



# The Prevalence of *Salmonella* Bacteria in Captive Reptiles and Amphibians found in New York City

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## Abstract

Our objective for this experiment was to investigate how widespread *Salmonella* and other pathogenic bacteria are in the captive reptile and amphibians in NYC. This is important because the Center for Disease Control (CDC) has documented several ongoing nationwide *Salmonella* outbreaks, with most victims being under 5 years old. We carried out our experiment by screening samples for a *Salmonella* associated gene (hil-A), and found that only 1/51 tested reptiles and amphibians carried *Salmonella*. We also assessed the microbiome of 18 turtle samples, and found an additional sample contained *Salmonella* and that most of the bacterium contained in the samples were non-pathogenic. These results suggest that pet reptiles and amphibians pose little threat to humans.

## Introduction

- *Salmonella* is a bacterium occurring in the intestine that causes a type of food poisoning called Salmonellosis.
- Previous studies have shown that *Salmonella* can be spread to humans through contact with pet reptiles and amphibians like turtles, lizards, and frogs. However, these studies have also indicated that *Salmonella* infection rates in reptiles and amphibians are low (usually around 5–6%).
- There are two species of *Salmonella*: *Salmonella bongoria* and *Salmonella enterica*, of which there are around six subspecies and innumerable serovars. *S. enterica* is the species that is usually found in reptiles (Trafny *et al.*, 2006). The gene most commonly used to discern *Salmonella* from other forms of bacteria, and to identify subspecies and serovars, is the hilA gene (Pathmanathan *et al.*, 2003).
- In this project, we tested the following two hypotheses:
  - 1) *Salmonella* infection rates in turtles and lizards will be low (5–6%)
  - 2) Turtles will harbor mostly non-pathogenic bacteria.

## Example Species used in this Study



Leopard gecko



Gray tree frog



Snapping turtle



Ball python

## Materials and Methods

- Samples were obtained through oral or fecal swabs of the reptile or amphibian.
- Genomic DNA was extracted using a Mol Bio PowerSoil Kit.
- Using PCR, we amplified the 16S and HIL-A gene
- We confirmed presence of a band of the appropriate size (~600 – 800 bp) using gel electrophoresis. We then sent away any positive HIL-A samples for sanger sequencing.
- 18 turtle samples were amplified for the V3-V4 region of the 16S gene, and sent away for illumina sequencing to assess the entire microbiome of these samples.

## Sample collection and PCR success

- 56 samples were obtained for DNA extraction (33 turtles, 12 lizards, 4 frogs, 1 gecko and 1 snake). 51 out of the 56 samples were successful DNA extractions because we were able to amplify the 16S gene.

## Salmonella results

- In the screening process using the HIL-A primer, only one sample (#60) came up positive for *Salmonella* (see Fig. 1).
- For the 18 samples analyzed with MiSeq sequencing, we were able to identify small traces of *Salmonella enterica* that existed in three other samples (samples 31, 39, 48), which was not identified with the HIL-A primer. Samples 31 and 39 had a very low read count (<8 reads) while Sample 48 (a red foot tortoise) had more reads (>8 reads), so we only considered Sample 48 as a confirmed *Salmonella* infection.

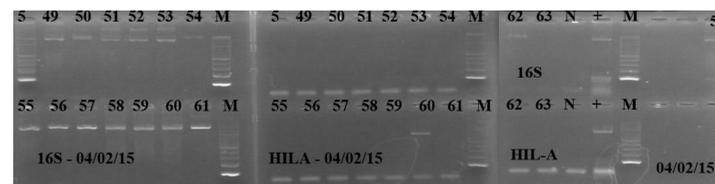


Figure 1. Gel results for several reptile and amphibian samples. The 16S gene was used to confirm successful DNA extractions, and the HIL-A gene was used to screen for positive *Salmonella* results.

## Results

### Turtle Microbiome Results

- Almost all the bacteria identified in the turtle samples were nonpathogenic, and only approximately 11% of the most prevalent bacteria in the samples were found to be potentially pathogenic to humans or plants.
- Samples 36, 38, 47 did not harbor any pathogenic bacteria.
- The most prevalent potentially pathogenic bacteria was *Citrobacter freundii* which is associated with respiratory infections (see Fig 2.).

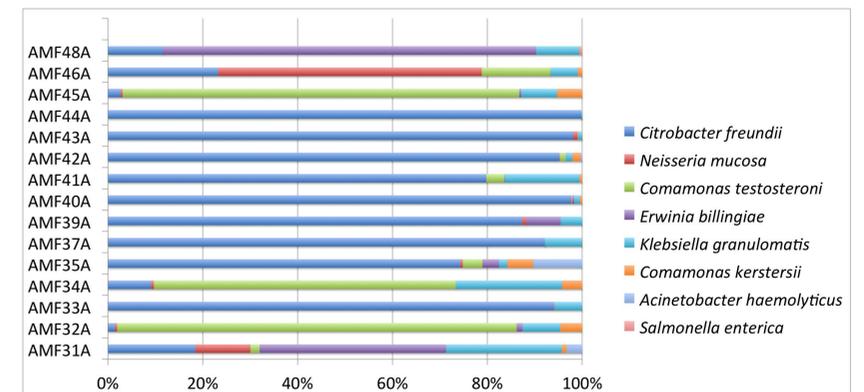


Figure 2. Stacked bar graph showing the samples with bacteria that are potentially pathogenic to either plants or humans.

## Discussion

- We supported our hypothesis that *Salmonella* infections in reptiles and amphibians are low, with only 2/51 samples (4%) showing indication of some *Salmonella* infection. We also found that pathogenic bacteria infection in our samples were low (8/75; 11%), but they did contain 8 species (including *Salmonella*) associated with human or plant disease. The most common pathogenic bacteria is associated with respiratory disease, and this is not surprising since many of the turtles had respiratory infections.
- It is interesting to note that the HIL-A screening gene did not indicate any *Salmonella* infection in samples 31, 39, and 48, but some sequence reads were recovered as *Salmonella* in the miSeq sequencing results. We think the hil-A primer did not detect *Salmonella* infection due to the very low count of *Salmonella enterica* in these samples.
- Only a bearded dragon and a red foot tortoise came up as infected with *Salmonella* (Fig. 3). Captive turtle pets have been a problem for young children who become infected with *Salmonella* (Van Meervenne *et al.*, 2009). The CDC created a rule in 1975 that makes it illegal to sell any turtle with a carapace (shell) of less than 4 inches to help prevent young children from contracting *Salmonella* (Gerner-Smidt and Wichard, 2010). While the focus has been on turtles transmitting *Salmonella*, bearded dragons have also recently been linked to a multistate outbreak of *Salmonella* infection in humans (Center for Disease Control and Prevention report, 2014).



Figure 3. A red foot tortoise (left) and a bearded dragon (Peanut McBrien – right) were the samples showing indication of *Salmonella* infection.

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