

Diagnostic Exercise

From The Davis-Thompson Foundation*

Case #: 65 Month: February Year: 2016

Answer Sheet

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Clinical History: An approximately 4-year-old, male, blind Virginia opossum (*Didelphis virginiana*) was found in the woods and brought to a wildlife rehabilitator. The rehabilitator brought the opossum to the Texas A&M Zoological Medicine service for further treatment. Due to the extent of the skin condition, blindness and the advanced age of the opossum, humane euthanasia was elected and the animal was submitted for necropsy.



Figures 1 & 2

1. Morphologic diagnoses:

- a. Pinna: Multifocal protozoal cysts with minimal surrounding histiocytic and lymphoplasmacytic dermatitis, and epidermal hyperplasia and hyperkeratosis.
- b. Kidney: Multifocal protozoal cysts with minimal surrounding histiocytic and lymphoplasmacytic interstitial nephritis.

2. Cause: *Besnoitia darlingi*

3. Microscopic findings:

- a. Multifocal, very large (300 μm to 1 mm in diameter) round protozoal cysts delimited by a 10-20 μm clear, hyaline wall and containing myriad, tightly packed, crescent shaped, basophilic, 10-15 μm bradyzoites. The protozoal cysts are within the cytoplasm of the host cell, leading to peripheralization of the host cell nucleus. The surrounding dermal collagen is compressed.
- b. Minimal histiocytic and lymphoplasmacytic inflammation surround the cysts. The inflammatory infiltrate can become granulomatous if degenerate cysts rupture.

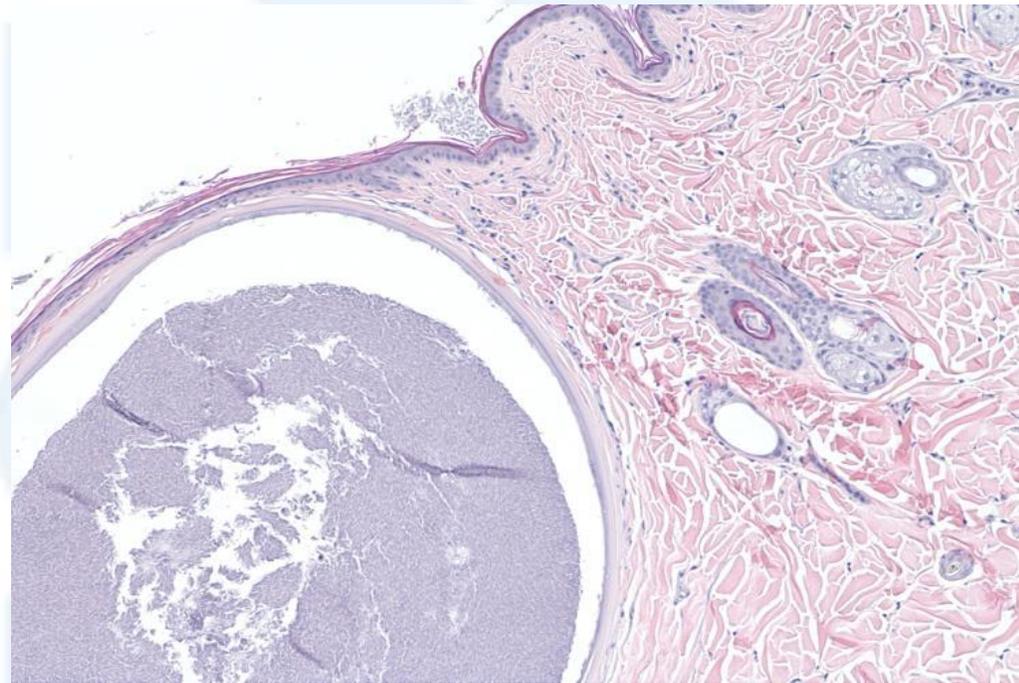


Figure 3 - Intact protozoal cyst within the superficial dermis. There is minimal inflammation around the cyst and the epidermis is mildly hyperkeratotic, H&E, 2X.

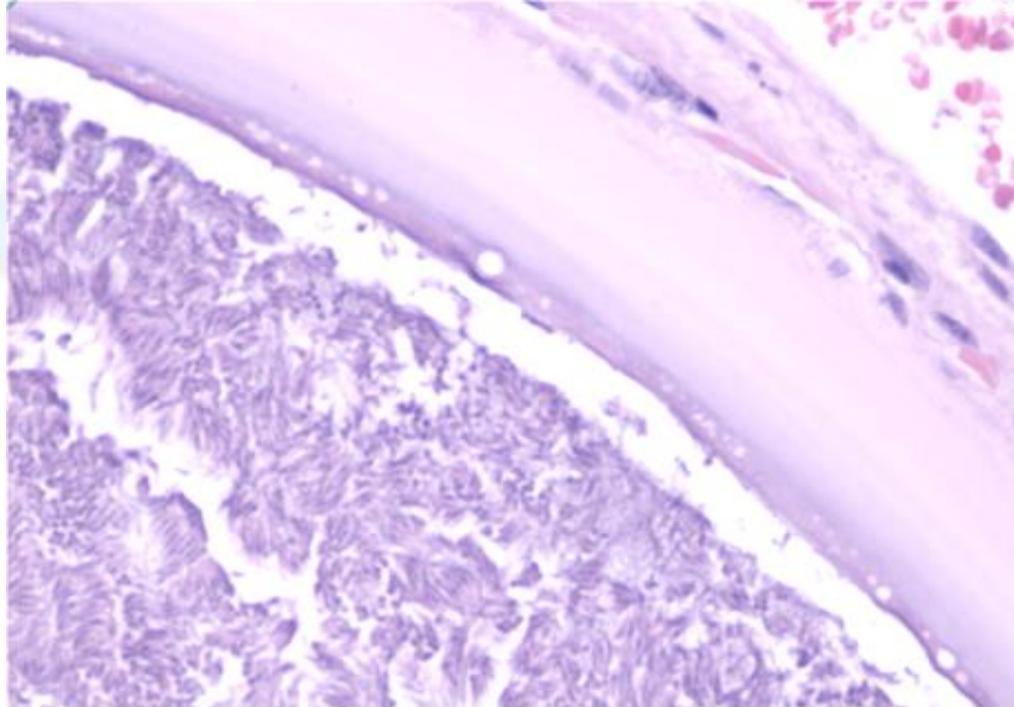


Figure 4 - Detail of the hyaline cyst wall that surrounds numerous bradyzoites, H&E, 40x.

4. **Differential diagnoses:**

- a. Granulomatous furunculosis and dermatitis can also be caused by *Demodex* or dermatophytes, but the size of the pustules would not be as uniform as observed in this case. Nonetheless, this opossum also had mild concurrent demodicosis.
- b. Systemic fungal or parasitic infection, though a more severe granulomatous response would be expected. This opossum was also incidentally infected with the schistosome *Heterobilharzia americana*.

While cysts were found in the brain, the histologic lesions in the eyes consisted mostly of severe, neutrophilic keratitis and uveitis with rupture of Descemet's membrane and extensive corneal pigmentation and neovascularization. The inciting cause of the keratitis was not evident histologically, but the blindness was not directly related to the besnoitiosis as no cysts were found in the ocular chambers or optic nerve.

Discussion: *Besnoitia* is a genus comprised of several species of parasitic, cyst forming, apicomplexan protozoa that typically infect the skin, blood vessels and mucous membranes of humans and animals. Besnoitiosis in Virginia opossums is attributed to infection with *Besnoitia darlingi* and has been reported in opossums throughout the Southeastern United States. *B. darlingi* also infects lizards and snakes through ingestion of infected tissues or contaminated

water but has not been reported in humans. Cats are considered to be the definitive host of *B. darlingi*. Opossums are an intermediate host and consequently do not shed oocysts. Besnoitiosis typically does not cause severe clinical disease in opossums despite often widespread dissemination of tissue cysts. However, debilitation and death of severely affected opossums has been reported. Dissemination of the cysts may be secondary to immunosuppression from stress or concurrent parasitic infections.

References and Recommended literature:

1. Dubey, JP, DS Lindsay, BM Rosenthal, C Sreekumar, DE Hill *et al.* 2002. Establishment of *Besnoitia darlingi* from opossums (*Didelphis virginiana*) in experimental intermediate and definitive hosts, propagation cell culture, and description of ultrastructure and genetic characteristics. *Int J Parasitol.* 32:1053-1064.
2. Ellis, A, E Mackey, PA Moore, SJ Divers, P Hensel *et al.* 2012. Debilitation and mortality associated with Besnoitiosis in four Virginia opossums (*Didelphis virginiana*). *J Zoo Wildl Med.* 43:367-374.
3. Houk, A.E., Rosypal, A.C., Grant, D.C., Dubey, J.P., Zajac, A.M. *et al.* 2011. Serological response of cats to experimental *Besnoitia darlingi* and *Besnoitia neotomofelis* infections and prevalence of antibodies to these parasites in cats from Virginia and Pennsylvania. *Journal of Parasitology.* 97 (2), 259-261
4. Shaw, S, B Grasperge, J Nevarez, S Reed, L Long *et al.* 2009. *Besnoitia darlingi* infection in a Virginia opossum (*Didelphis virginiana*). *J Zoo Wildl Med.* 40:220-223

*The Diagnostic Exercises are an initiative of the **Latin Comparative Pathology Group (LCPG)**, the Latin American subdivision of The Davis-Thompson Foundation. These exercises are contributed by members and non-members from any country of residence. - Consider submitting an exercise! - A final document containing this material with answers and a brief discussion will be posted on the CL Davis website (http://www.cldavis.org/diagnostic_exercises.html).

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