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THE MORPHOLOGY AND PATHOLOGY OF *BESNOITIA* SP. IN REINDEER (*RANGIFER TARANDUS TARANDUS*)

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ABSTRACT: Four of five reindeer (*Rangifer tarandus tarandus*) obtained from a *Besnoitia* sp.-infected herd at the Assiniboine Park Zoo in Winnipeg, Manitoba, Canada, in October 1989, had evidence of mild dermatitis over the articular surfaces of carpal and tarsal joints. Cysts of *Besnoitia* sp., either surrounded by inflammatory reactions or without evident host response, were present within the dermis, submucosa of the nasal turbinates, periosteum, tendons, testes and hooves. The light microscopic and histochemical features of *Besnoitia* sp. from reindeer were indistinguishable from those of other *Besnoitia* spp. described in cattle, rodents and horses.The *Besnoitia* sp. cysts and organisms from reindeer were unique in that bradyzoite membrane micropores and cytoplasmic enigmatic bodies were not observed. Two cats were fed cysts of *Besnoitia* sp. but no oocysts were detected in feces for 90 days post-infection.

Key words: Reindeer, *Rangifer tarandus tarandus*, *Besnoitia*, protozoa, pathology, ultrastructure, life cycle, cat.

INTRODUCTION

Since the original description of besnoitiosis in cattle caused by *Besnoitia besnoiti* (Apicomplexa, Sarcocystidae) (Besnoit and Robin, 1912), infections due to protozoans of the same genus have been observed in horses and burros (*B. bennetti*; Terrell and Stookey, 1973), rodents (*B. jellisoni*; Senaud et al., 1974), goats and antelope (*B. besnoiti*; Pols, 1960), reindeer and caribou, *Rangifer tarandus* (*B. tarandi*; Wobeser, 1976; Rehbinder et al., 1981), and in mule deer, *Odocoileus hemionus* (*B. tarandi*; Glover et al., 1990). The taxonomy of this genus is poorly defined, and has been based largely on occurrence of characteristic bradyzoite-filled cysts in different species of intermediate host (Pols, 1960; Terrell and Stookey, 1973; Wobeser, 1976), and on the ultrastructural characteristics of bradyzoites (Van Heerden et al. 1993). In *Rangifer* sp., taxonomic convention has been to refer to the parasite as *B. tarandi*. In the absence of sound taxonomic criteria for species identification, however, it is preferable to refer to this organism only as *Besnoitia* sp.

The translocation of *Besnoitia*-infected reindeer from arctic to southern Canada has generated concern regarding the possible spread of besnoitiosis to cattle and local wild ungulates (Glover et al., 1990). Our objective was to provide a detailed anatomical and pathological description of besnoitiosis in *Rangifer* sp. Experimental infection of domestic cats as definitive hosts also was attempted.

MATERIALS AND METHODS

Five captive reindeer (*Rangifer tarandus tarandus*), of Eurasian source, a 7-yr-old male and four females aged 9, 8, 3 and 2 yr, respectively, were donated to the Western College of Veterinary Medicine, Saskatoon, Saskatchewan, Canada, by the Assiniboine Park Zoo, Winnipeg, Manitoba, Canada, and transported under permit in October 1989 to the Health of Animals Laboratory, Agriculture Canada, Saskatoon, Saskatchewan. The Canadian Council of Animal Care (CCAC) guidelines were followed in the care and maintenance of animals. Because of the occurrence of besnoitiosis at the zoo in 1983 (Glover et al., 1990), the two younger animals which were born at the zoo, had been separated from their mothers since birth. All five animals had been kept in fly-proof barns for the entire fly season (April to October) dur-

ing the two previous years. Small pale masses on the sclera, interpreted as cysts of *Besnoitia* sp., had been identified in all animals at some time during the past 2.5 yr. However, only the male and oldest female had such lesions at the time of donation.

Each animal was given 20 mg of xylazine (Rompun, Haver Lockhart, Etobicoke, Ontario, Canada) intramuscularly, allowed to become sedated and then was euthanized in October 1989 with 10 cc of sodium pentobarbital (Euthanyl Forte, Canada Packers, MTC Division, Cambridge, Ontario) administered intravenously. Following death, 500 cc of blood were collected immediately and the lower portions of the limbs were clipped, severed proximal to the carpus and tarsus, and surgically scrubbed with iodine soap and 70% ethanol. Surgical incisions were made in the skin of the lower limbs, and portions of fascia, free of muscle and containing visible cysts, were placed in sterile phosphate buffered saline (pH 7.4). A complete post-mortem examination then was performed on each animal. Portions of the organs evaluated were fixed in 10% neutral buffered formalin; eyes were placed in Bouin's fixative (Luna, 1968) for 24 hr and then were transferred to 70% ethanol. Fixed tissues including skin, bone, flexor tendon, nasal turbinate, eye, skeletal muscle, brain, testicle, uterus, lung, liver, intestine, rumen, abomasum, lymph node, spleen, heart, kidney, and hoof were embedded in paraffin, and 5 μm thick sections were cut, stained with hematoxylin and eosin (H&E), and examined. Other stains used on selected sections were Brown-Brenn (B&B), Alcian Blue (AB), Gomori's methenamine silver (GMS) (Luna, 1968); periodic acid-Schiff (PAS), Ziehl Neelsen (ZN) (Culling, 1963); and Masson's Trichrome (MT) (Scheehan and Hrapschak, 1980).

For transmission electron microscopy, 1 mm blocks of subcutaneous tissue containing individual cysts were placed in s-collidine buffered 5% gluteraldehyde (J.B.EM Services Inc., Pointe Claire, Quebec, Canada). Tissues were post-fixed in osmium tetroxide, dehydrated in alcohols and propylene oxide, and embedded in epoxy resin (Epon 812, Electron Microscopy Sciences, Fort Washington, Pennsylvania, USA). Ultrathin sections were stained with uranyl acetate and lead citrate and mounted on copper grids (Bennent and Luft, 1959). They were examined using a Philips electron microscope (Philips 410LS, Philips Electronics Inc., Scarborough, Ontario).

Two adult domestic male cats raised in isolation, were obtained from the Animal Resources Centre, University of Saskatchewan, Saskatoon, Canada, and were housed in separate cages following CCAC guidelines, with no direct contact with other animals. Upon receipt, a fecal flotation using a saturated sucrose solution (specific gravity = 1.28) was done on 1 g of feces (Dunn, 1978). Ten days after arrival, each cat was fed fresh muscle-free connective tissue containing between 25 to 100 *Besnoitia* sp. cysts obtained from two reindeer at necropsy. The cysts had been stored in PBS for 15 hr on ice between removal from reindeer and ingestion by the cats. The cats ate the cysts and there was no vomiting. All feces were collected from each cat daily for 30 days and every 3 days for an additional 60 days. A fecal flotation was done on one gram of feces from each collection.

At the end of the 90 day test-period, blood was collected from each cat, smears were made, and animals were killed in January 1990 with 3 cc of sodium pentobarbital (Euthanyl, Canada Packers) given intravenously. The intestine was immediately removed; the lumen was infused with 100 ml of Bouin's fixative, then immersed in Bouin's fixative for 24 hr and transferred to 70% ethanol. A complete post-mortem examination was performed on each animal and portions of brain, skin, lung, heart, small and large intestine, stomach, liver, skeletal muscle, spleen, lymph node, and kidney were fixed in 10% neutral buffered formalin. Samples of all fixed tissues were embedded in paraffin and 5 μm sections were stained with H&E and examined.

RESULTS

All reindeer were in excellent nutritional condition. Two animals had mild alopecia over the nasal area and four had mild thickening and encrustation of the skin over the carpus and tarsus. Low to moderate numbers of 0.5 to 1.0 mm firm translucent spherical nodules were present in the subcutaneous fascia, around and between tendons, and over the periosteal surfaces of limb bones in the three oldest animals. These were most numerous from the carpal and tarsal bones distally. Numerous very small depressions could be felt on periosteal surfaces in these same areas. No such nodules were seen anywhere in the two youngest animals.

Histologically, the nodules noted grossly were typical of the intracellular bradyzoite-filled cysts of the genus *Besnoitia* and were present in variable numbers in the skin and nasal turbinates in the three older animals only. Cysts also were present in periosteum, tendons, tendon sheaths, hoofs,

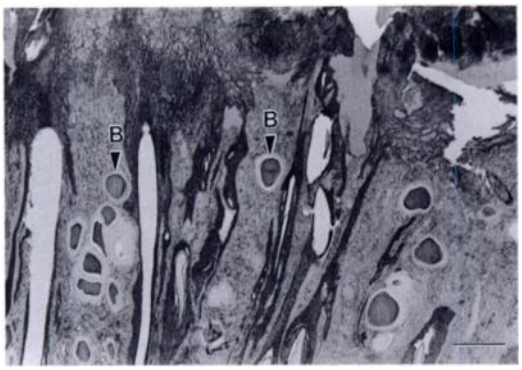

FIGURE 1. Section of lower limb skin of a reindeer with marked acanthosis, hyperkeratosis and numerous degenerated *Besnoitia* sp. cysts (B) within the dermis. Some cysts are surrounded by a granulomatous inflammatory reaction. H&E. Bar = 300 μm.

FIGURE 2. A single *Besnoitia* sp. cyst (BC) present within the corium of the hoof (HC) of a reindeer. Note close association of *Besnoitia* sp. cyst to blood vessel (BV) wall. H&E. Bar = 200 μm.

and testes in one or more of these animals. In all tissues examined, host reaction to the cysts varied from a total absence of inflammatory cells around smoothly contoured cysts to inflammation consisting of numerous neutrophils, lymphocytes, macrophages and multinucleated giant cells around irregularly shaped or dead and degenerate cysts.

In the skin, cysts were most numerous in the middle to deep dermis within alopecic areas of the nasal area and distal limb (Fig. 1). Those within the deep dermis commonly were present in clusters within the media of blood vessel walls and frequently were surrounded by large numbers of lymphocytes, macrophages and multinucleated giant cells. Partial obstruction of blood vessel lumina was evident in severely affected areas. Cysts were fewer in number and usually were free of inflammatory cells in the superficial dermis up to the dermal-epidermal junction. The overlying epidermis had varying degrees of orthokeratotic hyperkeratosis, parakeratotic hyperkeratosis, acanthosis and pseudoepitheliomatous hyperplasia.

The mucosa of the nasal turbinates contained cysts that frequently were within walls of blood vessels of the lamina propria. Numerous cysts were surrounded by a nonsuppurative, often granulomatous, reaction. In all sections examined, nasal epithelium was normal.

Besnoitia sp. cysts surrounded by lymphocytic, plasmacytic, or granulomatous inflammation commonly were present within periosteum, tendon, and tendon sheaths. Cysts occupied superficial pits of their own dimensions within the cortex of limb bones, especially metacarpus, carpus, metatarsus, and tarsus. Cysts surrounded by granulomatous inflammation also were present within the corium of the hoof (Fig. 2) and, in one animal, within the marrow cavity of a third phalanx.

In the male reindeer, *Besnoitia* sp. cysts were present within the testicular tunics (Fig. 3) as well as within the pampiniform plexus. Cysts at these sites were attached to the intima of blood vessels with a resulting decrease in luminal diameter. A marked granulomatous reaction to some cysts was present in these locations. No cysts were noted within the testicular parenchyma. Low numbers of mature sperm were present within the epididymis. Several small cerebral granulomas were present in the male, but no causative organisms could be demonstrated in serial sections of these lesions.

The remainder of the tissues examined were normal and no cysts were seen in any of the visceral organs.

By light microscopy, cysts had four distinct layers when viewed in cross section:

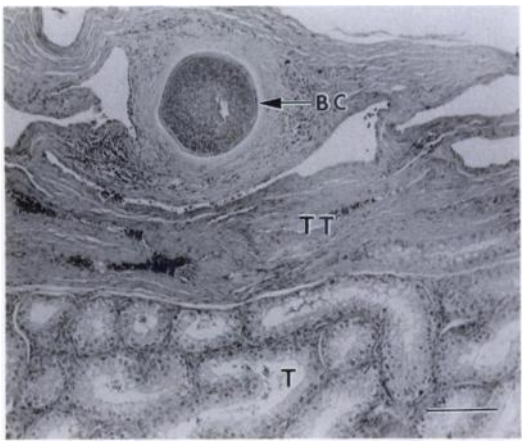

FIGURE 3. Section of testicle (T) of a reindeer with a single, possibly viable, *Besnoitia* sp. cyst (BC) within a blood vessel wall in the testicular tunics (TT). H&E. Bar = 200 μm.

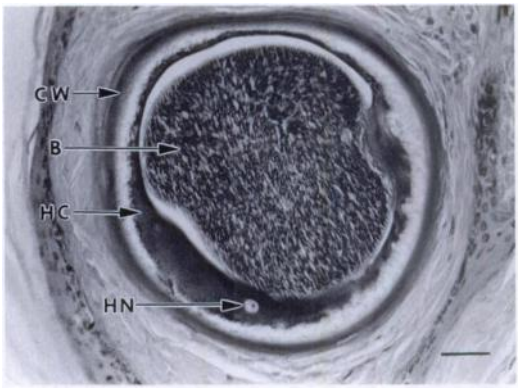

FIGURE 4. Detail of a *Besnoitia* sp. cyst in the dermis of a reindeer. CW, capsule; B, parasitophorous vacuole containing bradyzoites; HC, host cell cytoplasm; HN, host cell nucleus. Periodic acid-Schiff. Bar = 50 μm.

an external capsule, the host cell cytoplasm and nucleus, a membrane surrounding the parasitophorous vacuole, and the parasitophorous vacuole and its contents (Fig. 4). Cysts were approximately 200 to 400 μm in diameter and were circular in cross-section. The cyst capsule always was thick but was not uniform in thickness; it stained pale red with H&E, blue with MT and AB, and red with PAS. The capsule surrounded a host cell which, in turn, contained one or more flattened nuclei. An intracytoplasmic parasitophorous vacuole, usually circular, contained numerous fusiform bradyzoites and was enclosed by a thin vacuolar membrane. This membrane, which separated the vacuole from the host cell cytoplasm, stained pale blue with MT. Small intracellular granules that were black with GMS and red with PAS were present within the bradyzoites (Table 1).

Ultrastructurally, the external capsule contained a dense outer layer of circumferentially-arranged collagen fibers and granular, electron-dense material. The inner portion adjacent to the host cell plasma membrane was composed of loosely arranged fibrillar material. The infected host cell had a thin (13 to 20 μm) layer of cytoplasm in direct contact with the capsule. The plasma membrane had numerous folds which formed interdigitations with the inner portion of the capsule (Fig. 5).

The vacuolar membrane was 1 to 2 μm thick and was composed of an external thin

TABLE 1. Histochemical properties of *Besnoitia* sp. cysts and bradyzoites from reindeer, Assiniboine Park Zoo, Winnipeg, Manitoba, Canada, 1989.

Stain	Outer capsule	Inner capsule	Host cell cytoplasm	Vacuole membrane	Bradyzoites
Periodic acid-Schiff	+*	+++	+	+/−	++
Ziehl-Neelsen	−	−	−	−	−
Masson's trichrome	+++	+	−	++	−
Gomori's methenamine silver	+	+	+	−	++
Brown-Brenn	−	−	−	−	−
Alcian blue	+	+++	−	−	−

* Intensities of positive stain reaction: − = negative; + = weakly positive; ++ = moderately positive; +++ = strongly positive.

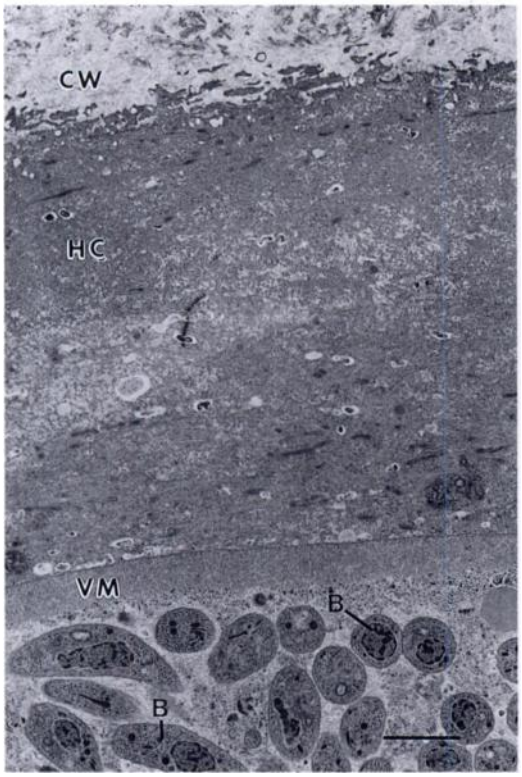

FIGURE 5. Transmission electron photomicrograph of a portion of a *Besnoitia* sp. cyst from a reindeer. CW, capsule; HC, host cell cytoplasm; VM, vacuole membrane; B, *Besnoitia* sp. bradyzoites. Uranyl acetate and lead citrate. Bar = 3 μm.

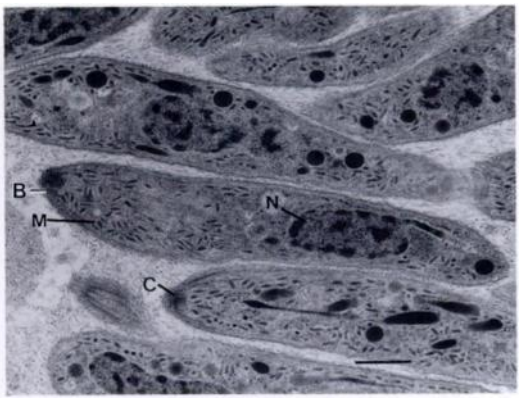

FIGURE 6. Transmission electron photomicrograph of the parasitophorous vacuolar contents of a *Besnoitia* sp. cyst from the skin of a reindeer. Numerous crescentic or piriform bradyzoites (B) are present within the vacuole. C, conoid; M, microneme; N, bradyzoite nucleus. Uranyl acetate and lead citrate. Bar = 1 μm.

rim of electron dense material and a highly folded internal portion of moderate electron density. The parasitophorous vacuole contained numerous organisms, an electron lucent background, short pieces of membranous material, and small electron-dense particles (Fig. 6). Bradyzoites measuring from 8.0 to 10 μm × 2.0 to 2.6 μm were piriform or crescentic, and had an outer double membrane (Figs. 6 and 7). The inner membrane appeared to terminate in a polar ring at the anterior end of the bradyzoite (Fig. 7). A cone-shaped structure (conoid), was within the polar ring. Surrounded by the conoid were two to four rod-shaped, moderately electron-dense rhoptries interspersed with short, moderately electron dense structures interpreted to be micronemes (Fig. 7). There were 22 anterior subpellicular microtubules which extended posteriorly along the inner membrane of the zoite (Fig. 8). At least 30 micronemes were present throughout the cytoplasm (Figs. 6 and 7). Other structures within the bradyzoite cytoplasm included free ribosomes, rough

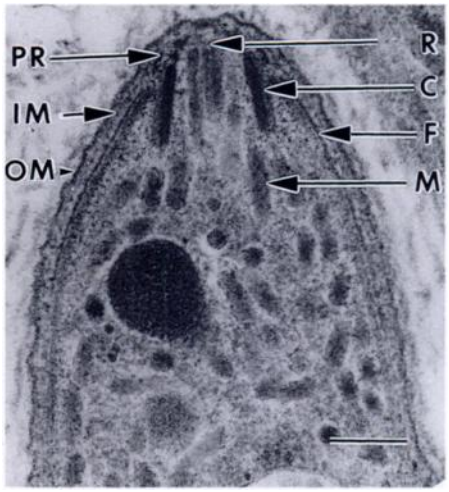

FIGURE 7. Transmission electron photomicrograph of the anterior end of a *Besnoitia* sp. bradyzoite from a reindeer. OM, outer membrane; IM, inner membrane; PR, polar ring; C, conoid; F, subpellicular microtubule; R, rhoptrie; M, microneme. Uranyl acetate and lead citrate. Bar = 0.2 μm.

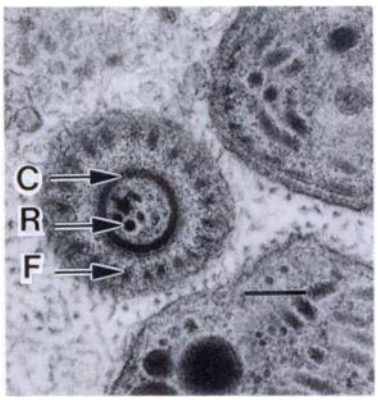

FIGURE 8. Transmission electron photomicrograph of a cross section through the anterior end of a *Besnoitia* sp. bradyzoite from a reindeer. F, subpellicular microtubule; C, conoid; R, rhoptrie. Uranyl acetate and lead citrate. Bar = 0.3 μm.

endoplasmic reticulum, and a mitochondrion with microvillar cristae. The nucleus was located in the mid- to caudal portion of the zoite (Fig. 6).

No oocysts were found in the cats' feces obtained prior to experimental infection with *Besnoitia* sp. organisms. No oocysts were detected in feces from either cat during the 90 days post-infection. No protozoan parasites were detected in blood, intestine or other tissues examined histologically from either cat.

DISCUSSION

Lesions in the *Besnoitia* sp.-infected reindeer were similar to those previously described in *Rangifer* sp. (Wobeser, 1976; Glover et al., 1990) as well as in cattle (Pols, 1960). Clinical disease was limited to focal, mild thickening of the skin with some encrustation and hair loss. These areas corresponded to dense accumulations of *Besnoitia* sp. cysts with surrounding inflammatory responses. This is in agreement with some descriptions of besnoitiosis in *Rangifer* sp. (Lewis, 1989); however, severe clinical disease also has been described in this species and in mule deer (Glover et al., 1990). The factors that determine whether infection with *Besnoitia* sp. will cause disease remain unknown (Lewis, 1992).

Besnoitiosis in male cattle has been reported to cause sterility by initiating severe scrotal dermatitis and orchitis, and by reducing the blood supply to the testicles due to formation of cysts in the lumina of arteries (Kumi-Diaka et al., 1981). Since cysts were present in the vessel walls of the pampiniform plexus and tunics in the male reindeer in this study, it is conceivable that sterility in male reindeer also could result from infection with *Besnoitia* sp.

The light-microscopic and histochemical properties of *Besnoitia* sp. cysts from the reindeer were similar to those of *B. besnoiti* (Pols, 1960) and *B. bennetti* (Terrell and Stookey, 1973). Other genera within the Sarcocystidae that form large intracellular cysts containing bradyzoites, including *Sarcocystis* spp., *Toxoplasma gondii*, and *Frenkelia* spp., lack the thick connective tissue capsule present around *Besnoitia* spp. cysts (Gardiner et al., 1988). The ultrastructural features of *Besnoitia* sp. cysts and bradyzoites from reindeer are similar to those of other species in the genus (Scholtyseck et al., 1974). However, ultrastructural differences between *Besnoitia* spp. bradyzoites have been described. Single-membrane micropores consistently are observed in bradyzoites of *B. jellisoni* and *B. bennetti* (Van Heerden et al., 1993). Membrane-bound electron-dense intracytoplasmic structures called enigmatic bodies are reliable characteristics of *B. jellisoni*, but are not a feature of *B. bennetti* (Van Heerden et al., 1993). Neither of these structures were observed in bradyzoites of *Besnoitia* sp. of reindeer. The specific type of host cell infected with bradyzoites could not be determined. Based on the presence of an external collagenous capsule and the location of cysts in fibrous connective tissue, we believe that the host cells were fibroblasts.

A two-host life cycle, with sexual stages and oocyst development in the domestic cat and zoite-filled cysts in tissues of intermediate hosts, has been described for a

Besnoitia sp. (Wallace and Frenkel, 1975), *B. wallacei* (Frenkel, 1977), and *B. darlingi* (Smith and Frenkel, 1977). Possible explanations for our negative parasitological findings in the two cats used in our study include that the cats were fed too few organisms or dead organisms because most of the cysts had degenerated, the cats were immune or too old, intermittent shedding occurred, oocysts were present in portions of feces not tested, infection occurred but was not detected, or that domestic cats are not suitable definitive hosts for this *Besnoitia* sp. Diesing et al. (1988) fed cats and many other species of carnivore cysts of *B. besnoiti*, but were unable to demonstrate oocysts in feces. Similar results were obtained by Glover et al. (1990). Thus, the role of cats and other carnivores in the life cycle of *Besnoitia* spp. of ruminants remains to be clarified.

No *Besnoitia* sp. cysts were found in the two youngest animals that had been separated from their infected mothers at birth and were never exposed to biting flies. These animals previously were thought to have had *Besnoitia* sp. cysts within the sclera; however, there were no scleral cysts at the time of necropsy. While the previously-evident cysts may have regressed by the time of study, other anatomical structures of very similar appearance, such as lymphoid nodules, may have been mistaken for *Besnoitia* sp. cysts in clinical examinations. The absence of *Besnoitia* sp. cysts in these two younger animals, together with epizootiological findings of Glover et al. (1990), are consistent with the observation that biting flies may serve as vectors of *Besnoitia* sp. between ungulate intermediate hosts (Pols, 1960).

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LITERATURE CITED

BENNENT, H. S., AND J. H. LUFT. 1959. s-Collidine as a basis for buffering fixatives. Journal of Biophysics, Biochemistry and Cytology 6: 113.

BESNOIT, C., AND V. ROBIN. 1912. Sarcosporidiose cutanée chez une vache. Revue Vétérinaire 37: 649.

CULLING, C. F. A. 1963. Handbook of histopathological techniques. Butterworth and Company, London, England, 553 pp.

DIESING, L., A. O. HEYDORN, F. R. MATUSCHKA, C. BAUER, E. PIPANO, D. T. DE WAAL, AND F. T. POTGIETER. 1988. *Besnoitia besnoiti*: Definitive host and experimental infections in cattle. Parasitology Research 75: 114–117.

DUNN, A. M. 1978. Veterinary helminthology. William Heinemann Medical Books Ltd., London, England, 323 pp.

FRENKEL, J. K. 1977. *Besnoitia wallacei* of cats and rodents: With a reclassification of other cyst-forming isosporid coccidia. The Journal of Parasitology 63: 611–628.

GARDINER, C. H., R. FAYER, AND J. P. DUBEY. 1988. An atlas of protozoan parasites in animal tissues. Agriculture Handbook No. 651, U.S. Department of Agriculture, U.S. Government Printing Office, Washington, D.C., 83 pp.

GLOVER, G. J., M. SWENDROWSKI, AND R. J. CAWTHORN. 1990. An epizootic of besnoitiosis in captive caribou (*Rangifer tarandus caribou*), reindeer (*Rangifer tarandus tarandus*) and mule deer (*Odocoileus hemionus hemionus*). Journal of Wildlife Diseases 26: 186–195.

KUMI-DIAKA, J., S. WILSON, A. SANUSI, C. E. NJOKU, AND D. J. K. OSORI. 1981. Bovine besnoitiosis and its effects on the male reproductive system. Theriogenology 16: 523–529.

LEWIS, R. 1989. Besnoitia infection in woodland caribou. Canadian Veterinary Journal 30: 436.

———. 1992. Besnoitia infection in game-farmed reindeer: A trial to determine susceptibility of domestic cattle and mule deer. Canadian Veterinary Journal 33: 76.

LUNA, L. C. (editor). 1968. Manual of histologic staining methods of the Armed Forces Institute of Pathology. McGraw-Hill Book Company, New York, New York, 258 pp.

POLS, J. W. 1960. Studies on bovine besnoitiosis with special reference to its aetiology. Onderstepoort Journal of Veterinary Research 28: 265–356.

REHBINDER, C., M. ELVANDER, AND M. NORDKVIST. 1981. Cutaneous besnoitiosis in a Swedish reindeer (*Rangifer tarandus t*). Nordisk Veterinaer Medicin 33: 270–272.

SCHEEHAN, D. C., AND B. B. HRAPSCHAK. 1980. Theory and practice of histotechnology. The C. V. Mosby Company, St. Louis, Missouri, 481 pp.

SCHOLTYSECK, E., H. MEHLHORN, AND B. E. G. MULLER. 1974. Feinstruktur der Cyste und Cystenwand von *Sarcocystis tenella, Besnoitia jellisoni, Frenkelia* sp. und *Toxoplasma gondii*. The Journal of Protozoology 21: 284–294.

SENAUD, J., H. MEHLHORN, AND E. SCHOLTYSECK, 1974. *Besnoitia jellisoni* in macrophages and cysts from experimentally infected laboratory mice. The Journal of Protozoology 21: 715–720.

SMITH, D. D., AND J. K. FRENKEL. 1977. *Besnoitia darlingi*. (Protozoa: Toxoplasmatidae): Cyclic transmission by cats. The Journal of Parasitology 63: 1066–1071.

TERRELL, T. G., AND J. L. STOOKEY. 1973. *Besnoitia bennetti* in two Mexican burros. Veterinary Pathology 10: 177–184.

VAN HEERDEN, J., H. J. ELS, E. J. RAUBENHEIMER, AND J. H. WILLIAMS. 1993. Besnoitiosis in a horse. Journal of the South African Veterinary Medical Association 64: 92–95.

WALLACE, G. D., AND J. K. FRENKEL. 1975. *Besnoitia* species (Protozoa, Sporozoa, Toxoplasmatidae): Recognition of cyclic transmission by cats. Science 185: 369–371.

WOBESER, G. 1976. Besnoitiosis in a woodland caribou. Journal of Wildlife Diseases 12: 566–571.

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