

Case #3

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SIGNALMENT: Female, adult, green and black poison dart frog (*Dendrobates auratus*).

HISTORY: A collection of captive-bred, green and black poison dart frogs (*Dendrobates auratus*) and Panamanian golden frogs (*Atelopus zeteki*) were housed together in an exhibit at a zoological institution. Two female Panamanian golden frogs were found dead unexpectedly on exhibit and no clinical signs were noted in the remaining animals. One was submitted for necropsy. At that time, the green and black poison dart frogs were removed from the exhibit and placed in a quarantine tank. Five days later, the green and black poison dart frog of this report died without premonitory signs. Histologic examination of the Panamanian golden frog revealed chytridiomycosis, and all remaining frogs were treated with itraconazole and ciprofloxacin. Approximately eight days after initiation of quarantine, the remaining green and black poison frogs were swabbed and tested for *Batrachochytrium dendrobatidis* via polymerase chain reaction (PCR), which was negative. The quarantine was removed after negative PCR results and no further losses in 32 days.

GROSS FINDINGS: No gross lesions were noted during post-mortem examination. The body was submitted whole in formalin for processing.

HISTOPATHOLOGIC/CYTOLOGIC FINDINGS:

Skin: The epidermis had multifocal mild to moderate hyperplasia with overlying orthokeratotic hyperkeratosis. Embedded within the superficial layers of the epidermis (ie stratum corneum) and in the orthokeratotic crust were numerous fungal organisms, including 7-10 µm diameter thalli and zoosporangia with internal, 2-3 µm diameter zoospores. Thalli were either empty with 1-3 internal septations, uninucleate with a large basophilic nucleus, or multinucleate. Organisms were most abundant in sections of the feet. Lymphocytic inflammation and single cell epidermal necrosis was minimal.

Large Intestine (not present on submitted slide): The lamina propria was mildly expanded by lymphocytes. In the lumen, there was a single transverse section of a nematode with lateral alae.

MORPHOLOGIC/ETIOLOGIC DIAGNOSIS:

1. Skin: mild to moderate, chronic, multifocal, hyperplastic dermatitis with intralesional fungal thalli and zoosporangia (chytridiomycosis).
2. Large intestines: mild, chronic, regionally extensive enteritis with intralesional nematodes.

DISCUSSION:

Chytridiomycosis, a devastating fungal infection caused by *Batrachochytrium dendrobatidis* (Bd), has been linked to amphibian population declines worldwide.^{2,4,8} Bd has

distinct life stages with both a zoospore and thallus.³ Aquatic flagellated zoospores embed in host epidermis and form a thallus. Within the thallus, zoospores divide asexually and mature before being released into the moist or aquatic surrounding environment via a discharge papilla, a flask-like opening in the thallus wall.

Clinical signs in infected animals vary widely and can include lethargy, weakness, loss of righting reflex, abnormal posturing, brown discoloration, erythema with excessive skin shedding, or no apparent signs.^{1,4} If present, gross lesions are frequently confined to the ventrum of the skin of the body, feet, and toes. Uncommonly, concurrent secondary fungal or bacterial infections can exacerbate clinical signs and gross lesions. Definitive diagnosis is based on cytology or histopathology demonstrating fungal thalli.^{1,4} Polymerase chain reaction testing of live frogs has been effective as a screening procedure in live frogs. Thalli occur within the stratum corneum or keratin crust, are 7-20 µm in diameter, and have multiple forms, including empty thalli with one to multiple internal septae, multinucleate thalli with microvacuolated basophilic cytoplasm, and zoosporangia with internal, 2-µm-diameter, discrete, round to oval, basophilic zoospores.^{1,6} A uninucleate thallus form has also been identified but is rare.⁶ Organisms can be seen on skin sheds or skin scraping using Diff-Quick stain or in skin sheds or in the histologic sections of epidermis.

Samples for histology should include at least three sections of skin from the ventrum.¹ Histologically, epidermal hyperplasia and orthokeratotic hyperkeratosis are present with minimal to no inflammation or necrosis in cases without secondary infections.⁵ Walls of the thalli stain with periodic acid-Schiff and Gomori methenamine silver stains and spores are Gram-positive and stain weakly with Giemsa. HE stain alone is frequently sufficient to identify thalli in heavy infections.⁴

Successful treatment with daily itraconazole baths has been described in poison dart frogs.⁴ Amphibian skin is unique when compared to other vertebrates. It is thin and highly permeable, and plays a key role in various physiologic processes, including water absorption, osmoregulation, and respiration.⁴ The cause of death in chytridiomycosis is believed to be related to massive depletion of electrolytes and osmotic imbalance secondary to disruption of cutaneous epithelial functions.^{1,3,7,8} Some affected frogs have substantial renal tubular necrosis, likely related to hypoxia associated with the skin lesion, and the renal lesion may contribute to the electrolyte imbalances (M. Garner, pers. comm.).

REFERENCES:

1. Baitchman EJ, Pessier AP. Pathogenesis, diagnosis, and treatment of amphibian chytridiomycosis. *Vet clin North Am exot anim pract*. 2013. 16:669-685.
2. Kilpatrick AM, Briggs CJ, Daszak P. The ecology and impact of chytridiomycosis: an emerging disease of amphibians. *Trends ecol evol*. 2009. 25:109-118.
3. Rosenblum EB, Voyles J, Poorten TJ, Stajich JE. The deadly chytrid fungus: a story of an emerging pathogen. *PLoS pathog*. 2010. 6:1-3.
4. Pessier A. An overview of amphibian skin disease. *Sem avian exotic pet med*. 2002. 11:162-174.
5. Pessier A. Chytridiomycosis. In: Miller RE, Fowler M (eds.). *Fowler's zoo and animal medicine current therapy*, volume 7, 1st edition. St. Louis (MO): Saunders Elsevier. 2011. p. 255-270.

6. Pessier A, Nichols DK, Longcore JE, Fuller MS. Cutaneous chytridiomycosis in poison dart frogs (*Dendrobates spp.*) and White's tree frogs (*Litora caerulea*). J vet diagn invest. 1999. 11:194-199.
7. Voyles J, Berger L, Young S, Speare R, Webb R, et al. Electrolyte depletion and osmotic imbalance in amphibians with chytridiomycosis. Dis aquat org. 2007. 77:113-118.
8. Voyles J, Young S, Berger L, Campbell C, Voyles WF, Dinudom A, Cook D, Webb R, Alford RA, Skerratt LF, Speare R. Pathogenesis of chytridiomycosis, a cause of catastrophic amphibian declines. Science. 2009. 326:582-585.