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SHORT COMMUNICATIONS

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Mortality of Captive Canadian Toads from *Basidiobolus ranarum* Mycotic Dermatitis

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ABSTRACT: Twenty-six adult free-ranging Canadian toads (*Bufo hemiophrys*) were collected from northeastern North Dakota (USA) during the last week of August 1994 and placed in captivity. During late December and January 1995, 21 Canadian toads died. Clinical signs included increased time sitting in water bowls, darkened dorsal skin, constant arching of their backs, and hyperemia and sloughing of ventral epidermis. The condition progressively worsened until death occurred within 5 to 7 days after onset of clinical disease. Mycotic dermatitis due to *Basidiobolus ranarum* was diagnosed in all toads and the fungus was isolated from 11 (52%) of 21 toads. Histology of the ventral skin and digits revealed numerous fungal spherules and occasional hyphae without significant inflammatory reaction. This condition clinically resembled red leg associated with *Aeromonas hydrophila* and many other bacterial organisms, and the diseases could be confused without appropriate diagnostic tests. This also is the first report of *B. ranarum* causing clinical disease in a toad species.

Key words: Amphibian, *Basidiobolus ranarum, Bufo hemiophrys,* Canadian toad, mortality, mycotic dermatitis, red leg.

Amphibian numbers and species are declining worldwide (Pechmann et al., 1991). Use of pesticides, changes in agricultural practices, increased predation, disease, increased ultraviolet radiation, decreased suitable habitat, and climatic changes have been suggested as possible causes of these declines (Carey, 1993; Scherman and Morton, 1993), but little research has been done to determine specific causes (Rabb, 1990; Bishop and Pettit, 1992; Wake, 1992).

Mycotic infections in mammalian species manifest almost exclusively in those which have undergone physical stress, injury, and/or immunosuppression (Rippon, 1982; Koneman and Roberts, 1985, Roberts, 1986; Ajello, 1988). While there has been little research to evaluate potential predisposing factors, most amphibian mycoses also are believed to occur in compromised individuals (Anver and Pond, 1984; Blaustein et al., 1994). Amphibians frequently inhabit the moist soils of the aquatic-terrestrial interface in which a diversity of potentially pathogenic fungi thrive, however, there are relatively few reports of amphibian mycoses (Reichenbach-Klinke and Elkan, 1965).

A widespread saprophytic fungus, *Basidiobolus ranarum,* is a member of the family Entomophthoraceae and has caused disease in humans (Davis et al., 1994; Drechsler, 1952, 1964; Coremans-Pelseneer, 1973). When cultured, it is easily identified based on its distinctive "beaked" zygospores and *Streptomyces*-like odor (Drechsler, 1958). This organism was first isolated from healthy frog intestines by Eidam in 1886 and has since been cultured from feces and the intestinal lining of many species of amphibians (Drechsler, 1956; Robinow, 1963; Hutchinson and Nickerson, 1970; Nickerson and Hutchinson, 1971; Tills, 1974; Gugnani and Okafor, 1980; Okafor et al., 1984; Zahari et al., 1990). Groff et al. (1991) reported the first case of *B. ranarum* causing disease in amphibians by inducing fatal mycotic dermatitis in captive post-metamorphic dwarf African clawed frogs (*Hymenochirus curtipes*). This epizootic resulted in morbidity and mortality of almost 100% and affected

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10,000 animals. Studies conducted during this outbreak indicated that transmission to healthy frogs occurred from infected moribund animals but not after exposure to fungal broth culture suspensions. The first free-ranging species found to be lethally affected by a *B. ranarum* mycotic dermatitis was in Wyoming toads (*Bufo baxteri*) (Taylor et al., 1994).

During late August 1994, 26 adult free-ranging Canadian toads (*Bufo hemiophrys*) were collected by hand from along the roadside in northeastern North Dakota (USA; 47°56′N, 97°4′W). Toads weighed 35–55 gm. Toads were transported and kept at the Wyoming State Veterinary Laboratory (Laramie, Wyoming, USA) in peat moss lined glass terrariums in groups of four. The peat moss had been initially autoclaved for 15 min at 121 C. The amount of peat moss in each terrarium was weighed to standardize quantity. Terrariums were spot cleaned as needed. Toads were provided free-choice live 2 to 3-wk-old crickets obtained from a commercial vendor (Top Hat Cricket Farm, Kalamazoo, Michigan, USA). Crickets were provided with free-choice commercial rodent chow (Purina, St. Louis, Missouri, USA). A large glass water bowl was placed in each terrarium. Water in each tank was changed every other day. Tap water was dechlorinated naturally by letting it sit out for 24 hr at room temperature before use in water bowls. The room was on a 12 hr light/12 hr dark cycle, room temperature ranged between 20 to 22 C, and the humidity in the room was approximately 40%. Disposable premoistened powder-free latex gloves were worn when handling toads and gloves were changed between groups.

One adult male died of emaciation 3 wk after capture. All other toads were observed eating on a regular basis. By November, the remaining 25 toads had increased in weight by 10 to 15 g, were calling, and most were observed in amplexus. During the last week of November, toads in amplexus were placed in plastic containers with dechlorinated water and areas with dry peatmoss for 72 hr. No eggs were laid and toads were returned to their original terrariums. From the last week of December through the end of January, 21 toads developed clinical signs of disease that included sitting in water bowls for prolonged periods, darkening of dorsal skin, constant arching of their backs, and hyperemia and sloughing of the ventral epidermis and toes. The condition progressively worsened until death occurred within 5 to 7 days from onset of clinical signs in all the toads. Other toads in the same room, being fed from the same food and water sources were not affected.

Most dead toads were evaluated fresh, however, some dead toads were placed in sterile plastic bags and frozen at either −20 or −70 C until postmortem evaluation. Body condition was subjectively evaluated by size of intestinal fat bodies and amount of pericardial fat as poor, fair, or excellent. Sections of ventral abdominal skin, digits, tongue, gluteus muscle, lung, heart, liver, kidney, stomach, intestine, fat bodies, and gonads were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 to 7 μm, and stained with hematoxylin and eosin. Periodic-acid Schiff (PAS) and Gram's stain were used on selected sections of skin (Chandler et al., 1980). Tissues were evaluated by light microscopy.

Swabs of ventral abdominal skin, subcutaneous fluid, and liver were collected and placed in modified Stuart's bacterial transport medium (S/P Brand Culturette Systems, Baxter Diagnostics, Deerfield, Illinois, USA). These were plated onto Columbia agar with 5% sheep blood (Acumedia Manufacturing, Inc., Baltimore, Maryland USA). Plates were incubated at 35 C in atmospheric air for 96 hr. Each day plates were examined for bacterial growth. Bacterial isolates were inoculated into a commercial aerobic bacterial identification system which characterizes microbes based on carbon metabolic tests

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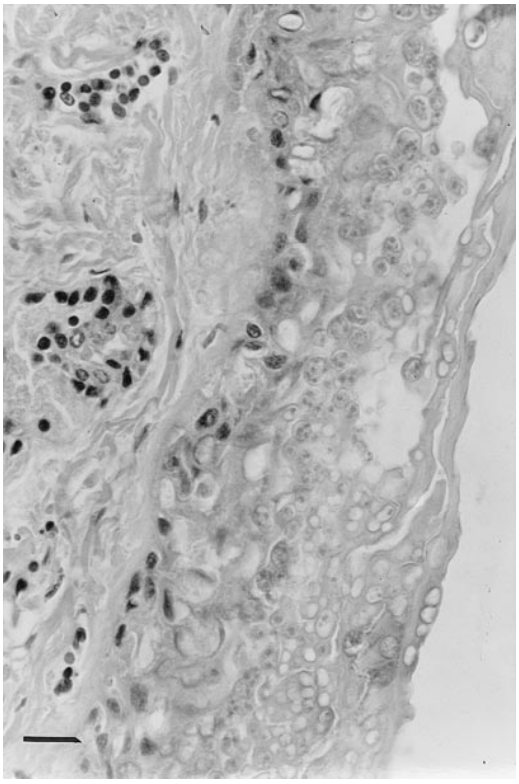


FIGURE 1. Light microscopy of ventral abdominal skin from a Canadian toad infected with *Basidiobolus ranarum* which is characterized by erosions with numerous fungal spherules measuring 8.8 (SD = 1.6) × 7.4 μm (SD = 1.2) in the superficial layers of the epidermis without significant inflammatory reaction. Periodic-acid Schiff stain. Bar = 10 μm.

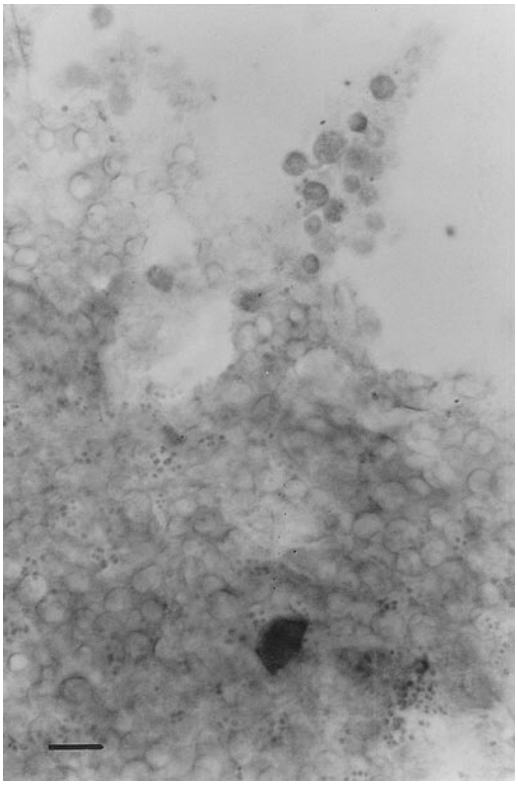


FIGURE 2. Impression smear of ventral skin stained with Periodic-acid Schiff (PAS) demonstrating the spherical fungi in the epidermis from a Canadian toad infected with *Basidiobolus ranarum.* Periodic-acid Schiff stain. Bar = 10 μm.

(Biolog Panels, Biolog, Inc., Hayward, California, USA).

Fungal cultures were conducted by placing sections of ventral abdominal skin and digits with the internal skin side down towards the agar on Sabouraud agar slants and grown at room temperature (22 C) for 1 mo (Rippon, 1982). Isolates were stained with lactophenol cotton blue and identified microscopically by morphology (Rippon, 1982). Impression smears of ventral skin were air dried, heat fixed, stained by PAS, and examined by light microscopy.

All toads were judged to be in excellent fat body condition upon postmortem evaluation based on plump fat bodies and the quantity of pericardial fat. Gross examination revealed ventral abdominal hyperemia and sloughing of superficial layers of epidermis were present in all toads that died. Similar lesions occurred on the toes. Livers were enlarged, mottled and gray. Histologically, the ventral abdominal and digit skin from all toads was characterized by erosions with numerous fungal spherules measuring 8.8 μm (SD = 1.6) × 7.4 μm (SD = 1.2) and occasionally hyphae in the superficial layers of the epidermis without a significant inflammatory reaction (Fig. 1). Impression smears of the ventral skin over the abdomen stained with PAS demonstrated spherical fungi in the epidermis (Fig. 2). Hepatocytes were swollen and aggregates of pigment bearing macrophages were present in some of the livers. While some toads were frozen, the lesions seen were identical to those toads

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not frozen; thus, freezing artifact is not a significant factor.

Bacterial cultures of skin, celomic fluid, liver, and lungs revealed *Aeromonas hydrophila* from six (29%) of 21 and *Pseudomonas aeruginosa* from five (24%) of 21 toads. In addition, *Flavobacterium indolgenes, Citrobacter freundii, Acinetobacter lwoffi, Pleisomonas shigelloides, Bacillus spericus, Enterobacter cloacae, Escherichia coli, Klebsiella ozaenae, Proteus mirbilis, Proteus vulgaris, Serratia liquefaciens,* and *Vibrio mettchnikovii* were cultured from the internal organs of <4 toads. *Aeromonas* spp. are frequently thought to be the predominant cause of redleg in captive amphibians (Gibbs, 1963; Nyman, 1986). However, *Aeromonas* spp. are common in aquatic environments (Hazen et al., 1978; Palumbo, 1993). In experimental studies, extremely high numbers of bacteria (1.5×10^9) were required to induce mortality in leopard frogs (*Rana pipiens*) (Rigney et al., 1978). *Aeromonas hydrophila* was isolated from 94 (32%) of 294 clinically healthy free-ranging leopard frogs, thus demonstrating that it is not always pathogenic (Hird et al., 1981). Other bacteria including *Pseudomonas* spp. (Brodkin et al., 1992), *Staphylococcus* sp., *Citrobacter* sp. (Gibbs, 1963), *Klebsiella* sp., and *Flavobacterium* spp. (Olson et al., 1992) have been associated with red leg in anurans. *Citrobacter freundii* and *Acinetobacter lwoffi* have been reported to cause localized infections in amphibians (Li & Lipman, 1995). The other cultured bacterial species have not yet been reported to cause amphibian morbidity or mortality.

Basidiobolus ranarum was cultured from 11 (52%) of 21 toads (Fig. 3). We were able to increase recovery of this organism by deviating from traditional skin scrapings and placing whole sections of digits and ventral skin inner side down on the agar. African clawed frog skin has been found to contain antimicrobial peptides (Zasloff, 1987). If this were true with the Wyoming toad skin then by placing it internal side down we may have avoided this antimicrobial effect and thus increased organism recovery. Fungal species identified from <5 toads included *Mucor* sp., *Aspergillus* spp., *Penicillium* sp., *Rizopus* sp., and *Fusarium* sp. The lesions of *B. ranarum* infection in the skin may have provided an avenue for secondary bacterial invasion. Other fungi isolated from these toads were considered nonpathogenic contaminants (Rippon, 1982; Koneman and Roberts, 1985).

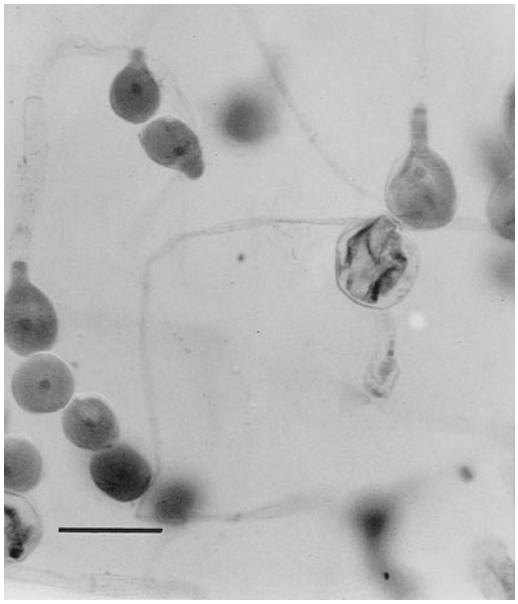


FIGURE 3. *Basidiobolus ranarum* cultured from the ventral skin of a Canadian toad that died of mycotic dermatitis on a wet mount. Lactophenol cotton blue stain. Bar = 30 μm.

Changing husbandry, for 72 hr to allow for attempted breeding, approximately 4 wk prior to the epizootic could have contributed to increased stress and transmission of *B. ranarum* among animals. Woodhouse's toads (*Bufo woodhousi*) kept in the same room as the Canadian toads under the same husbandry protocol and food and water source did not demonstrate any signs of clinical disease or mortality. However, the Woodhouse's toads did not display any amplexus and thus were not put in breeding tanks. Histologic evidence of the fungal spherules in the epidermis sup-

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ported this diagnosis. *Basidiobolus* sp. has chitinolytic activity (Gugnani and Okafor, 1980). This may explain why it has an affinity for insect cuticle and has been isolated frequently from insects. Thus, the toad's food base may actually be a source of infection.

Relatively little is known about the amphibian immune system's response to mycotic organisms. Stresses such as those related to husbandry (humidity, temperature, water source, handling) are often generically implicated in infectious disease cases in amphibians. However, while this is probably true, there is minimal physiologic data on amphibians to document this. There is some evidence to support seasonal variation in the immune system of amphibians. Zapata et al., (1992) reported that in frogs of unreported species, maximal thymus development occurs in the summer with marked regression as winter approaches. Even when the frogs were maintained in an active state in a thermoregulated environment and not hibernated, thymuses regressed. Zapata et al., (1992) showed toads of unreported species to have lower humoral responses to heterologous erythrocytes in the autumn as compared to the spring and summer. This phenomena may have contributed to the increased susceptibility of the Canadian toads to *B. ranarum* infection. We were unable to detect any immunological response upon necropsy or histologic evaluation. The significance of malanomacrophages in amphibians is unknown. In fish, this has been reported to be a sign of chronic inflammatory response (Ferguson, 1989). While this histologic finding in the toads may fit with this sign of chronic disease in fish, further investigation is warranted before the significance of the malanomacrophages are known.

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