Protozoal Disease in a California Opossum (Didelphis Virginia)

Besnoitia darlingi

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Abstract

Internal parasitic infections are common in the Virginia opossum (Didelphis Virginia). Besnoitia darlingi is parasitic coccidia protozoa found in North American opossums. This article provides an overview of clinical signs, pathologic findings, transmission, and treatment options. The life cycle involving the cat and the opossum is discussed. The following is a first recognized case of *Besnoitia darlingi* diagnosed in a Southern California opossum.

Key words: *Besnoitia darlingi, Apicomplexa, Didelphis virginiana,* Intermediate host, Bradyzoites, Sporulated oocysts,

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Virginia opossums (Didelphis virginiana) acquire parasites because of their foraging and scavenging behavior. The type of parasitic infection depends on various environmental factors, including weather, seasonal temperature and food availability. Besnoitia darlingi is known to affect only opossums and transmitted by felids. An opossum in Southern California was infected with a coccidian protozoan parasite.

Introduction

Besnoitia Darlingi belongs to the group of protozoal cyst forming extra intestinal coccidia closely related to Toxoplasmosis. Besnoitia darlingi has been recognized for over 80 years in North America with a reported infection rate ranging from 10-60% depending on location. [1] This infection has been reported in opossums from Kentucky, Missouri, Illinois, Texas, Louisiana, Indiana, Michigan and Kansas. [2,3] This case describes first reported B. Darlingi infection found in Los Angeles, California. This study reviews clinical diagnosis, pathologic findings, life cycle, and potential modes of transmission and significance of disease found in a naturally infected V. Opossum.

Case study

An adult male Virginia opossum was brought into the Jones Animal Hospital for surgical evaluation. The opossum needed to have its tail amputated.(Fig. 1)

FIGURE 1. Appearance of the tail at presentation.

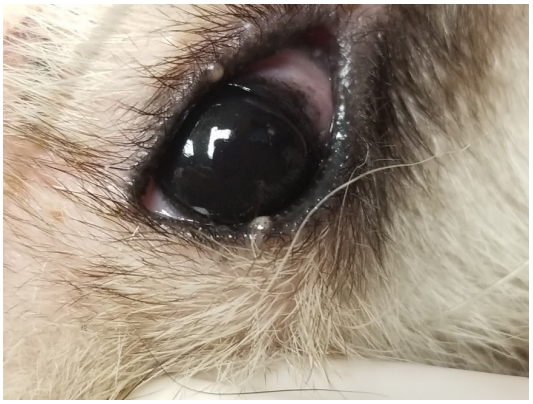

FIGURE 2 B. Example of lesions found on the eyelid margins.

On presentation the opossum appeared healthy, was aggressive, and alert. The opossum was anesthetized for a thorough examination. While preparing the opossum for surgery pinpoint white bumps were noticed around the eyelids and pinna. The author initially believed these lesions were reactions from insect bites. (Fig. 2A,B)

During surgical preparation additional small white papules were noticed widely distributed throughout the dermis. (Fig. 2C)

FIGURE 2 A. Protozoal cysts seen on the pinnae

FIGURE 2 C. Typical distribution of protozoal cysts seen on the skin with Besnoitia darlingi infection.

During intubation additional white bumps were noticed on the tongue and oral mucosa. (Fig. 2D)

FIGURE 2 D. Additional nodules seen on the mucosal membranes of the mouth and tongue.

CBC and general chemistry were evaluated using the Abaxis blood and chemistry equipment. Following routine blood draw, blood was also submitted to IDEXX lab for evaluation. The blood smear of the peripheral blood showed the presence of occasional pyknotic cells. (Fig. 3) The tail was repaired and samples of the skin were submitted for biopsy.

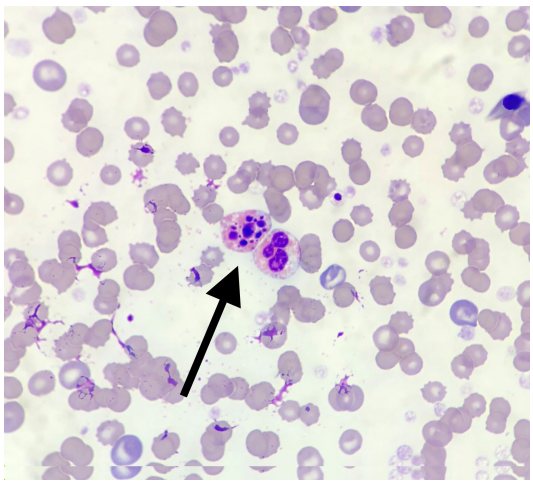

FIGURE 3. Pyknotic neutrophils seen in the peripheral blood smear.

Following recovery, the opossum was released to an experienced caregiver pending results of biopsy. The opossum was confined to a large cage, was eating well, was active but had severe diarrhea. Despite diet modification and de-worming the diarrhea could not be controlled. Fecal analysis showed infection of gastro intestinal ova of *Turgida turgida* and rhabidiform larvae that is an indication of lungworm infection.

Histopathologic finding of the dermis confirmed the presence of Besnoitia darlingi protozoal cysts. (Fig. 4A,B,C)

FIGURE 4 A. Histology appearances of 1 mm diameter protozoal cysts in the dermis.

FIGURE 4B. Besnoitia darlingi cysts have

a pale cell wall with minimal inflammatory reaction.

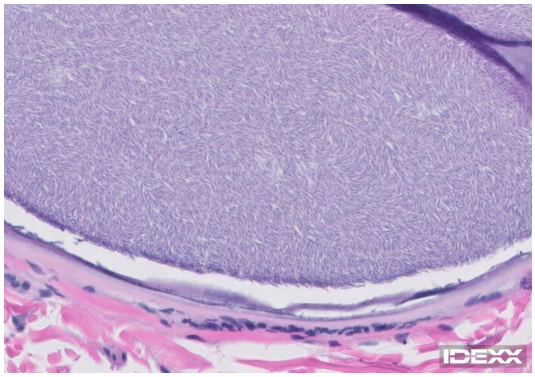

FIGURE 4 C. Protozoal cyst with myriad of spindle shaped bradyzoities.

The appearance or these lesions are uniquely diagnostic of Besnoitia darlingi infection containing bradyzoites. Based on the diagnosis, and uncontrollable diarrhea the opossum was returned to the hospital and was euthanized upon request of the caregiver.

Gross pathology revealed nodules on both kidneys, on the gastrointestinal serosa and urinary bladder. Similar nodules were also found on the pericardium, myocardium and endocardium. Kidney and heart were submitted for biopsy (Fig. 5 A,B,C,D).

FIGURE 5 A. Protozoal cysts seen on the surface of kidney.

FIGURE 5 B. Lesions seen on the pericardium.

FIGURE 5 C. Papules seen in the heart muscle.

FIGURE 5 D. Cysts located on heart valve.

The stomach contained a large number of Physaloptera nematodes. (Fig. 6)

FIGURE 6. The common opossum stomach nematode, *Turgida turgida*.

Histopathology report:

Protozoal cysts were identified in the kidney and were distributed throughout the heart, including in the myocardium and in the heart valves. (Fig. 7 A,B,C) There is a lack of inflammatory response surrounding Besnoitia d. cysts with lack of leukocyte response. [4]

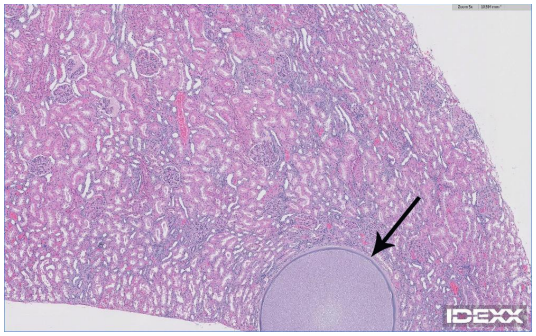

FIGURE 7 A. One mm diameter protozoal cyst identified in the kidney with normal cortical and medullary architecture with small numbers of lymphocytes and plasma cells.

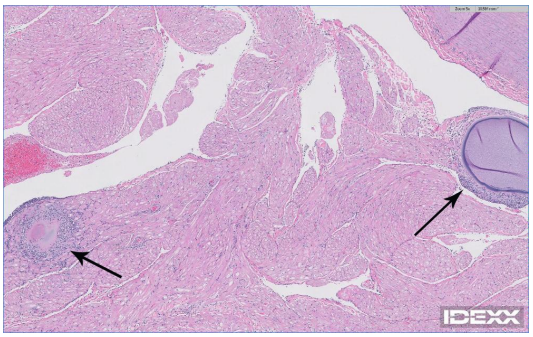

FIGURE 7 B. Representative multifocal cysts distributed throughout the myocardium.

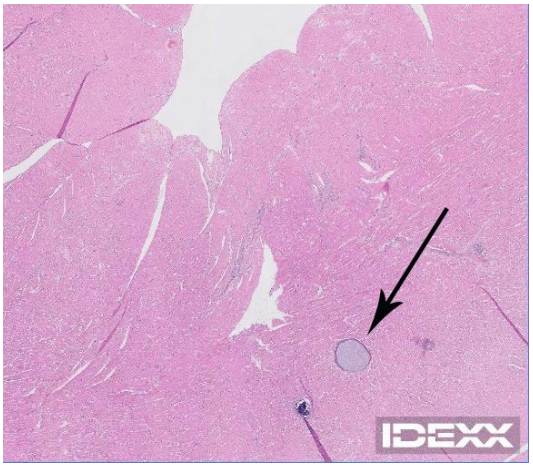

FIGURE 7 C. Cysts are seen within the ventricular and atrial myocardium within the valves and pericardial connective tissue.

The lung section shows *Capillaria sp.* in the airways along with other significant disease in in the lung, indicating broncho-interstitial pneumonia. (Fig. 7D)

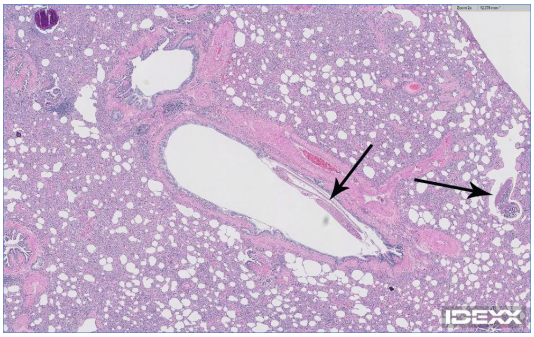

FIGURE 7 D. Lung with broncho-interstial pneumonia with bronchiolar nematodes consistent with Capillaria both male and

female nematodes are seen. With female containing ova with developing larvae.

The spleen demonstrates white pulp hyperplasia, likely secondary to systemic antigenic stimulation, which can be related to pulmonary disease and/or parasitism. (Fig. 7E)

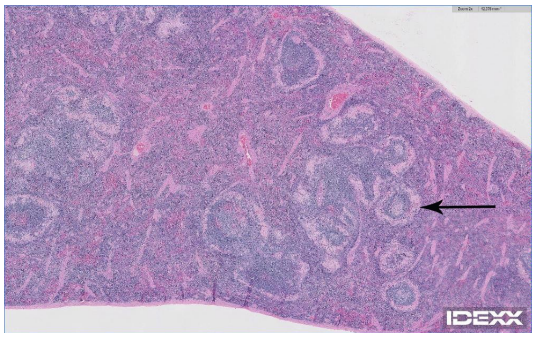

FIGURE 7E. Spleen with white pulp hyperplasia.

There are mild portal infiltrates in the liver, which may reflect some degree of ascending infection in the gastrointestinal tract. (Fig. 7F)

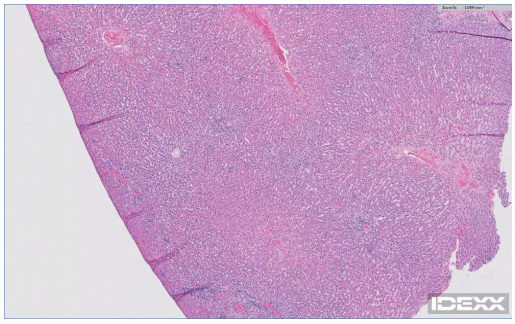

FIGURE 7 F. The liver demonstrates a infection within normal limits for non-domestic animals.

Clinical Diagnosis

Definitive diagnosis of *Besnoitia d.* is based on the anatomic lesions and histopathology. A presumptive diagnosis of *Besnoitia d.* should not be confused with granulomatous furunculosis or dermatitis caused by Demodex or dermatophytes.

Finding tens to hundreds of non-pruritic, white 1-3 mm pinpoint papular lesions in the skin and mucous membranes, especially over the nares, conjunctiva, sclera, pinnae, limbs, and perineum are patho-mnemonic for this disease. The presence of multifocal protozoal cysts with minimal surrounding histiocytic and lymphoplasmacytic dermatitis is seen histologically with epidermal hyperplasia and hyperkeratosis results in the morphological diagnosis. Finding crescent or spindle shape bradyzoites in crushed skin and conjunctival scrapings, confirms the suspected diagnosis. [4]

The appearance of pyknotic neutrophils cell in the circulation has been reported in chronic inflammatory reactions, toxicity and may be related to the severity of concurrent disease.[5,6] The presence of pyknotic cells found in the blood smear of this opossum can indicate severity of *Besnoitia darlingi* infection.

This is the first reported case of Besnoitia darlingi in a California opossum. *Besnoitia darlingi* is the only species of Besnoitia known to infect marsupials. No possum in Australia has been reported infected. The definitive lifecycle for many *Besnoitia* spp. is still not known. The following is a discussion of what is known about the life cycle of *Besnoitia darlingi* affecting opossums.

Besnoitia darlingi

Life Cycle

Besnoitia darlingi follows a heteroxeneous life cycle similar to Toxoplasmosis requiring both an intermediate and a definitive host. The domestic cat, *(Felis catus)* the Bobcat *(Lynx rufus)* and other felids serve as primary host while the opossum serves as the intermediate host. The transmission of *Besnoitia darlingi* between opossums and felids is well-documented [7,8]

Feline

When cyst-containing tissue is ingested by the cat bradyzoites are released and penetrate enterocytes of the intestinal tract forming the sexual stage. Schizonts and gamonts form the sexual stage undergoing one generation, forming gametes. Once fertilization occurs there is a prepatent period of 11-14 days when the oocysts are shed in the feces. Cats do not develop extra intestinal infection with Besnoitia as they do with Toxoplasmosis. [9] Cat feces are not routinely examined for Besnoitia oocysts which closely resemble Toxoplasmosis oocysts. Prevalence of these *Besnoitia darlingi* cysts in cats is unknown. Cats do not become ill, and no treatment for infection is needed. [10]

Opossum

When the opossum becomes infected with sporulated oocysts, the oocysts replicate by a form of asexual reproduction known as endodyogeny. Sporozoites are released during the prepatent period after about 10 days. Sporozoites encyst and invade intestinal cells, become tachyzoites. This patent period lasts about 6 days. The parasites penetrate and feed on various host cells in the intestine, undergo schizogony, develop into schizonts, and release merozoites. First-generation meronts develop in the endothelial cells of blood vessels, while later generations develop primarily in fibroblasts and connective tissue of various organs producing nodules and papules containing bradyzoites.

More Besnoitia oocysts are shed in the summer. Studies show *Besnoitia darlingi* has a shorter incubation period with increase production during warm weather [4.] Opossums are more active foraging and scavenging in the spring and summer increasing exposure to oocysts.

Opossums become exposed to sporulated feline oocysts in several ways. Opossums are not coprophagic and do not intentionally ingest cat feces. They may become infected through ingesting infected animals such as lizards, snakes or rodents, or by ingesting coprophageous insects such as beetles, crickets, and cockroaches, or through ingesting contaminated food or water. Some authors believe that *Besnoitia sp* can be transmitted though bites of hematophagous insect vectors as flies or ticks. [8,11]

Finding *Capillaria sp*. in the intra-bronchial and bronchiolar lung tissue indicates this opossum ingested infected feces of a mammalian species known to transmit Capillaria sp. such as bobcats *(Lynx rufus)* or domestic cats (Felis catus) Further study is needed to ascertain correlation between *Capillaria* and *Besnoitia* infection in opossums.

Discussion

Besnoitiosis does not typically cause clinical disease in opossums despite widespread systemic dissemination of bradyzoites cysts. More severe clinical manifestation of disease may be secondary to compromised immune suppression, ongoing comorbidities, environmental stress, and concurrent parasitic infection. Few opossums were reported to have severe clinical disease and inflammation associated with *Besnoitia d*. Reported signs include trembling, incoordination, circling, blindness, poor body condition and sudden death. [12]

No routine test is available and there is no known specific treatment for an infected opossum. Few treatments have been suggested based on treatment of toxoplasmosis and other coccidia species using sulfadiazine or Ponzuril. In limited studies of experimental induced Besnoita infection in rabbits, antimony and sulfanilamide complex was found to prevent cyst development. Oxytetracycline also may have some therapeutic value if give early in the course of the disease. External lesions may be treated with 2% tincture of iodine. [13- 15]

Summary

Clinical recognition and pathology, gross, and histology of Besnoitia darlingi have been discussed. It is know that opossums like to hang out with feral cats where they are attracted to easily available food. Probable life cycle and transmission between the cat and opossum has been shown. *Besnoitia darlingi* infection generally results in minimal disease in opossums but severity increases depending on co-morbidities and degree of parasite load. Every rescued opossum needs to be examined thoroughly, treated with parasiticides and antibiotics. Rescuers need to recognize B. Darlingi and not confuse this newly emerging disease with other skin diseases.

Comments

There is still much to be learned about the life cycle of Besnoitia in opossums and its clinical significance. The significance of finding Besnoitia darlingi in a local Southern California opossum is not clear. At this time no *Besnoitia* species are considered zoonotic. Opossums have adapted well to urban areas. With increasing interaction between humans, domestic animals, feral cat populations, and wildlife the potential exists for creating new zoonoses.

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