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DIAGNOSTIC CHALLENGE

The Journal of Herpetological Medicine and Surgery, Volume 35, No. 4, 2025 pp. 249–251
DOI: 10.5818/Diagnostic_challenge

Diagnostic Challenge: Skin Touch Imprints from an Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*)

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Signalment

Adult, eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*) under managed care found deceased along with multiple other animals deceased or with blue-gray skin coloration. A touch imprint of the skin was collected for diagnostic purposes (Fig. 1).

Interpret the image, formulate differential diagnoses, and continue reading

Figure 1. Touch imprint, skin, wet mount (phase contrast), eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*). Sample was collected by touching a glass slide to the dorsal skin of a deceased hellbender that was one of several to unexpectedly die in a captive collection. Scale bar = 50 μm.

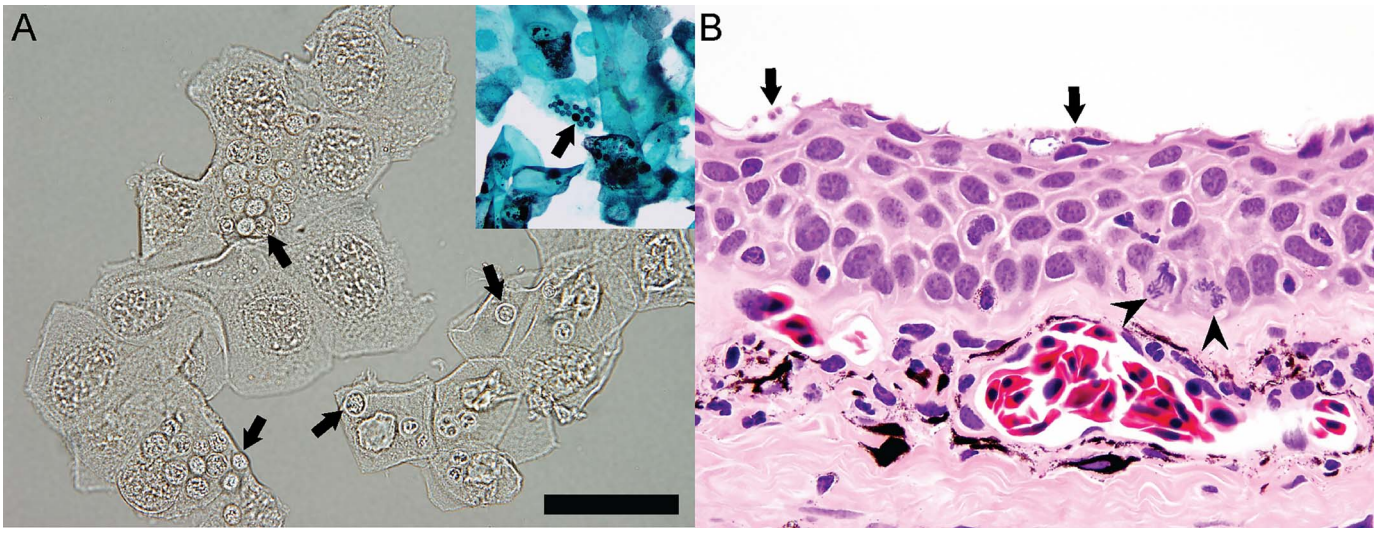

Figure 2. (A) Touch imprint, skin, wet mount (phase contrast) and Grocott's methenamine silver (GMS) staining (inset), eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*). The touch imprint cytology consists of frequent keratinocytes (consistent with hyperkeratosis) that contain or are associated with numerous, round, 3–4 μm fungal sporangia (arrows) that are highlighted with Grocott's methenamine silver (GMS) staining (arrows, inset). Scale bar = 50 μm. (B) Microscopically, the hellbender had marked epidermal hyperplasia with prominent mitotic figures in the basal epidermis (arrowheads) and hyperkeratosis with similar fungal sporangia (arrows). Hematoxylin and eosin stain.

The touch imprint cytology comprised markedly increased numbers of keratinocytes, consistent with hyperkeratosis (Fig. 2). The keratinocytes often contained or were associated with numerous, round, 3–4 μm fungal sporangia. The combination of hyperkeratosis and fungal sporangia in amphibian skin are consistent with a diagnosis of chytridiomycosis. *Batrachochytrium dendrobatidis* infection was confirmed by quantitative polymerase chain reaction of skin swabs collected at necropsy and microscopic examination–confirmed concurrent epidermal hyperplasia.

Summary

Batrachochytrium dendrobatidis is a major cause of morbidity and mortality in both free-ranging and captive amphibians globally. Infection is restricted to the skin of amphibians and causes epidermal hyperplasia and hyperkeratosis (Berger *et al.*, 1998; Pessier *et al.*, 1999). Experimental infections in frogs showed that *B. dendrobatidis* infections inhibit electrolyte transport across the epidermis, resulting in hyponatremia, hypokalemia, and ultimately asystolic cardiac arrest (Voyles *et al.*, 2009). For species that respire primarily across their skin, such as eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis*), epidermal changes also likely decrease blood oxygenation because of the increased distance between dermal and intraepidermal (if present) capillaries. Gross signs of chytridiomycosis can be limited but include "graying" or cloudiness of the skin due to epidermal thickening and hyperkeratosis. In aquatic species that respire primarily across their skin, such as hellbenders, infected individuals may also exhibit altered behavior with more time at the surface gulping air, presumably for increased pulmonary respiration. The most sensitive and accurate diagnostic method is polymerase chain reaction of skin swabs to confirm the presence of *B. dendrobatidis* (Hyatt *et al.*, 2007); pathologic assessment of the skin is also required to associate the presence of *B. dendrobatidis* with skin lesions for a true diagnosis of chytridiomycosis, if necessary. However, simple in-house screening of sick animals by cytology, as demonstrated here, may facilitate more rapid diagnosis to facilitate quicker therapeutic administration. Effective *B. dendrobatidis* treatment options include select antifungal drugs and heat therapy (Woodhams *et al.*, 2012).

Keywords: *Batrachochytrium dendrobatidis*, chytridiomycosis, cytology, *Cryptobranchus alleganiensis alleganiensis*, eastern hellbender

Disclaimer: The authors have nothing to disclose.

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