PRELIMINARY PATHOLOGIC FINDINGS IN BULLFROG (*Rana catesbiana*) AND GREEN FROG (*Rana clamitans*) LARVAE COLLECTED FROM FARM PONDS IN TENNESSEE

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ABSTRACT

Amphibian populations are declining worldwide, with anthropogenic stressors as a common mechanism among leading hypotheses. An anthropogenic stressor that has not been explored in detail is the influence of cattle grazing in farm ponds. In particular, cattle may introduce pathogens or change the aquatic environment such that resident larval amphibians become stressed and immunity is compromised. Amphibians also may function as reservoirs for pathogens (e.g., *Cryptosporidium parvum*, *Leptospira* spp., *Salmonella* spp., *Escherichia coli*, *Mycobacterium paratuberculosis* and *Listeria monocytogenes*) that are associated with cattle and could be ultimately transferred to humans as a foodborne illness. Thus, our objective was to compare pathogen prevalence in bullfrog and green frog tadpoles inhabiting farm ponds with and without cattle access. We collected 101 bullfrog and 80 green frog tadpoles from eight farm ponds in February, June, and October 2005, and compared prevalence between cattle-access treatments. Iridovirus, *Aeromonas hydrophila*, *Pseudomonas* spp., *Corynebacterium* spp. and *Mycobacterium* spp. were isolated from tadpoles in both treatment groups. All individuals were negative for cattle-associated pathogens. These findings suggest that cattle may not influence pathogen prevalence in amphibians; however, our results may be a consequence of uninfected cattle or relatively low grazing intensity in our cattle-access ponds. A more extensive and prolonged study is planned involving controlled experimental infections of tadpoles with pathogens of concern to amphibians and humans.

Introduction

Amphibian populations are declining and, given that amphibians are considered excellent ecologic indicators, these trends are troubling.1,6 There are numerous hypotheses for global amphibian declines, most which are considered to be anthropogenically induced.1,3 Habitat destruction and degradation are considered the most influential of amphibian stressors, and may influence populations by causing mortality or reducing immunocompetence and thereby increasing susceptibility to pathogens.4,7 Although grazing cattle in wetlands is generally
considered a subtle anthropogenic stressor, its influence on the prevalence of amphibian pathogens has not been documented extensively. Further, the role of amphibians in the epidemiology of pathogens commonly associated with cattle and potentially harmful to humans (e.g., Cryptosporidium parvum, Leptospira spp., Salmonella spp., Escherichia coli, Mycobacterium paratuberculosis and Listeria monocytogenes) has not been explored.

**Methods**

Two treatments, cattle access and non-access (control), exist at eight wetlands (four per treatment) at the University of Tennessee Plateau Research and Education Center (PREC). The PREC is located on Cumberland Plateau near Crossville, Tennessee. Cattle have had access to cattle-access wetlands for >10 yr, whereas non-access wetlands have never been exposed to grazing cattle. Two species of anuran larvae (Rana clamitans and R. catesbiana) common throughout Tennessee and the Southeast were used as study organisms. Larvae were collected (ideally five individuals per species per wetland per sampling event) using seine and dip nets and placed individually in jars containing sterile water. Sampling took place on 15 February, 15 June, and 12 October in 2005.

Once collected, the larvae were humanely euthanatized using benzocaine hydrochloride and necropsied. An intracoelomic swab was collected from each larva for bacterial culture. Tissue from the brain, heart, skeletal muscle, skin, respiratory organs (lung and gills), spleen, liver, kidney, reproductive tracts, adrenal glands, stomach, intestines, bone marrow, sinonasal cavity, and eye were fixed with 10% buffered formalin, embedded in paraffin wax, sectioned, stained by hematoxylin and eosin and examined by light microscopy. Additionally, a subset of the above tissues was collected for bacterial and viral analysis. Feces were collected for parasite screening via fecal flotation and PCR for Cryptosporidium spp.

**Results and Discussion**

A total of 181 larvae were collected. Although rare erythematous cutaneous gross changes were noted, only incidental or mild histologic changes were seen and did not correspond to gross findings. Iridovirus was detected with virus isolation and/or PCR in larvae from two of the ponds with no cattle access and one pond with cattle access. The organisms Aeromonas hydrophila, Pseudomonas spp., Corynebacterium spp. and Mycobacterium spp. were isolated from routine and special bacterial cultures from individuals of both treatments. All the samples were negative for Leptospira spp., Salmonella spp., Escherichia coli, Mycobacterium paratuberculosis and Listeria monocytogenes. Acid-fast stains of the fecal preparations were positive, but negative for Cryptosporidium spp. based on tissue PCR. Microsporidiosis was suspected after histologic examination of the corresponding tissues.

Although we documented the occurrence of pathogens associated with worldwide amphibian declines in our tadpoles (Iridovirus, Aeromonas hydrophila), prevalence was not greater in cattle-
access ponds. This may have been a consequence of relatively low grazing intensity in our farm ponds (35-40 cattle/ha), which possibly did not compromise amphibian immunity or health. Even though, cattle did not increase pathogen prevalence, they can trample egg masses and decrease shoreline vegetation and water quality, which can negatively affect amphibian demographics (Gray, unpublished data). We also did not detect pathogens of concern to humans in our tadpoles; however, this could have been a consequence of uninfected cattle at our study site. Given that controlled studies have isolated some foodborne pathogens from amphibians, we maintain that amphibians could play a role in the epidemiology of foodborne pathogens associated with cattle. For example, Graczyk et al. experimentally infected adult amphibians with \textit{C. parvum}, and documented shedding of this pathogen in amphibian feces up to 12 days post inoculation.\(^5\) This duration is certainly sufficient time for an amphibian to release this pathogen in a farm pond, and possibly disperse it overland to adjacent farm ponds and cause infection of naïve individuals. Therefore, to expand upon our preliminary findings, a more extensive study involving multiple study sites and years and controlled experimental infections is planned.

**LITERATURE CITED**