Differentiating between *Besnoitia besnoiti* from cattle and *Sarcocystis hoarensis* from rodents

**Abstract**
To provide a biological basis for studies designed to establish the mode of transmission of the veterinary pathogen *Besnoitia besnoiti*, we compared salient features of this pathogen in cattle with those of *Sarcocystis hoarensis* in rodents. The cysts and cystozoites of these organisms can readily be distinguished morphologically. In contrast to *S. hoarensis*, which is well adapted to rodents, *B. besnoiti* fails to mature in jirds or mice and generally is lethal in jirds. Serological reagents discriminately detect these pathogens. *B. besnoiti*, therefore, can unambiguously be differentiated from *S. hoarensis* either by morphological or serological methods or on the basis of experimental comparisons of virulence in laboratory rodents.

**Introduction**
Although bovine besnoitiosis, due to *Besnoitia besnoiti*, burdens cattle-raising economies in Africa and the Middle East (Pols 1960; Bigalke 1981), the mode of transmission of this sporozoan pathogen of connective tissue remains unknown. Its host range also includes impala, kudu, and wildebeest (Hofmeyr 1945; Basson et al. 1965). Although cats are said to shed oocysts after feeding on tissues infected with *B. besnoiti* cysts (Peteshev et al. 1974), attempts to confirm this observation have failed (Rommel 1975; Uvaliev et al. 1979; Diesing et al. 1988). Other *Besnoitia* species that have been described (Fayer 1980) include *B. wallacei* and *B. jellisoni* of rodents (Frenkel 1953; Wallace and Frenkel 1975), *B. darlingi* of lizards and opossums (Schneider 1967), *B. benneti* of horses (Bennett 1939) and burros (Smith and Jones 1957), and *B. tarandi* of caribou and reindeer (Hadwen 1922; Choquette et al. 1967). None of these other *Besnoitia* affects agriculture, and none occurs in Africa or the Middle East.

The only other Ethiopian sporozoan that forms connective-tissue cysts is *Sarcocystis hoarensis*. Sporozoites, shed in the feces of the snake definitive host of this parasite, are ingested by the rodent intermediate host (Matuschka and Häfner 1984; Matuschka et al. 1987). This demonstration of a small mammal reservoir for cyst-forming connective-tissue sporozoan provides a model for efforts to define the mode of transmission of bovine besnoitiosis.

To provide a biological basis for identifying the cycle of bovine besnoitiosis in nature, we compared salient features of *B. besnoiti* in naturally infected cattle with those of *S. hoarensis* in rodents experimentally infected with snake-derived sporozoites. Transmission experiments served as a basis for comparing antigenicity and virulence in laboratory rodents. The structure of cystozoites was compared by light and electron microscopy.

**Materials and methods**

**Organisms**
Throughout the study, 4-month-old white mice and Tristram’s jirds (*Meriones tristrami shawii*) of both sexes, bred at the Kimron Veterinary Institute, were used. White mice were infected with *Sarcocystis hoarensis* sporocysts shed in the feces of an experimentally infected *Bitis* sp. (Matuschka and Häfner 1984). The opalescent cysts, grossly visible in the subcutaneous and connective tissues of the snout, ears, tail, legs, and footpads of mice, were injected into jirds and mice. *Besnoitia besnoiti* were obtained from a naturally infected bull. Cyst-containing connective, subcutaneous, and fascial tissues from cattle and mice were disrupted to release cystozoites as described elsewhere (Shkap et al. 1987). To prevent possible contamination by naturally occurring coccidians, the life cycle of *S. hoarensis* was maintained in a laboratory located in Germany, where such parasites do not appear to occur.

**Microscopy**
Giemsa-stained parasites were observed and measured at x1000 magnification using an ocular micrometer in which the smallest...
readable unit was 1.3 μm. For histology studies, samples of cyst-infected tissues were fixed in buffered neutral 10% formalin. Paraffin-embedded tissues were sectioned at 5 μm and stained with hematoxylin and eosin. Specimens of Besnoitia from cattle and mice were also prepared for transmission electron microscopy according to Perk et al. (1980) and examined with a Jeol JEM-101 electron microscope.

Infection of animals

In all, 12 mice and 12 jirds were divided into 4 groups, respectively. Each of six mice and six jirds was inoculated intraperitoneally with $2 \times 10^7$ cystozoites of B. besnoiti. Another six jirds received a similar inoculum of S. hoarensis. The peritoneal washings of two jirds and two mice that had been inoculated with the S. hoarensis isolate were examined individually for parasites after 6 days. Surviving experimental animals were observed for more than 8 months. Moribund animals were necropsied and their peritoneal washings were examined microscopically for parasites.

Serology

Mice and jirds in these experiments were bled from the retro-orbital sinus before and at 8 months after inoculation with parasites. Sera were tested for antibodies by indirect immunofluorescent assay (IFA) with homologous or heterologous antigen (Shkap et al. 1984). Serum from a mouse infected with snake sporozoites served as the positive control in the mouse system. Serum from a