Mass Mortality of the Mountain Chicken Frog

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The Commonwealth of Dominica is a small island nation in the southern Caribbean Sea. It is the home to *Leptodactylus fallax,* a large terrestrial frog commonly known as the *crapaud* or *mountain chicken frog* (Figure 1). In the past, this species may have inhabited up to eight islands in the Caribbean, however, it is currently found only on Dominica and Montserrat.

In the fall of 2002, Dominica's Forestry and Wildlife Division (DFW) began receiving reports from the Southern Forest Range of sick and dead frogs (Figure 2). These reports corresponded with the time of year when the local people generally hunt the frogs. Residents of the area also said they had been hearing fewer frog calls.

Several dead frogs were collected and taken to the Animal Health Care Unit of the Agriculture Division (Figure 3). Necropsies were performed, and samples submitted to the local medical hospital for bacteriology and histopathology. A diagnosis of septicemia was made in 11 of 12 frogs.

Shigella, which is generally considered nonpathogenic in amphibians, was present.[1,2] *Shigella* was isolated from the stomachs, small and large intestines, lungs, and hearts of four frogs. {Sample?} Contamination was not suspected.

Diagnostic Workup

The director of DFW, several foresters, the veterinarians from the Agriculture Division, and the author met to determine a plan of action. Nine sites were examined for signs of contamination or disturbance. Soil and water samples, as well as sick and dead frogs were collected. Rainfall and temperature data were obtained and interviews with local residents were conducted when possible.

Site Visits

Each site was visited by the author and two foresters. Findings at the sites included:

- **Bois Cotelette** had several old stone buildings that were under renovation. Sick and dead frogs had previously been observed in a small pond. On this visit, the author found one living frog and two dead frogs in a stone cistern. It appeared that the frogs had been unable to escape, and two died from starvation.
- **Dublanc** consisted of a new dirt road, a river, and a recently cleared field. While exploring the river, the author caught a sick frog which was approximately 10 yards from human feces. It died 30 minutes later. A forester found three decomposing frogs another 10 yards upstream. The owner of the field claimed that he did not use herbicides or pesticides, and that he had only heard a few frogs calling recently.
- **Elmshall** is a subdivision east of the capitol. A sick frog had been collected from a drainage gutter beside a house. No toxic substances were found.
- **Forest Bistro** is a guest house surrounded by forest, citrus crops, and livestock. The owner reported that during the past several months, an undetermined number of dead frogs were found near a pond in a goat enclosure, as well as near a leaking outdoor aquarium. Stone water troughs were present throughout the property. The death of lime trees, while no other citrus species was showing signs of disease, was notable.

  In the small community of **Gallion,** goats and cows were grazing among the ruins. The area was extremely dry, yet was reportedly home to a large frog population. Numerous dead and dying frogs, including frogs infected with *Shigella,* had been found in old stone cisterns or metal troughs.
- **La Haut** had several houses with forested and open areas. Four dead baby frogs were found in a water container outside a chicken coop.
- **Petit Coulibri** had a guest house that was under renovation, and a new road surrounded by a forest and fields with water troughs. Sick and dead frogs were found in the vicinity of a small man-made pond. During the visit, a young, healthy frog was seen sitting in the same pond. A woman claimed that pesticides were not used on the property. While exploring the property, numerous old broken fertilizer containers were found in the ruins of a shed (Figure 4). Though fertilizers were not currently being used, accidental

Figure 1. Mountain chicken frog (*Leptodactylus fallax*).

Figure 2. Dead mountain chicken frog.

contamination was possible. This demonstrates the importance of investigators visiting sites of disease.

- In **Ravine Zombie,** a remote area with a few outbuildings, water was diverted to irrigate dasheen and other crops. Fertilizer pellets were scattered among the plants, and several sick and dead land crabs were present in the vicinity. A large, sick, female frog was captured, but it died a few days later.
- **Springfield Estates,** historically the home of a large frog population, was visited by the author in 1999, 2000, and 2001. In 1999, the frogs were plentiful, but no frogs were seen or heard during the past two trips. The estate's manager claimed to have not heard any frogs calling for the past 2 years. The fields that had been cleared had become overgrown, and the streams more polluted.

Histopathology

Though a diagnosis of septicemia was made, it should be noted that initial histopathology was performed by a pathologist in human medicine who may have been unfamiliar with some of the normal findings in amphibians. The same slides were also submitted to a veterinary diagnostic laboratory. Parasites were found in all animals, in the lungs, digestive tracts, and/or peritoneal surfaces. These parasites included nematodes and *Acanthocephalus spp.* These were considered normal species and of a "manageable" load for frogs in the wild. Some frogs also had changes in the dermis. The fungus *Batrachochytrium dendrobatidis* was identified in one individual.

Bacteriology

Early during the outbreak, samples from four dead frogs were submitted by the Agriculture Division to the local human medical hospital for bacteriologic culturing. Because of the poor economy, no other frogs were

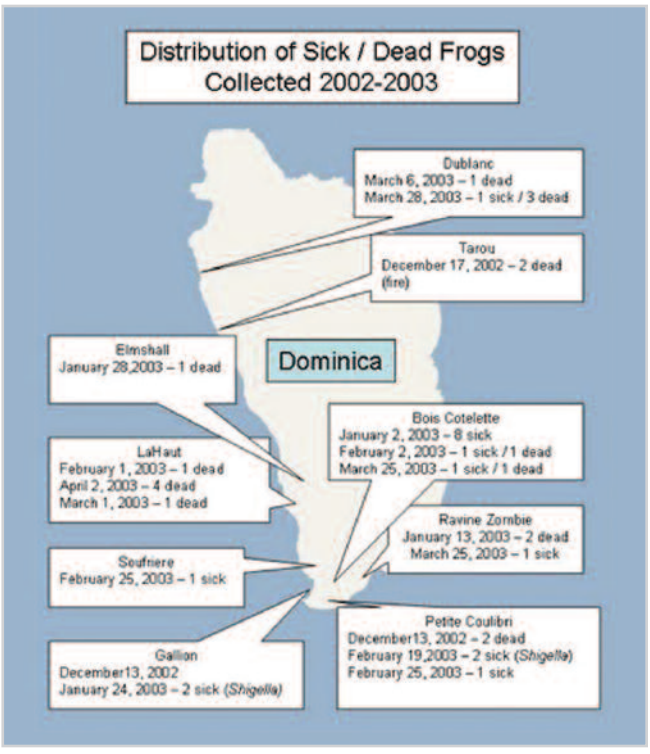

Figure 3. Distribution of sick and dead frogs during the 2002-2003 outbreak.

sampled. Samples were plated on blood agar, MacConkey agar, and *Salmonella-Shigella* (SS) agar. Primary isolates were normal enteric bacteria (Table 1). As stated earlier, the only unexpected finding was *Shigella* in multiple organs.

Toxicology

Water, soil, and tissue samples were submitted to a diagnostic laboratory. Limited toxicology was performed because of funding issues. Pesticides and fertilizers were the primary concern. All samples tested were negative.

DNA Testing

Tissue samples from ten frogs were submitted to Pisces Molecular LLC for PCR DNA analysis for the fungus *B. dendrobatidis.* Seven samples tested positive. One of the four frogs found in a small water container tested positive for *B. dendrobatidis.* There is the possibility that the other three frogs within that container may have also been infected and produced false negative results.

Discussion

No common factors among the visited sites could be determined. Some sites were agricultural, some were forest, and others were residential. All were along the west coast, but this is the normal range; frogs are not found on the east coast due to climatic conditions. It is possible

that there may have been multiple causes of deaths during the outbreak, although the primary supported etiology is that of chytridiomycosis.

Chytridiomycosis is caused by *B. dendrobatidis*. Chytrids, the common name for fungi in the phylum Chytridiomycota, are microscopic, zoospore-producing fungi found in soil and water. In 1998, *B. dendrobatidis* was identified in wild frogs in Australia and Panama, and captive frogs in Washington, DC.[3,4] Since that time, it has been identified in more than 100 species of amphibians in Africa, Australia, Europe, North America, Central America, and South America.[1] It is the only species of chytrids to infect vertebrates.

Figure 4. Though it was reported that fertilizers were not being used at Petit Coulibri, old broken fertilizer containers could be found on the property.

The pathogenesis of chytridiomycosis is not understood. Chytrids infect keratinized tissue; superficial layers of the skin and the mouthparts of tadpoles. It does not affect internal organs. The exact mechanism of death is unknown, but is suspected to affect hydration, osmoregulation, respiration, or a combination of these factors. It may also affect immunity or be involved in toxin production.

Infected individuals often show no clinical signs until shortly before death. Anorexia, lethargy, and excessive skin shedding are the most consistent findings. Tadpoles may show loss of pigmentation in tooth rows, swollen and pinkish or reddish labial papillae, or lost, interrupted, or misshapen toothrows.[5]

Histologically, lesions are described as a diffuse epidermal hyperplasia, hyperkeratosis, with colonization of the keratinized layers of the epidermis.[6] Small, scattered foci of epidermal degeneration are present in some frogs and are occasionally accompanied by infiltration of the underlying dermis by low numbers of neutrophils and macrophages. Organisms are located within the cytoplasm of epidermal keratinocytes and their morphology typically consists of 10 to 15 µm diameter, round to oval, fungal cell bodies (thalli), many of which contain internal septa [auth: septa ok?] and/or zoospores.

Diagnosis of chytrids is done by histology or PCR. In this study, the Dominican human medical pathologist did not identify any chytrids, but the veterinary pathologist identified one infected individual. PCR was the most successful means of diagnosis.

In 2006, skin scrapings were obtained from 90 preserved *L. fallax* held at the Smithsonian's Museum of Natural History in Washington, DC. These specimens had been collected in Dominica between 1929 and 1980 with the majority in the mid-1960s. All samples tested negatives for chytrids. This suggests that chytrids are new pathogens to Dominica, although it is possible that the virulence of a pre-existing fungus may have increased. It is also possible that the frogs' immune system may have been suppressed and thus allowed *B. dendrobatidis* to spread through the population. Taylor, et al, stated, "Brief exposure to even minor adverse environmental conditions may prove disastrous to amphibians, altering their immune response and metabolism beyond their ability to cope with infectious disease."[7]

There are environmental concerns in Dominica that may have contributed to immunosuppression. The Careme, or dry season, of 2001 was reported as being the worst drought in 30 years.[8] This dry season was followed by a 10-year high amount of rainfall in 2002.[9] Extremes in precipitation may affect the immune system.

Pollution in streams appears to have increased during the past several years, and human feces were found in two streams during site visits. This may account for the presence of *Shigella* in several frogs and *E. coli* (too numerous to count) in a water sample collected in an isolated area.

Chemical contamination could be a contributory factor. Dominica's economy is primarily agricultural—bananas are the largest crop produced. Great amounts of fertilizers and herbicides have been used in the past. Sick frogs and land crabs were found in streams that contained fertilizer pellets.

Since chytrids are limited to keratinized tissue, topical medications can be used to eliminate the fungus.[1,10] Medications that have been reported to successfully treat infected frogs include itraconazole, miconazole, fluconazole, ketoconazole, formalin/malachite green, and chloramphenicol.[1,11,12] Elimination may require treatment for 10 days. Individual and small groups of frogs can be easily treated, but there are currently no means available to treat large populations in the wild.

Even if mass treatment was possible, chytrids have been shown to remain infective in organic material for at least 7 weeks and can be transmitted by fomites.[13] Therefore, it is important to prevent contamination between sites. Field equipment, shoes, and clothing should be completely dried or rinsed with a disinfectant solution of 1 part bleach per 16 parts water.

Table 1. Bacteriology of dead *Leptodactylus fallax*.

specimen site	frog 1*	frog 2*	frog 3*	frog 4*
oral cavity	gram negative bacilli	mixed gram negative bacilli *Enterobacter* spp *Streptococci*	no growth	no growth
stomach	Bacillus species *Shigella* mixed gram negative organisms	no growth	*Enterobacter agglomerans*	*Shigella* spp
small intestines	*Shigella* serogroup A,B,C gram negative organism			
large intestines		mixed gram negative *Proteus* *Shigella* serotypes		
peritoneal cavity	*Shigella* serogroup A,B,C mixed gram negative organisms	no growth		
caecal swabs			Citrobacter	mixed gram negative rods
vent swab			gram negative bacilli	mixed growth
kidney	gram negative organism	mixed gram negative bacilli		no growth
liver	gram negative organism	mixed gram negative bacilli	no growth	no growth
lung	*Shigella* serogroup A,B,C gram negative organism	*Enterobacter agglomerans*	no growth	no growth
heart	*Shigella* serogroup A,B,C	*Shigella* serotypes		

***Frogs 1 and 2 were collected at Gallion on January 24, 2003.**
Frogs 3 and 4 were collected at Petite Coulibrie on Feburary 19, 2003.

It has been suggested that chytrids spread through a population by physical contact when groups of frogs converge in a common breeding area.[14] Mountain chicken frogs are not "communal" breeders; two or three males compete for a single female. Because of this behavior, chytrids may be limited to small pockets of Dominica.

Another issue of concern is the presence of *Shigella,* a gram-negative aerobic bacteria. It is highly communicable (as few as ten bacteria is an infective dose[15]) and can be transmitted by contact with infected individuals, contaminated premises, food, water, or by vectors. It can survive for 3 to 8 days in the feces, on the body surfaces, or in the intestinal tract of cockroaches,[16] and for extended periods in a moist environment.

Shigella gains access to the intestines via the fecal–oral route. It penetrates intestinal epithelial cells and undergoes intracellular replication in the submucosa or lamina propria. Deeper penetration into the bloodstream and resulting bacteremia is possible. Diarrhea is the primary symptom in humans, but symptoms can range from gingivitis to seizures or death[17] (Table 2).

Shigellosis is a disease of public health concern and is most prevalent in children under 10 years of age, the elderly, and debilitated people. Each year, an estimated 130–140 million cases of diarrheal disease and 576,000–585,000 deaths associated with *Shigella* occur in children under 5 years of age.[18]

Only two reports of *Shigella* in frogs were found in the literature.[2] Neither case involved clinical signs of infection or illness. Dominican frogs had shown clinical signs (hyperemic skin, emaciation) consistent with chytridiomycosis, but most also had hemorrhagic/congested organs, which usually are not seen with this disease. The latter findings may be a result of a secondary bacterial infection, such as *Shigella.*

Frogs can serve as vectors for zoonotic disease. One study examined the role of frogs infected with *Shigella* and the impact on public health.[2] In the paper, the au-

Table 2. Syndromes and symptoms associated with Shigellosis in humans/primates.

Enteric Complex	Neurological Syndrome
Sitting forward with head between the hunched hind limbs	Lethargy
Drawn facial expression	Confusion
Tenesmus / painful, often ineffectual attempts at defecation	Severe headache
Abdominal pain (mild to severe) / cramps	Hallucinations
Edema of the facial and neck	Obtundation
Depression / lethargy / weakness	Frontal lobe signs
Prostration	Decorticate or decerebrate posturing
Anorexia	Scissoring
Weight loss / emaciation	Coma
Fever	Meningitis (extremely rare)
Feces may contain blood, mucus, and pus	
Diarrhea	
Dehydration	
Rectal prolapse	
GI ulceration	
Toxic megacolon	
Shock	

Hemolytic-Uremic Syndrome	Gingivitis Syndrome
Hemolytic anemia	Spontaneous bleeding of gingival surfaces
Acute renal failure	Gingival / periodontal recession
Oliguria to anuria	Alveolar attachment loss
Cardiac failure	Loss teeth

Bacteremia Syndrome	Death (due to)
Cecal perforation	Toxic megacolon
Toxic megacolon	Intestinal perforation
Bone marrow aplasia	Sepsis
Septicemia	Pneumonia
	Hemolytic anemia
	Hyponatremia
	Hypoglycemia
	Seizures
	Encephalopathy
	Malnutrition

thors stated, "Species like the common toad are recognized as important vectors in the epidemiology of Caribbean islands," and "the habitat and migration of frogs might facilitate the transfer of potential human pathogens from one water reservoir to another and thereby disseminate human pathogens."

So, how did the frogs become infected? The main hospital of Dominica reported 18 cases of *Shigella* in hospitalized patients in 2002. These cases did not include those treated in local villages. A majority of the cases occurred during the end of the year—approximately the same time *Shigella* was found in the frogs. Because *Shigella*'s natural hosts are humans, animals that are infected with or serving as vectors of *Shigella* must first contact human waste or another animal that had contacted human waste.

Treatment

Numerous factors were involved in this outbreak. Some of the factors can be addressed, such as pollution. Others, such as weather conditions, are out of our control. A partial list of recommendations presented to the

Dominican government included:

Samples should be collected from multiple locations and from all species of Dominican frogs (*Eleutherodactylus amplinympha, E. johnstonei, E. martinicensis, and Leptodactylus fallax*) to determine the extent of the disease (chytridiomycosis and shigellosis) in the country.

Foresters should thoroughly dry or disinfect field equipment, shoes, and clothing during daily activities.

Public service announcements should address potential human health hazards resulting from the handling and consumption of frogs.

A hunting moratorium should be enacted.

A "clean stream" program should be developed with three components; education about the importance of stream health (Figure 5), monitoring of streams for water quality, and cleaning of polluted areas.

Figure 5. Roadside sign warning of threats to mountain chicken frogs.


Figure 6. Baby mountain chicken frogs leaving the nest.

Monitor the use of chemicals in the environment and educate the public about proper chemical use and disposal.

If a chytrid-free area is found, frogs should be quarantined, examined for signs of disease, and treated for chytrids as a preventative. The frogs should then be relocated.

An off-island captive breeding program should be developed. An on-island breeding project could be attempted, but issues regarding adequate supplies of food for frogs and re-infection should be addressed first.

The local medical community should closely monitor *Shigella* in the human population. Rural areas are of most concern.

These efforts are necessary for the preservation of the frogs and for the welfare of the environment and the human population of Dominica.

Follow-up

In 2003, thirteen Dominican frogs were captured and samples were collected. No signs of chytrids were noted. In 2006, it was estimated that 95% of *L. fallax* had died. By 2007, only occasional calls were being heard. The Montserrat population had declined because of volcanic activity in the area. In 2009, chytrids reached Montserrat and frog populations were decimated.

There are twelve institutions in the US and Europe holding Montserrat frogs in captivity. They have been bred in three zoos. Dominican frogs are being held in two institutions. They have been successfully bred on several occasions (Figure 6). The fate of this species remains in question.

This case is outside the realm of typical small animal practice, but it shows that veterinarians should not be afraid to step out of the clinic. By using the procedures and practices that veterinarians use on a daily basis, the profession can make an impact on a global level. **vF**

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