 1 \times 10^5$ sporocysts. Blood samples were taken from the infected rabbits periodically. The animals were slaughtered at different intervals and their muscles were examined for the presence of cystozoites. In rabbit K_I (slaughtered 14 days p.i.) internal organs were examined microscopically.

RESULTS

1. Serological examination of a normal rabbit population

A total of 117 domestic rabbits were serologically examined by IFAT. Out of 92 sera of rabbits grown at a farm (Group I), 28 sera (= 29 %) were positive, mostly in the serum dilution 1 : 10. Sera of some animals reacted in the dilution up to 1 : 40 (see Fig. 1A). Out of 25 sera of rabbits grown by amateur breeders (Group II), about half

of the sera showed the presence of *Sarcocystis* antibodies. Highest titres recorded in this group were between 80 and 160 (see Fig. 1B).

2. The dynamics of antibodies in experimental infection

The dynamics of the antibody response in rabbits fed *Sarcocystis* sporocysts is shown in Fig. 2. First antibody response was observed 20 days p.i. when the rabbit K_{II} was positive in low serum dilution 1 : 10. Later the antibody level raised to titre 40 reaching this peak 50 days p.i. The peak was maintained for about 25 days after which a gradual decline in the antibody level occurred. 85–100 days p.i. all experimental animals were serologically negative (see Fig. 2.). However, the animals still harboured cystic stages of *Sarcocystis* as demonstrated by the presence of cystozoites in muscle tissue homogenates and in histological preparations from oesophagus and diaphragm. The cystozoites were fully viable and infective for kittens.

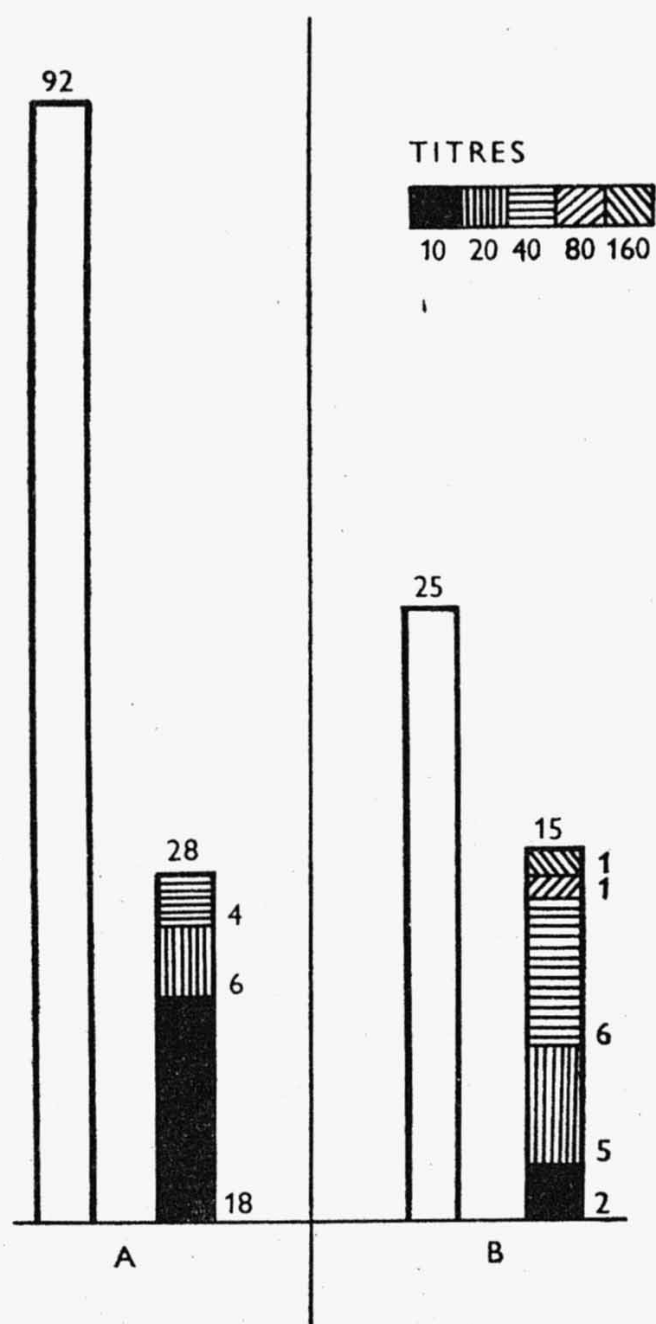


Fig. 1. Antibodies to *Sarcocystis* in two normal domestic rabbit populations as revealed by IFAT. A. Animals from an experimental farm. B. Animals bred by amateur breeders. The height of the left column indicates the total number of examined animals. The right column indicates the number of serologically positive animals and the distribution of their titres.

3. Experimental transmission of *Sarcosporidia* from rabbits to kittens

Infection in cats. Four kittens were fed the meat of a rabbit which was serologically positive in the IFAT in the serum dilution 1 : 40 and in muscles of which cystozoites were found on microscopic examination. The faeces of the kittens were examined coprologically starting on day 2 after infection. One week after infection, all kittens showed symptoms of indigestion. First sporulated oocysts and sporocysts (Fig. 4) were found 2 weeks after the infection. On day 18 p.i. one of the kittens died. Both non-sporulated and sporulated oocysts were found in duodenal and jejunal scrapings of this animal (Fig. 3). The oocysts measured $12.9\text{--}14 \times 18.5\text{--}19.6 \mu\text{m}$, the sporocysts were $9.2\text{--}10.4 \times 13.0\text{--}14.4 \mu\text{m}$.

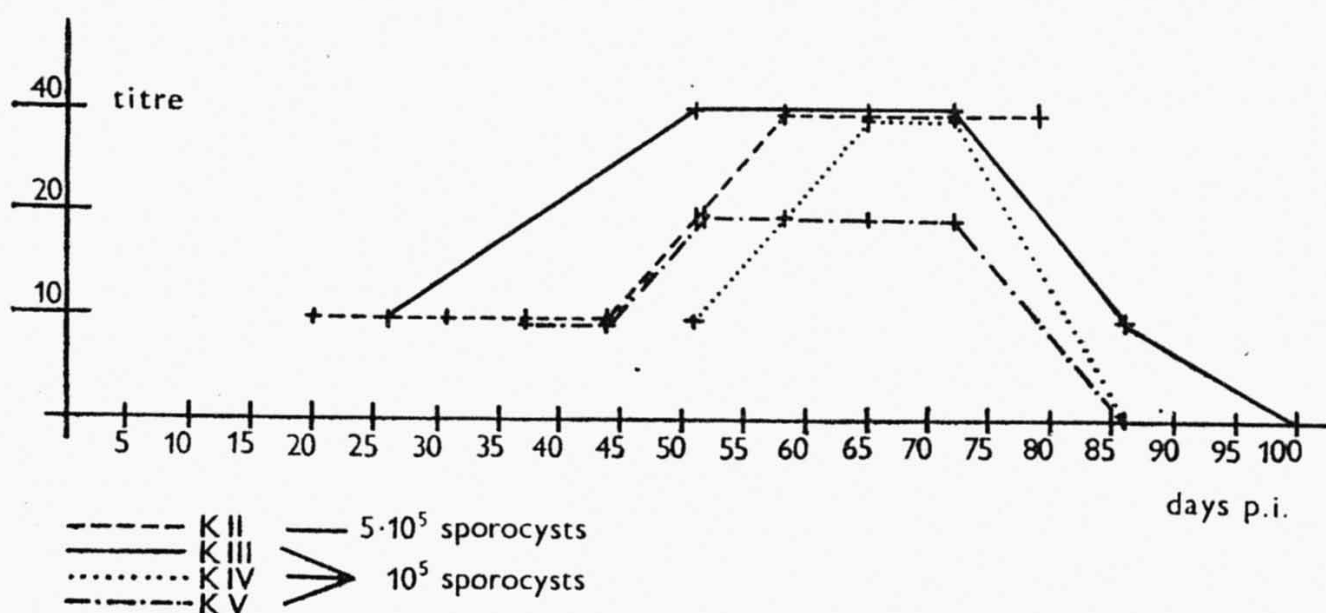


Fig. 2. The dynamics of antibodies in experimentally infected rabbits K_{II} — K_V.

Infection in rabbits. The sporocysts isolated from experimentally infected kittens were used for the infection of rabbits K_I—K_V as described in Material and Methods. K_I, infected with the highest dose (1×10^6), was killed 14 days p.i. However, no extramuscular stages were found on smears made from its organs. In other animals, killed at a later period (K_{II}-79 days p.i.; K_{III}-128 days p.i.; K_{IV}-131 days p.i.; K_V-300 days p.i.) cystic stages of *Sarcocystis* were found.

Table 1. Comparison of *Sarcocystis* from Czechoslovakia with *S. cuniculi* Brumpt, 1913 as studied by Tadros and Laarman (1977, 1978)

	Tadros and Laarman	Our material
Intermediate host	<i>Oryctolagus cuniculus</i>	<i>Oryctolagus cuniculus</i>
Definitive host	<i>Felis catus</i>	<i>Felis catus</i>
Cysts	macroscopic	macroscopic
Cyst wall	with finger-like protrusions	with finger-like protrusions
Length of protrusions (in μm)	11	8—10
Cystozoites (size in μm)	$10.8\text{--}12 \times ?$	$13\text{--}15 \times 3\text{--}4$
Oocysts (size in μm)	?	$18.5\text{--}19.6 \times 12.9\text{--}14.0$
Sporocysts (size in μm)	12.6×9.6	$13.0\text{--}14.4 \times 9.2\text{--}10.4$

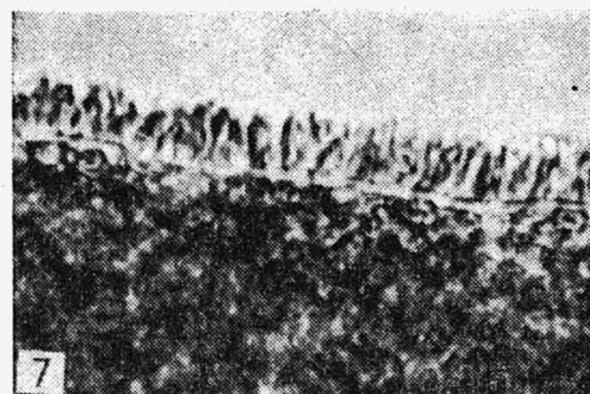
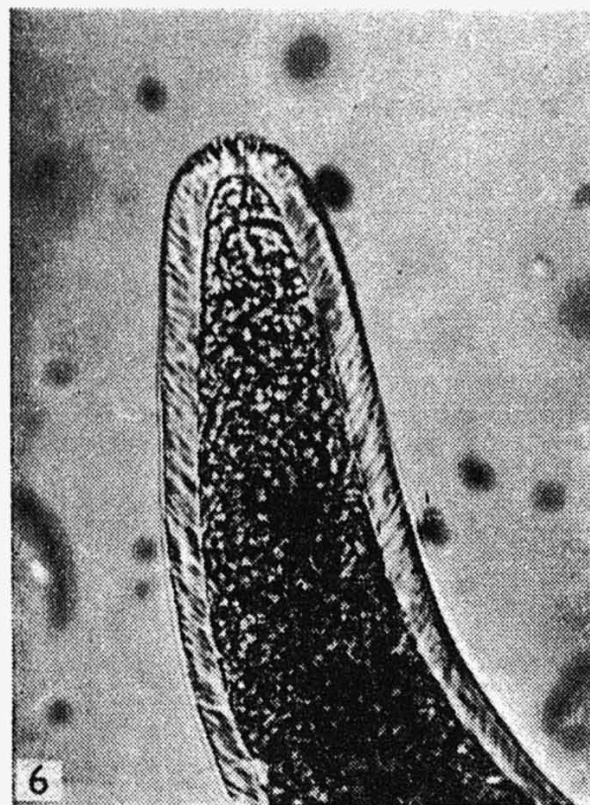
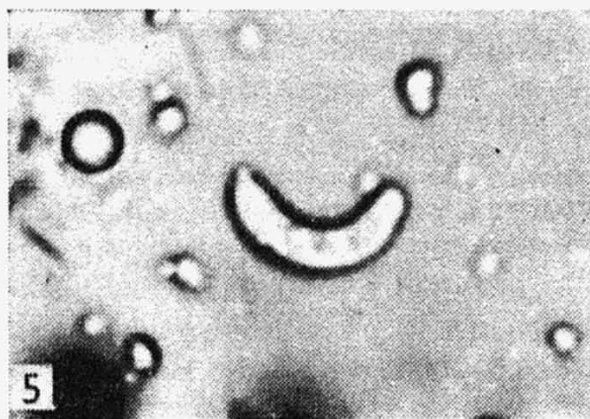


Fig. 3. Non-sporulated (single arrow), sporulating (double arrow) and fully sporulated oocysts of *S. cuniculi* in a jejunal scraping from an experimentally infected cat. ($\times 1000$). Fig. 4. Fully sporulated oocysts in the form in which they are excreted in cat's faeces. The oocysts wall is nearly invisible, while the wall of the sporocysts is clearly shown. ($\times 1500$). Fig. 5. Cystozoite of *S. cuniculi* in a rabbit muscle tissue homogenate. Unstained. ($\times 1250$). Fig. 6. Low magnification view of a portion of the *S. cuniculi* muscle cyst. ($\times 500$). Fig. 7. Detail of the cyst wall showing the finger-like protrusions forming the outer layer of the wall. ($\times 700$).

Cystic formations. In rabbits K_{II}—K_{IV} cystic stages were found mostly in the muscles of oesophagus and diaphragm. The homogenates of skeletal muscles contained only a few cystozoites (Fig. 5). On Giemsa stained smears the cystozoites measured 13—15 × 3—4 µm. The cystic stages recovered 130 days p.i. had a cyst wall displaying a conspicuous radial striation. This striation was caused by the presence of a compact layer of 8—10 µm long finger-like protrusions in the outer layer of the cyst wall. In older, macroscopically visible, cystic stages the protrusions were less compact (Figs. 6, 7).

Table 1 compares our data on *Sarcocystis* from rabbits with those obtained by Tadros and Laarman (1977, 1978).

DISCUSSION AND CONCLUSIONS

Experimental transmission of Sarcosporidia by cystozoites from a spontaneously infected rabbit to cat and the reinfection of rabbits by sporocysts excreted by cat confirms that cat is the definitive host of rabbit *Sarcocystis*. As it can be concluded from a comparative Table 1, the species which we have found in domestic rabbits in this country is conspecific with *Sarcocystis cuniculi* described by Brumpt (1913) and later studied in Holland by Tadros and Laarman (1977, 1978).

Our results confirm that IFAT is a suitable method for the detection of *Sarcocystis* antibodies in the intermediate host, the rabbit. However, the character of the antibody response in experimental infected rabbits shows the dependency of this response on both the infective dose and the duration of infection. Single infective dose of 1×10^5 sporocysts provokes a low production of antibodies detectable by IFAT (titres 20—40).

Table 2. Sporocysts of different species of *Sarcocystis* having cat as definitive host. (According to the data summarized by Černá 1981)

Species	Intermediate host	Sporocysts (size in µm)
<i>S. cuniculi</i> Brumpt, 1913	<i>Oryctolagus cuniculus</i>	13—14 × 9—10
<i>S. cymruensis</i> Ashford, 1978	<i>Rattus norvegicus</i>	11 × 8
<i>S. fusiformis</i> (Railliet, 1897) Bernard et Bauche, 1912	<i>Bubalus bubalis</i>	13 × 8
<i>S. hirsuta</i> Moulé, 1888	<i>Bos taurus</i>	11—14 × 7—9
<i>S. leporum</i> Crawley, 1914	<i>Sylvilagus floridanus</i>	13—17 × 9—11
<i>S. muris</i> (Blanchard, 1885) Labbé, 1899	<i>Mus musculus</i>	8—12 × 7—9
<i>S. porcifelis?</i> Dubey, 1976	<i>Sus scrofa</i>	13—14 × 7—8
<i>S. tenella</i> (Railliet, 1886) Moulé, 1886	<i>Ovis aries</i>	11—14 × 8—9
<i>Sarcocystis</i> sp. Janitschke, Protz et Werner, 1976	<i>Gazella granti</i>	11—15 × 8—12

Because such low antibody levels are reached in experimental infections, the relatively low titres (positivity in serum dilution 1 : 10) found in some examined animals should be considered as indicative of sarcosporidiosis.

The duration of infection is the factor determining whether the antibodies *Sarcocystis* can be detected by IFAT. As the experiments show, in infected rabbits, still harbouring viable parasites in their muscles, antibodies decline to non-detectable level less than 100 days p.i. Thus, only relatively recent infections can be detected by IFAT. Yet, a considerable number (36 %) of serologically positive rabbits occur among animals kept in a usual way. Also the titres reached in some animals from amateur breeders' colonies are higher (80—160) than those reached in experimental infections. From this we can conclude that *Sarcocystis* is a rather frequent parasite of rabbits. In spontaneous infections the parasite is evidently acquired both repeatedly and in higher doses.

As far as the practical diagnosis is concerned, IFAT can be used in living rabbits with the limitations discussed above. In post mortem diagnosis the examination of muscle tissue homogenates, especially of those of oesophagus and diaphragm, is recommended. In the definitive host, the cat, the sporocyst morphology is of no diagnostic value as other *Sarcocystis*, namely *S. hirsuta*, *S. leporum*, *S. tenella* and *Sarcocystis* sp. from *Gazella granti*, have all the same size and structure (see Table 2). Thus the identification experiment is the only means of species identification in sporocysts excreted by cats.

СПОНТАННАЯ И ЭКСПЕРИМЕНТАЛЬНАЯ ЗАРАЖЕННОСТЬ ДОМАШНИХ КРОЛИКОВ ВИДОМ *SARCOCYSTIS CUNICULI* BRUMPT, 1913

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Резюме. На зараженность саркоцистами исследовано с помощью непрямой реакции иммунофлуоресценции антител (НРФА) 117 домашних кроликов. Антитела к саркоцистам выявлены у 43 животных (36 %). При помощи экспериментальной передачи паразита от кролика на кошку и обратно на кролика доказано, что паразит *Sarcocystis cuniculi* Brumpt, 1913. Уровень антител, определенный с помощью НРФА, был низок и антитела исчезли в течение менее чем 100 дней после заражения, тогда как паразиты в мышечной ткани хозяина остались жизнеспособными. Обсуждается диагноз кроличьего саркоспориоза в дефинитивном и промежуточном хозяевах.

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