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Infectivity of Sarcocystis from Donkey for Horse Via Sporocysts from Dogs

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Abstract. The dog is the final host for sarcosporidia cysts from the oesophagus and diaphragm of donkeys from Sardinia. The prepatent period lasted 9 to 10 days. Sporocysts measured 12.2–13.8 × 9.2–9.9 µm. Infection of a horse with 10^5 donkey/dog sporocysts increased the rectal temperature to more than 40° C on days 10 and 20 after infection. On day 138 p.i. predominantly immature cysts containing metrocytes were found, especially in the oesophagus. Infection on day 117 p.i. with 2×10^5 horse/dog sporocysts did not give rise to a temperature increase during the following 21 days.

The final host of sarcosporidia cysts from the oesophagus and diaphragm of horses from Sardinia is the dog. The prepatent period lasted 9–10 days. Sporocysts measured 12.2–13.8 × 9.2–9.9 µm. The rise in the rectal temperature of three foals infected with horse/dog sporocysts did not differ from that of the foal infected with donkey/dog sporocysts. In both cases rectal temperature increased to more than 40° C on days 10 and 20 following infection with 10^5 sporocysts. Because of the occurrence of two temperature peaks following infection, two generations of schizogony are postulated. The presence of a sarcosporidia species occurring in the donkey only is doubtful.

Introduction

Sarcosporidia cysts in horses were mentioned by Gerlach (cited by Bertram 1892) as early as 1866. Siedamgrotzky (1872) described cysts of 3–4 mm, in the oesophagus up to 12 mm long and 0.3 mm broad, with cilia of the cyst membrane 2 µm in length. Tubes 9–10 mm long with a cyst wall of rod-like structure from the musculature of the horse were named by Doflein (1901) as *Sarcocystis bertrami.*

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The discovery that the life cycle of sarcosporidia requires a host change necessitated study of the developmental biology of sarcosporidia in horses (Fayer 1970, 1972; Rommel et al. 1972; Heydorn and Rommel 1972a, b; Rommel and Heydorn 1972). Rommel and Geisel (1975) were the first to reveal the dog as the final host of a sarcosporidia species occurring in the horse. For the species described they proposed the name *Sarcocystis equicanis*. Two years later Dubey et al. (1977) described *S. fayeri* as a further species of sarcosporidia of the horse transmitted by the dog. Neither of the authors undertook experimental retransmission. Erber and Geisel (1981) reinfected two ponies with sporocysts, obtained from puppies fed horse musculature containing sarcocysts. In one of the animals, the authors found cysts of *S. equicanis* and *S. fayeri* 167 days after infection. After feeding this musculature to two dogs the prepatent period was 11 and 17 days. They did not find any cysts in the second pony and even feeding musculature to a dog did not lead to excretion of sporocysts.

In this paper the first experimental results are described on infectiousness of the donkey sarcosporidia transmitted by the dog to the horse and on the development of sarcosporidia of the horse transmitted by dog.

Materials and Methods

Test Animals

Three beagle puppies, two cats and four ponies were reared at the institute. Two raccoons were bought from a fur farm. The animals were kept conventionally: the ponies were fed with hay and usual fodder, the puppies, cats and raccoons were fed with canned food as well as water ad libitum.

Infectious Material

For the production of sporocysts, oesophagus and musculature from the diaphragm of naturally infected donkeys and horses from Sardinia served as original material. Pooled samples of musculature of about 10 donkeys and 20 horses of different ages were fed separately to each puppy, i.e., musculature of donkeys to one puppy and musculature of horses to another puppy on 2 days. Additionally for two consecutive days, one cat and one raccoon were fed with musculature of donkey and horse. Prior to the feeding experiments neither the dogs, the raccoons nor the cats had ever been fed with raw meat. The feces of the carnivores were tested daily up to 28 days with the $ZnCl_2$-NaCl-flotation method to determine the presence of sporocysts and oocysts.

Animals that excreted sporocysts after feeding them with infected muscles of donkey or horse were killed after a 12–14 h starvation period. The sporocysts were removed from the small intestine according to the method described by Heydorn et al. (1981) and were preserved at 4° C.

Infections in Horses

One foal was administered about 100,000 sporocysts from a puppy fed on musculature of a donkey infected naturally with sarcocysts. On days 44 and 59 after infection, biopsies of the thigh muscle were carried out. The same foal received about 200,000 sporocysts on day 117 after infection from a puppy nourished on musculature of a horse infected with sarcosporidia. The foal was killed on day 138 after infection.

The rest of the foals were infected only with sporocysts that originated from the puppies fed on horse musculature containing sarcocysts. The second foal was infected with 100,000 spor-

ocysts and killed on day 197 after infection. Biopsies were carried out on days 50 and 89 after infection. Musculature of this animal was fed to a puppy. The third foal was infected with 10,000 sporocysts and killed on day 212 after infection. The fourth foal, killed on day 21 after infection, was infected orally with 200,000 sporocysts.

In all the experimentally infected horses, the temperature was measured twice daily – morning and evening – before, during and at least 21 days after infection.

Histology

Muscle specimens naturally infected or muscle specimens of horses infected experimentally with sarcosporidia were minced in a mixer by addition of physiological NaCl solution and examined microscopically using a Zeiss photomicroscope.

Results

Experimental Infection of the Final Hosts

All puppies that had been fed on musculature containing sarcocysts of donkeys or horses infected naturally or experimentally excreted sporulated sporocysts after a prepatent period of 9–10 days. The dogs, which were killed to isolate the sporocysts, revealed almost regularly distributed infection especially in the apical villous region of the small intestine. The sporocysts were about 12.2–13.8 × 9.2–9.9 µm (Figs. 1–4). However, no sporocysts were found in the cats or raccoons.

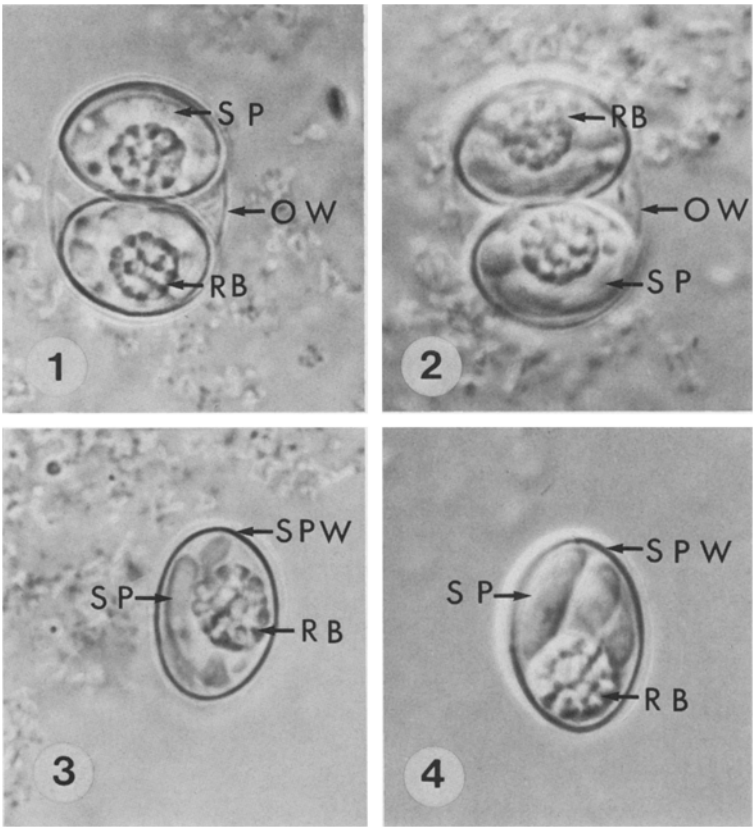
Experimental Infection of the Intermediate Hosts with Donkey/Dog Sporocysts

Foal 1 was successfully infected with sporocysts from the intestine of a dog that had been fed on musculature of donkeys infected with sarcocysts. The temperature of this animal increased from 38.4° C up to 40° C on days 10 and 11 and up to 40.4° C on days 19, 20 and 21 after infection. It was possible to prove the presence of sarcocysts post-mortem on day 138 after infection. The sarcocysts, which were only microscopically detectable, still contained almost exclusively metrocytes. No sarcocysts were found in biopsies of the thigh muscles carried out on days 44 and 59 after infection.

Experimental Infection of the Intermediate Hosts with Horse/Dog Sporocysts

Foals 2, 3 and 4 were infected with sporocysts from the small intestine of a dog that had been fed on musculature of horses infected with sarcocysts. There was only a slight rise in temperature on days 10 and 11 in foals 2 and 4, e.g. in foal 2 an increase from 37.8° C to 38.8° C, but up to 41.2° C on days 19, 20 and 21 after infection. There was no obvious change in the temperature of foal 3, which had been infected with only 10,000 sporocysts.

Sarcocysts were found in the musculature of skeleton and oesophagus of foals 2 and 3 on days 197 and 212 after infection respectively. The sarco-



Figs. 1–4. *Sarcocystis sp.* Sporulated oocysts **1** and **2** and sporocysts **3** and **4** from the small intestine of a dog eleven days after feeding donkey muscles (unstained). Abbreviations: *OW* oocyst wall; *RB* granular residual body; *SP* sporozoite; *SPW* sporocyst wall Illumination **2** and **4** Differential-interference-contrast (DIC) ×1,600

cysts mainly contained mature cystozoites. Sarcocysts were not found in biopsy material of the second foal on days 50 and 89 after infection. The feeding of oesophagus to the dog again caused an excretion of sporocysts after 9 days.

Foal 4 was killed on day 21 after infection after its temperature rose to 40.5° C, but no schizonts or sarcocysts were found.

Discussion

The demonstration of the infectiousness of sarcosporidia of the donkey which is transmitted by dogs to horses makes the existence of a sarcosporidia species only for the donkey improbable. The size of the sporocysts as well as the absence of fever on days 10 and 20 after infection with horse/dog sporocysts in foal 1 showed that the species in donkey and horse may be identical. In accordance with Levine and Tadros (1980), the occurrence of the species *S. asinus* described by Gadaev (1978) is doubtful.

According to the results of Rommel and Geisel (1975), Dubey et al. (1977) and Erber and Geisel (1981), only the dogs excreted sporocysts after feeding on donkey and horse musculature containing sarcocysts. These authors give different data on the duration of prepatent period as well as on the sizes of sporocysts. Rommel and Geisel (1975) established a prepatent period of 7 days for the species *S. equicanis*. Dubey et al. (1977) determined a prepatent period for *S. fayeri* of 11–14 days, while Erber and Geisel (1981) found a prepatent period of 11–17 days. By infecting two foals, they found cysts of *S. equicanis* and *S. fayeri* in a pony on day 167 after infection.

The prepatent period in the three dogs, described in this paper, was 9–10 days. All the animals were killed to obtain sporocysts. No immature sporocysts were found in the small intestine of the animals. The sporocysts were measured in fresh smears and in water. The comparatively uniform size of the sporocysts (12.2–13.8 × 9.2–9.9 µm) partly deviates from the data of other authors. Rommel and Geisel (1975) gave 15–16.3 × 8.8–11.3 µm for *S. equicanis*, Dubey et al. (1977) 11–13 × 7–8.5 µm for *S. fayeri*, and Erber and Geisel (1981) 12–14.4 × 9.3–10.5 µm for a mixed population of both species. Erber and Geisel (1981) did not find any clinical symptoms in two foals that were infected with 100,000 sporocysts each. No clinical symptoms were observed in this investigation when the animal was infected with only 10,000 sporocysts. In the three foals infected with 100,000 and more sporocysts, there was an obvious increase in the temperature with peaks on about days 10 and 20 after infection. The animals were in poor general health, had anorexia and behaved apathetically especially during the second fever attack, regardless of whether the sporocysts originated from a donkey/dog or horse/dog feeding.

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