

Salmonella enterica in semi-aquatic turtles in Colombia

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Abstract

Introduction: Turtles can be hosts of *Salmonella enterica* serovars which can cause disease both in the animals themselves and in people they come into contact with, especially when the turtles are kept as pets. To investigate the prevalence of *Salmonella* in turtles in Colombia, we studied animals at a wildlife protection centre. The turtles had either been confiscated or donated to the centre.

Methodology: Detection of *Salmonella* spp. was conducted in feces samples using bacteriological cultures and polymerase chain reaction to identify genus and serovar.

Results: By PCR and culture, 30/110 samples (27%) were positive while by PCR alone eight further samples were positive (total of 38/110 (35%) positive). The most common serovar was *S. Enteritidis* (26/38 (68%)) with only one isolate being *S. Typhimurium* (3%). Four (11%) samples were positive for both serovars and seven (18%) could only be identified as *Salmonella enterica* spp.

Conclusions: These results show that turtles in Colombia are commonly infected with *Salmonella* and are a risk for infection to people who come into contact with them.

Key words: Colombia, turtles, *Salmonella*

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Introduction

In Colombia different species of wildlife including turtles are captured and kept as pets. These animals can be reservoirs of zoonotic microorganisms such as *Salmonella* which may affect the health of the animals and their owners [1]. Studies in other countries have shown that up to 94% of turtles may be infected with *Salmonella* and it is estimated that every year 93,000 people are infected with *Salmonella* as a result of contact with reptiles [2]. This prevalence is increasing as more and more people keep reptiles as pets.

To obtain information on *Salmonella* in semi-aquatic turtles in Colombia we tested animals held in a centre of wildlife protection.

Methodology

Turtles and sample collection

Semi-aquatic turtles were studied at the Centro de Atención y Valoración del Area Metropolitana del Valle del Aburrá in Medellín, Colombia. These

animals had either been confiscated because they were possessed illegally or were voluntarily surrendered between April and November of 2007. Information on size, gender, and species was obtained and feces collected from the cloaca onto a sterile swab moistened with saline after the pericloacal area was disinfected with a 5% iodine solution. The feces on the swab were stored in 500 µl selenite broth at 4°C until culture and polymerase chain reaction (PCR) were performed.

Isolation and identification

A further 4.5 ml of selenite broth was added to the 500 µl containing the fecal sample and incubated for 18 hours at 37°C before subculture onto MacConkey and Hektoen agar which was incubated for 18 hours at 37°C. Non lactose fermenting isolates were identified using conventional biochemical tests with API 20E (Biomerieux Inc, Hazelwood, MO, USA) and tested by PCR for the *hilA* gene specific for *Salmonella enterica*.

Table 1. *Salmonella* serovars isolated according to the turtle specie.

Turtle species n and (%)	Number (%) of Samples positives only by PCR	Number (%) of positives by culture and PCR	Number and Serovar identified by Multiplex PCR
<i>Kinosternum dunni</i> n = 39 (35.4)	7 (6.4)	13 (11.8)	1 <i>S. Typhimurium</i>
			13 <i>S. Enteritidis</i>
			5 <i>S. enterica</i>
			1 <i>S. Typhimurium</i> and <i>S. Enteritidis</i>
<i>Trachemys scripta</i> n = 36 (32.7)	1 (0,9)	12 (10.9)	11 <i>S. Enteritidis</i>
			2 <i>S. enterica</i>
<i>Rinochlemys melanosterna</i> n = 23 (21)	0	2 (1.8)	1 <i>S. Enteritidis</i>
			1 <i>S. Typhimurium</i> and <i>S. Enteritidis</i>
<i>Podocnemis unifilis</i> n = 8 (7.3)	0	2 (1.8)	1 <i>S. Enteritidis</i>
			1 <i>S. Typhimurium</i> and <i>S. Enteritidis</i>
<i>Podocnemis lewyana</i> n = 2 (1.8)	0	0	-
<i>Chelus fimbriata</i> n = 1 (0.9)	0	0	-
<i>Chelidra serpentine</i> n = 1 (0.9)	0	1 (0,9%)	1 <i>S. Typhimurium</i> and <i>S. Enteritidis</i>
Total n = 110	8(7,3)	30 (27,3)	38 (34.5)

DNA extraction and PCR tests

Genomic DNA was extracted using a modified lyses buffer protocol [3]. Briefly, 500 µl of selenite broth was centrifuged at 13,680 g for 5 minutes and 1 ml of lysis buffer was added to the resultant pellet. After mixing and centrifugation at 13,680 g for 6 minutes the supernatant was discarded and the procedure repeated. Following a wash in distilled water the pellet was suspended in 100 µl of sterile water in a sterile glass tube and placed in boiling water for 20 minutes. Once the tubes had cooled to room temperature they were frozen at -20°C until PCR was performed.

The extracted DNA was amplified using the oligonucleotide primer set specific for *hila* gene of *Salmonella enterica* [4].

The sequences of the two primers were *hila*
Forward 5'-GCATGGATCCCCGCCGGCGAGATTGTG-3 and
hila Reverse 5'-CGGAAGCTTATTTGCGCCATGCTGAGGTAG-3'.

The negative control was a PCR mixture without DNA template, while the positive control was with DNA extracted from *Salmonella* Typhimurium ATCC14028. Thermal cycling was done in a thermo

cycler MWG (Biotech, High Point, NC, USA), using 4 ul of DNA template, 15 µl of PCR super mix Taq polymerase (Invitrogen, Sao Paulo, Brazil) and 0.5 µl each of primer (0.1 mM) in a volume of 20 µl of reaction mix [5]. The initial denaturation step was at 94°C for 5 minutes, followed by 30 cycles of 94°C for 1 minute, 65°C for 1 minute and 72°C for 1 minute followed by a final extension cycle at 72°C for 10 minutes.

The PCR products were electrophoresed in 1% agarose gel (Amresco, Solon, OH, USA), stained with SybrSafe (Invitrogen, Sao Paulo, Brazil), visualized with ultraviolet light, and photographed using the Epicemi3 Dark room (UVP Bioimaging Systems, Upland, CA, USA). Samples were considered positive when an 854-bp band was detected.

Samples positive for the *hila* gene were tested with a multiplex PCR to determine if the serovar was Typhimurium or Enteritidis. The primers used were those previously reported [6] as follows: for *S. Typhimurium* amplification we used Fli15 forward: 5'-CGGGTGTGCCAGGTTGGTAAT-3' and Tym reverse: 5'-ACTCTTGCTGGCGGTGCGACTT-3'. For *S. Enteritidis* detection we used *Sef 167* forward:

5'-AGGTTTCAGGCAGCGGTTACT-3' and *Sef* 478 reverse: 5'-GGGACATTTAGCGTTTCTTG-3'.

The multiplex PCR protocol was the same as for *hila* amplification but the annealing temperature was 55°C. Samples were considered positive for *S. Typhimurium* or *S. Enteritidis* when bands of 559-bp or 312-bp were detected, respectively.

Statistical analysis

Statistical analysis was performed using the Epi Info 6.0 (Centers for Disease Control and Prevention, Atlanta, GA, USA) statistical program.

Results

Turtles tested

Of the 110 animals studied, *Kinosternum dunni* was the most common (39; 36%) followed by *Trachemys scripta* (36; 33%), *Rinochlemys melanosterna* (23; 20.9%), *Podocnemis unifilis* (8; 7%), *Podocnemis lewyana* (2; 2%), *Chelus fimbriata* (1; 1%) and *Chelidra serpentina* (1; 1%). Fifty (45: 5%) were males and 60 (54: 5%) were females.

Salmonella detection

Positive cultures and PCR for *Salmonella* spp. were obtained on 30/110 (27%) turtles while 8/110 (7%) were positive by PCR but not by culture. Multiplex PCR revealed *S. Enteritidis* was present in 26/38 (68%) positives, *S. Typhimurium* in one (3%) and both in four (11%). The remaining seven samples (18%) could be identified only as *Salmonella* genus. Table 1 shows the *Salmonella* found in the different turtle species.

Discussion

Salmonellosis is endemic in Colombia but available surveillance measures are inadequate to determine sources of infection. We found that 35% of the semi-aquatic turtles confiscated from their owners and living in a centre for wildlife protection in Medellín-Colombia were infected with *Salmonella enterica*. *S. Enteritidis* was most frequently detected in the turtles and this serovar is also the most prevalent isolate from human clinical samples in Colombia [7]. Local experience suggests wild turtles are now quite commonly kept as pets in Colombia and health workers in the country dealing with cases of salmonellosis should be aware that turtles can be sources of infection. The prevalence we found was higher than that reported in other areas, mainly Italy (24%) [8], Korea (30% including turtles and other reptiles) [9], Spain (5%) [10], and the United States

(0%) [11]. Such differences may be due to epidemiological factors in the areas studied and the sensitivity of the tests used [12-14].

Our study shows that people who come into contact with turtles and their feces are at risk of contracting *Salmonella*. Infections can be limited by taking appropriate precautions when handling turtles and developing strategic plans to halt the trafficking of wild animals and their commercialization.

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