BESNOITIOSIS IN A SOUTHERN PLAINS WOODRAT (NEOTOMA MICROPUS) FROM UVALDE, TEXAS

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ABSTRACT: Recently, Besnoitia neotomofelis was described from a southern plains woodrat (Neotoma micropus) from southern Texas. During May 2010, 1 of 55 southern plains woodrats trapped in Uvalde County, Texas, was diagnosed with besnoitiosis. Grossly, the woodrat had bilateral swellings of the cheeks, and numerous Besnoitia sp.-like cysts were observed in the tongue, facial region, musculature of the limbs, and subcutis of the dorsum and flanks. Little to no inflammation was noted around cysts. The cysts were morphologically similar to B. neotomofelis based on light and transmission electron microscopy. The sequence of the internal transcribed spacer region-1 was identical to the type isolate of B. neotomofelis. Attempts to isolate Besnoitia sp. in laboratory mice failed; however, Toxoplasma gondii was isolated in a Swiss Webster mouse. This represents the first report of besnoitiosis caused by B. neotomofelis in a southern plains woodrat and the first concurrent Besnoitia sp. and T. gondii infection in any host species.

Species of Besnoitia are cyst-forming parasites that are members of the Sarcocystidae (Apicomplexa). There is a wide host range for Besnoitia species that includes mammals, birds, and reptiles. Common hosts include cattle, equids, goats, reindeer (known as caribou in North America [Rangifer tarandus]), opossums, rabbits, rodents, and lizards (Leighton and Gajadhar, 2001; Dubey et al., 2003). Currently, Besnoitia contains 10 validly described species, although there are probably numerous other species that have not been sufficiently morphologically, or molecularly characterized for definitive identification. The life cycles of most of these species are still poorly understood, although felids serve as definitive hosts for at least 4 of the species, including B. oryctofelis, B. darlingi, B. wallacei, and B. neotomofelis (Dubey and Yabsley, 2010). In a previous study of Besnoitia sp. from southern plains woodrats, a novel species (B. neotomofelis) was isolated from a single southern plains woodrat by inoculation of laboratory mice with tissue homogenates (Dubey and Yabsley, 2010). In that study, the positive woodrat was clinically normal at the time of capture, and no cysts were observed grossly or histologically (Dubey and Yabsley, 2010). In the current report, we describe an unusual case of clinical besnoitiosis in a southern plains woodrat caused by B. neotomofelis that had concurrent asymptomatic Toxoplasma gondii and Sarcocystis sp. infections.

MATERIALS AND METHODS

In May 2010, 55 wood rats (17 juveniles and 38 adults) were trapped at 2 sites in Uvalde, Texas, during an ongoing study of Trypanosoma cruzi in southern Texas. Animals were captured using large Sherman traps (H.B. Sherman, Traps, Tallahassee, Florida) or small squirrel cage traps (Havahart, Lititz, Pennsylvania) with dried apricots as bait. Upon capture, animals were anesthetized with intramuscular injection of 100 mg/kg ketamine (Fort Dodge Laboratories, Inc., Fort Dodge, Iowa), weighed, and examined for ectoparasites. Whole blood was collected immediately by intracardiac puncture, and animals were killed by cervical dislocation. Each rat was necropsied, and samples of quadriceps muscle, tongue, diaphragm, liver, spleen, kidney, lung, heart, stomach, small and large intestines, cecum, adrenal glands, urinary bladder, and gonads were fixed in 10% neutral buffered formalin for routine histology. Tissues were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (H&E) for light microscopy examination. Sections of brain and muscle were submitted to the Athens Diagnostic Laboratory (Athens, Georgia) for immunohistochemical staining for T. gondii. For transmission electron microscopy (TEM), tissues that were fixed in 10% neutral buffered formalin were post-fixed in osmium and processed for TEM. For isolation of Besnoitia sp., samples of brain, tongue, and heart were homogenized and aliquots of homogenates (some digested in acidic pepsin) were inoculated subcutaneously into outbred Swiss Webster (SW) and γ-interferon knockout mice (Dubey et al., 2008); grossly visible cysts from buccal mucosa were fed to a laboratory-raised domestic cat. Serum from the woodrat was tested for antibodies to T. gondii by the modified agglutination test as described by Dubey and Desmonts (1987). For polymerase chain reaction, DNA was extracted from a single cyst by using the DNeasy blood and tissue kit (QIAGEN, Valencia, California) following the manufacturer’s protocol. The internal transcribed spacer (ITS)-1 region was amplified with primers 15C and 13B as described previously (Bostrom et al., 2008), and amplicons were purified with a Gel Extraction kit (QIAGEN) and independently bidirectionally sequenced at The University of Georgia sequencing facility (Athens, Georgia).

RESULTS

A single adult female woodrat (262 g) was found to be depressed and lethargic within a cage and had markedly swollen cheeks and muzzle (Fig. 1A). The remaining 54 woodrats trapped from that site and a second site within the same county were bright, alert, and responsive. At necropsy, the fascia along the lateral aspects of the sinonasal area was reddened and edematous with numerous firm, white spherical cysts that were ~0.5 mm in diameter (Fig. 1B). Additional cysts were observed in the musculature on the skull, tongue, nasal cavity, and inner ear. Cysts were distributed throughout the skin and musculature of the dorsum, flanks, and the thighs. Cysts also were found in linear chains within the musculature of the distal fore- and hind limbs (Fig. 1C). Within the visceral cavity, only 2 small cysts were observed; both were located near the renal vein. Partial 18S rRNA, complete ITS-1, and partial 5.8S rRNA sequences from a single cyst were identical to sequences obtained from the type isolate of B. neotomofelis (Dubey and Yabsley, 2010). The sequences were submitted to GenBank with accession number HQ909085.

Histologically, cysts were morphologically consistent with Besnoitia sp. and measured between 224 and 634 μm, with a mean

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Figure 1. (A) External view of woodrat (*Neotoma micropus*) showing severe swelling of the face and a single superficial *Besnoitia* cyst (arrow). (B) Lateral view of the head with skin reflected. Exposed tissue is diffusely reddened and periocular tissue has a gelatinous appearance (edema). Numerous *B. neotomafelis* cysts are present in the muscles and fascia (arrow). (C) Rows of *B. neotomafelis* cysts present in the musculature of the lower leg (arrow). (D) Facial skin of woodrat. Numerous zoite filled cysts expand the dermis, ×20 magnification, H&E stain. (E) Parts of 2 *B. notomofelis* cysts are visible. Cysts have a thick capsule and contain numerous banana- or teardrop-shaped zoites. The lower cyst is within a cystic space containing erythrocytes and fibrin (blood vessel), ×200 magnification, H&E stain. (F) A single *S. neotomafelis* sarcocyst in skeletal myocyte. The cyst has a thin (indistinct) wall and is filled with deeply basophilic zoites. No inflammation or degeneration is associated with the cyst, ×100 magnification, H&E stain.
diameter of 463 μm. The majority of cysts were embedded in collagen or skeletal muscle, although some cysts were found within endothelial lined spaces (blood, or lymphatic vessels, or both; Fig. 1D, E). Tissue cysts contained numerous closely packed, crescent-shaped zoites. Cysts had double-contoured walls (5–15 μm) with a darkly eosinophilic inner layer and a thicker, paler eosinophilic outer layer covered by flattened hyperchromatic nuclei. Results of the TEM showed that cyst walls consisted of 3 distinct layers as described in Dubey and Yabsley (2010).

Within the dermis and subcutis of the face, there were scattered foci of mild edema and small aggregates of neutrophils, lymphocytes, and rare plasma cells, and some cysts were surrounded by small numbers of neutrophils. One cluster of cysts in the superficial dermis was surrounded by homogeneous eosinophilic material resembling amyloid. A single cyst in the cheek had central necrosis with proliferation of bacteria. No inflammation was noted around cysts detected in the tongue or skeletal muscle. In a section of skeletal muscle, a cyst morphologically consistent with Sarcocystis neotomafelis was observed with no associated tissue reaction (Fig. 1F; Galaviz-Silva et al., 1991).

Isolation attempts for Besnoitia sp. were compromised due to autolysis of samples during shipping to the laboratory (6 days before bioassay). The cat fed grossly visible cysts did not shed oocysts for 27 days after feeding tissues. Five SW and 4 γ-interferon gene knockout mice inoculated with undigested muscle cysts did not become infected with Besnoitia or T. gondii. However, 1 SW mouse inoculated with pepsin digest of brain, heart, and tongue became infected with T. gondii (Dubey et al., 2011). Genetic characterization of this T. gondii isolate is reported previously (Dubey et al., 2011). Serum from the woodrat was seropositive for T. gondii (titer, 1:25).

Histologic lesions not attributable to B. neotomafelis also were noted. Diffuse, mild-to-moderate lymphocytic proliferation was observed in the meninges and rare, loosely organized glial nodules were noted in the neuropil of the cerebrum. Immunohistochemical staining for T. gondii, which also reacts with Besnoitia sp. antigens, failed to detect any protozoal antigens in the brain. In addition, the cerebellar meninges contained sections of adult and larval nematodes morphologically consistent with Dunningharia meningoidea; no inflammatory response was noted. In the liver, there were several random foci of necrosis and inflammation composed primarily of eosinophils and epithelioid macrophages with occasional neutrophils, lymphocytes, or plasma cells. No causative agents, e.g., migrating parasitic larvae, were identified in association with the liver lesions. In the lungs, a focal granuloma containing numerous fungal hyphae was observed.

**DISCUSSION**

Historically, infections with Besnoitia spp. were considered to be nonpathogenic for the majority of hosts, although some Besnoitia species, e.g., B. tarandi, B. bennetti, and B. besnoiti, are recognized as important pathogens for their intermediate hosts. Infections with B. besnoiti of domestic cattle and wild bovids, B. bennetti of equids, B. caprae of caprids, and B. tarandi of caribou can cause disease and economic losses due to the induction of poor body condition, edema, thickened skin, hair loss, blindness, vascular obstruction, severe inflammation of affected internal organs, and secondary infections (Leighton and Gajadhar, 2001; Elsheikha et al., 2003). Recently, a series of clinical cases was noted in Virginia opossums (Didelphis virginiana) infected with B. darlingi, which has historically been considered nonpathogenic (A. E. Ellis, unpubl. obs.).

Among the 3 rodent Besnoitia species (B. jellisoni, B. wallacei, and B. neotomafelis), clinical besnoitiosis has only previously been associated with B. jellisoni. Besnoitia jellisoni has been reported from deer mice (Peromyscus maniculatus) and kangaroo rats (Dipodomys ordii and D. microps), and although most infected kangaroo rats did not exhibit any clinical signs in captivity (Ernst et al., 1968), disease has been reported in 4 naturally infected kangaroo rats (Chobotar et al., 1970). Similarly, clinical disease in woodrats infected with B. neotomafelis seems to be rare. The previous study of B. neotomafelis in woodrats from southern Texas only detected 1 of 42 being infected, and the infected woodrat had no gross or histologically visible cysts and was clinically normal (Dubey and Yabsley, 2010).

Interestingly, cysts of B. neotomafelis in this woodrat were significantly larger than the cysts described from experimentally infected laboratory mice and rats in Dubey and Yabsley (2010). In the laboratory mice and rats, the largest cyst observed was 210 μm compared with 634 μm in the present study (Dubey and Yabsley, 2010). Because B. neotomafelis was pathogenic for laboratory mice, the cysts may not have grown as large as they would have in an appropriate host, and infections in laboratory rats only resulted in 2 small cysts (Dubey and Yabsley, 2010). Our inability to isolate B. neotomafelis in known strains of susceptible mice in the current study was probably due to autolysis of infected donor woodrat tissues; it seems that this species of Besnoitia is more susceptible to autolysis than T. gondii.

There is currently only 1 report of S. neotomafelis; this report was also from the southern plains woodrat from Nuevo Leon, Mexico (Galaviz-Silva et al., 1991). Cysts of S. neotomafelis were numerous, macroscopic, and found in several tissues, with most occurring in the musser muscles. In that first report, 29% of woodrats (37/129) had grossly visible cysts of S. neotomafelis, but no clinical signs or lesions were reported (Galaviz-Silva et al., 1991). In the current study, only 1 S. neotomafelis cyst was found in the skeletal muscles of the hind limb of our besnoitiosis woodrat.

It is currently unknown why this woodrat developed clinical besnoitiosis from the B. neotomafelis infection as in the only previous report of B. neotomafelis, the animal was clinically normal and no cysts were observed (Dubey and Yabsley, 2010). In the current study, no grossly visible cysts were observed in the other 54 woodrats, and histologic examinations of numerous tissues from 28 woodrats were negative for Besnoitia cysts. This single clinically ill woodrat could have ingested an overwhelming number of oocysts, or it could have been immunosuppressed for some unknown reason. The ill woodrat had a fungal granuloma in the lung, suggesting it may have been immunosuppressed; however, if the woodrat was immunosuppressed, a subclinical T. gondii infection seems unlikely. Although speculative, the animal had a low serologic titer to T. gondii, so it is possible the infection was recently acquired.

Interestingly, the known definitive host for the 3 protozoal infections in this woodrat (T. gondii, B. neotomafelis, and S. neotomafelis) is the domestic cat (Galaviz-Silva et al. 1991; Dubey, 2009; Dubey and Yabsley, 2010). Feral domestic cats have been noted at the property where the 2 B. neotomafelis infections were
found, and it is possible that wild felids also could serve as definitive hosts because bobcats (Felis rufus) and cougars (Puma concolor) are present in the region, although both wild felids are rare (Schmidly, 2004). The risk of exposure to cat feces is not likely uniform across a landscape because woodrats, in general, are asocial and maintain territories that may result in focal infections (Conditt and Ribble, 1997). This may explain the low prevalence of B. neotomafelis noted in the current and previous study (Dubey and Yabsley, 2010) and why a particular woodrat may have been exposed to high numbers of parasites if a cat routinely defecated in this woodrat’s territory.

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LITERATURE CITED