CUTANEOUS CHYTRIDIOMYCOSIS IN AMPHIBIANS: AN EMERGING DISEASE?

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Abstract

Fungi in the Phylum Chytridiomycota (chytrids) are ubiquitous microscopic organisms that reproduce asexually by means of motile, uniflagellate spores (zoospores). Many chytrids are saprobes in aquatic and terrestrial habitats. Other species are obligate or facultative parasites of other fungi, algae, vascular plants or invertebrate animals but none has previously been recognized as a parasite or a pathogen of vertebrate animals.[1,2,6,7]

From September 1996 through October 1997, the deaths of 24 juvenile blue poison arrow frogs (*Dendrobates azureus)*, 4 juvenile green-and-black poison arrow frogs (*Dendrobates auratus),* 3 aged adult White's tree frogs (*Litoria caerulea),* and an adult ornate horned frog (*Ceratophrys ornata*) at the National Zoological Park (NZP) were associated with cutaneous infections by chytrids. Affected frogs were housed in close proximity in NZP's Reptile House. Anorexia and lethargy were noted in two blue poison arrow frogs 1 day before their deaths. Ventral erythema with multifocal patches of soft, brown shedding skin was seen in one White's tree frog the day prior to its being found dead. However, most frogs died without prior clinical signs of disease. At gross necropsy, the skin along the ventral aspects of the body of many of the frogs was granular and discolored brown. Histologically, cutaneous lesions were most prominent over the ventral abdomen, pelvis, and hind legs and were characterized by epidermal hyperplasia and hyperkeratosis associated with numerous, 10-30 μm chytrid thalli within the superficial epidermis. Multifocal epidermal degeneration with minimal to mild inflammation also was often present. The cause of death in affected frogs was attributed to disruption of normal cutaneous function, which in amphibians may include water absorption, osmoregulation, respiration, and electrolyte maintenance.[3]

Three forms of the chytrid thalli were identified in the skin by light microscopy: uninucleated forms with homogeneous basophilic cytoplasm, multinucleated forms containing lacey to microvesiculated cytoplasm, and thick-walled sporangia containing multiple 2-3 μm round spores. Sporangia usually had single tubular extensions (discharge papillae) directed toward the skin surface. Histologic special stains revealed that all forms of the fungus were periodic acid-Schiff (PAS) positive and stained with Gomori's methenamine silver (GMS). They did not stain with Gridley's fungal stain and were not acid-fast. The spores were gram-positive and stained weakly with Giemsa. Transmission electron microscopy demonstrated that spores had flagella and that these zoospores had features characteristic of chytrids, including kinetosome props, a terminal plate in the axoneme

core, and mitochondria with plate-like cristae. Other ultrastructural features, such as ribosomes grouped in a mass, placed the organism within the order Chytridiales.[2] Phase contrast microscopy revealed that living zoospores released from zoosporangia in fresh skin samples from infected frogs swam with a single, posteriorly-directed, whiplash flagellum. Pure cultures of the chytrid were established from the skin of a blue poison arrow frog and from a green-and-black poison arrow frog; the fungal isolates from the two species of frogs were morphologically indistinguishable and represent a new genus and species of chytrid fungi.

These findings indicate that chytrids can infect vertebrate tissue and act as significant pathogens in frogs. Although the infective agent has not previously been recognized as a member of the Chytridiomycota, a review of pathology records at NZP revealed other cases of cutaneous chytridiomycosis in a White's tree in 1988 and an ornate horned frog and another White's tree frog in 1990. The disease was previously described in captive arroyo toads (*Bufo microscaphus californicus),* amargosa toads (*Bufo nelsoni*) and a woodhouse toad (*Bufo w. woodhousei*) and the causative agents were characterized as "fungal-like protists."[5] A fatal dermatomycosis in captive dwarf African clawed frogs (*Hymenochirus curtipes*) was probably caused by chytrids instead of the zygomycete *Basidiobolus ranarum,* as previously reported.[4] Cutaneous chytrid infections have been recently identified in captive amphibians at several U.S. zoos, affecting species such as false tomato frogs (*Dyscophagus guineti*), Cowan's mantellas (*Mantella cowanii*), a Solomon's Island eyelash frog (*Ceratobatrachus guentheri*), an European green toad (*Bufo viridis*) and a giant palm salamander (*Bolitoglossa dofleini)* (T. McNamara, M. Greenwell, K. Wright and M. Garner, personal communication). Chytrid fungi have been associated with significant population declines in several species of wild frogs from Australia (L. Berger, personal communication) and leopard frogs (*Rana yavapiensis* and *R. chiricahuensis*) in Arizona (G. Bradley, personal communication). In most instances, the causative organisms were initially thought to be protozoa because of the morphology of chytrid thalli and zoospores on light microscopy.

The apparently sudden emergence of cutaneous chytridiomycosis in captive and wild amphibians is noteworthy, particularly in view of increasing recognition of worldwide declines in populations of amphibians. Further studies to better characterize this disease and the possible co-factors that predispose amphibian skin to chytrid infection are warranted.

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