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Author(s): Bridgid Lammers, David Bemis, Phil Colclough and Marcy J. Souza

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## Prevalence of *Salmonella* sp. Isolated from *Cryptobranchus alleganiensis alleganiensis* in Eastern Tennessee

Bridgid Lammers<sup>1</sup>, David Bemis<sup>2</sup>, Phil Colclough<sup>3</sup>, and Marcy J. Souza<sup>2,\*</sup>

**Abstract** - *Cryptobranchus alleganiensis alleganiensis* (Eastern North American Hellbender) were collected from the Little River of the Great Smoky National Park ( $n = 22$ ) and the Hiwassee River of the Cherokee National Forest ( $n = 30$ ), TN. The cloaca of each animal was swabbed for *Salmonella* culture and identification. No *Salmonella* was identified from any of the samples. The prevalence of *Salmonella* in Hellbenders may be higher than reported here since feces were not collected and animals were only sampled once. Park visitors should still take precautions to protect themselves from water-borne zoonotic pathogens.

### Introduction

The enteric bacterium *Salmonella* presents a challenge to public health (Herikstad et al. 2002). Many *Salmonella* serotypes have been isolated from a variety of reptile and amphibian hosts and have been linked to the occurrence of disease in humans (Burnham et al. 1998, CDC 2009, Clarkson et al. 2010, Lockhart et al. 2008, Mermin et al. 2004, Rosenstein et al. 1965, Srikantiah et al. 2004, Van Meervenne et al. 2009, Woodward et al. 1997). Annually in the United States, approximately 74,000 people are infected with *Salmonella* via contact with an amphibian or reptile.

Historically, amphibians have had a lower prevalence of *Salmonella* than reptiles (Chambers and Hulse 2006, Pflieger et al. 2003). However, amphibians worldwide have been shown to carry *Salmonella* at least occasionally (Bartlett et al. 1977, Chambers and Hulse 2006, Everard et al. 1979, Kourany et al. 1970, Pflieger et al. 2003, Sharma et al. 1974, Thomas et al. 2001, Trust et al. 1981). Studies have linked human salmonellosis with amphibian reservoirs (CDC 2009, Clarkson et al. 2010, Mermin et al. 2004, Pflieger et al. 2003, Srikantiah et al. 2004).

Although the occurrence of *Salmonella* infections in humans and domestic animal reservoirs has been monitored, few studies have examined the prevalence of this pathogen in wildlife, and in particular amphibians (Chomel et al. 2007). Even less is known about *Salmonella* in salamander species. Chambers and Hulse (2006) found that among wild-caught native amphibians, salamanders had a lower prevalence (11%) of *Salmonella* than did all amphibian species combined (39%). There are no data available on the prevalence of

<sup>1</sup>University of Tennessee College of Veterinary Medicine, Knoxville, TN, 37996. <sup>2</sup>Department of Biomedical and Diagnostic Sciences, University of Tennessee College of Veterinary Medicine, Knoxville, TN 37996. <sup>3</sup>Knoxville Zoological Gardens, PO Box 6040, Knoxville, TN 37914. \*Corresponding author - msouza@utk.edu.

*Salmonella* in *Cryptobranchus alleganiensis alleganiensis* (Daudin) (Eastern North American Hellbender).

The Eastern North American Hellbender may sit on the top of the food chain in the aquatic ecosystems that it inhabits (Nickerson and Mays 1973). A great diversity of *Salmonella* has been found in natural ecosystems, especially in rivers, and the cool, well-oxygenated, alkaline streams that Hellbenders are found in may provide an ideal media for the growth of *Salmonella* (Barzily and Kott 1991, Baudart et al. 2000, Morse and Duncan 1974, Nickerson et al. 2003). It has been demonstrated that direct contact with a shedding animal is not necessary to transfer *Salmonella* from animal to human, and the pathogen can remain viable in water even after the animal has been removed (Bartlett et al. 1977, Rosenstein 1965, Srikantiah et al. 2004, Van Meervenne et al. 2009).

There has been no published research examining the potential contamination of waterways with *Salmonella* from native amphibian species; this contamination could potentially lead to human salmonellosis in park visitors. This study examined the prevalence of cloacal *Salmonella* in two distinct populations of Hellbenders in East TN.

## Materials and Methods

### Study area

Hellbender populations are present in the Little River (LR) of the Great Smoky Mountain National Park (GSMNP), TN and Hiwassee River (HR) of the Cherokee National Forest (CNF), TN. Both rivers are used for many recreational purposes such as boating, fishing, swimming, and sightseeing/camping along the bank. The GSMNP and the CNF have millions of visitors annually, with an unknown portion of these visitors engaging in water-related activities.

### Study sites

River sites were chosen based on accessibility, appropriate habitat of shallow, cool, fast moving waters, and the presence of known Hellbender populations from historical surveys. Sites were surveyed from April to September 2010.

### Animal collection and sampling

Hellbenders were collected by hand in both rivers. Disposable nitrile gloves were worn by investigators and were changed after handling and sampling each animal. Animals were placed in cloth or plastic bags with water for holding. Cloth bags were not re-used until laundered with detergent in hot water; plastic bags were disposed of after one use. A swab (BBL™ Culture-Swab Plus™, Becton Dickinson and Co., Sparks, MD) was inserted through the vent into the cloaca and rotated 3–4 times. The swab was then placed into the sterile transport media and kept cool. Morphometrics (total length [cm], snout-to-vent length [cm], mass [g], and age class) were recorded. Age class (adult, sub-adult, juvenile, larva) determination was based on length, mass, and observer experience. PIT tags were placed in each animal over 40 g

for identification, and GPS coordinates were recorded for each animal's collection site.

### Bacterial cultures

Cloacal swabs underwent a routine bacteriology screen for *Salmonella*. This procedure included MacConkey's agar (MAC), Hektoen (HE) agar, and Selenite broth incubated at 35° Celsius. At 24 hours, any colonies seen were recorded; if no growth or only very small colonies were observed, the plates were incubated for an additional 24 hours. If lactose negative colonies were identified, they were tested for oxidase. All oxidase negative colonies were screened with Triple sugar iron (TSI) agar, urea agar, and Motility Indole Ornithine (MIO) agar. If the selenite broth was cloudy at 24 hours, the contents were subcultured to HE agar. If selenite broth appeared clear at 24 hours, it was incubated for 48 hours before subculture. All negative plates were held for 48 hours before discarding.

### Statistical analyses

Prevalence and 95% confidence intervals (CI) were calculated for each of the two Hellbender populations. Odds-ratio with confidence intervals was calculated to determine if significant differences in prevalence between the two populations were present ( $P < 0.05$ ).

## Results

A total of 52 Hellbenders were sampled from both rivers (LR,  $n = 22$ ; HR,  $n = 30$ ). Adults (LR,  $n = 8$ ; HR,  $n = 11$ ), sub-adults (LR,  $n = 0$ ; HR,  $n = 1$ ), juveniles (LR,  $n = 5$ ; HR,  $n = 17$ ) and larvae (LR,  $n = 9$ ; HR,  $n = 1$ ) were collected from both rivers. Morphometrics are shown in Table 1.

Table 1. Morphometric data of Eastern North American Hellbenders (*Cryptobranchus alleganiensis alleganiensis*) caught and sampled in two eastern Tennessee rivers, the Little and Hiwassee. Age class, snout-to-vent length (SVL; cm), total length (TL; cm) and mass (g) are reported according to which river animals were collected.

River	Age class ( $n$ )	SVL mean $\pm$ SD (range)	TL mean $\pm$ SD (range)	Mass mean $\pm$ SD (range)
Hiwassee	Adult (11)	20.2 $\pm$ 3.4 (15.6–26)	30.3 $\pm$ 4.4 (23.5–36)	183.8 $\pm$ 78.3 (86–330)
	Sub-adult (1)	19.1	33.0	197
	Juvenile (17)	13.8 $\pm$ 4.8 (3.2–22.2)	21.2 $\pm$ 5.9 (6.5–31.8)	80.2 $\pm$ 62.2 (8–235)
	Larvae (1)	2.5	4.0	1.0
Little	Adult (8)	21.0 $\pm$ 5.1 (12.7–26.7)	32.2 $\pm$ 8.5 (19.7–41.3)	206.0 $\pm$ 106.5 (36–337)
	Juvenile (5)	8.8 $\pm$ 3.4 (3.8–12.4)	13.7 $\pm$ 5.0 (6.4–19.7)	19.8 $\pm$ 14.6 (2–35)
	Larvae (9)	4.6 $\pm$ 1.3 (3–7.6)	6.7 $\pm$ 2.2 (4–11.4)	3.8 $\pm$ 2.5 (2–8)

The prevalence of cloacal *Salmonella* isolated from Hellbenders was 0% [95% CI = 0–6.85] from the Little River and Hiwassee River. Because no *Salmonella* was isolated from either population, no further statistics were performed.

### Discussion

The lack of cloacal *Salmonella* isolated from Hellbenders in eastern TN suggests a small risk of transmission to humans in the Little and Hiwassee Rivers. Although the prevalence was zero, various factors could have led to a lower measured level than the true prevalence.

*Salmonella* can be shed intermittently by a carrier animal, and fecal samples are typically collected over the course of 3–5 days for culture and identification. Fecal samples are generally preferred over swabs to isolate *Salmonella*. Sampling a wild animal over numerous consecutive days is not feasible, and fecal samples are not reliably produced by animals. Animals were only held captive for 10–15 minutes during this study. Therefore, single, cloacal swabs were collected from each animal. The true prevalence of *Salmonella* in Hellbender populations may be higher than that found in this study.

The total number of Hellbenders in either of the rivers is not known, and sampling a larger percentage of the population would certainly increase the power of the study. These animals were being collected as part of another study and were sampled opportunistically for *Salmonella*. It is difficult to know if the sampled population is representative of the entire population of Hellbenders in each river.

There are few studies examining the prevalence of *Salmonella* in wild amphibians, and none in Hellbenders. Future studies may keep animals captive for longer periods of time in order to collect a fecal sample; the stress associated with extended capture would need to be evaluated. Collecting more animals would increase the likelihood of accurately sampling the entire population. Outbreaks of human salmonellosis associated with amphibians have been reported, and monitoring of *Salmonella* in various amphibian species is warranted. Additionally, humans coming in contact with amphibians or water potentially contaminated by amphibians should be warned to wash themselves after contact and to not drink untreated water. These precautions will reduce the risk of contracting *Salmonella*, as well as other zoonotic pathogens found in untreated water such as *Giardia* and *Cryptosporidium*.

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