

Prevalence of *Salmonella* spp. in red-footed tortoises (*Chelonoidis carbonaria*) from Grenada, West Indies

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Abstract

Background and Aim: There is currently no published information on the prevalence of *Salmonella* spp. in pet red-footed tortoises in Grenada. Monitoring is essential to understand the dynamics of the epidemiology of *Salmonella*. This study aimed to estimate the prevalence of *Salmonella* spp. from pet red-footed tortoises from Grenada and to test for antimicrobial drug resistance of the isolates.

Materials and Methods: Established bacterial culture, serotyping, and antimicrobial susceptibility methods were used. Fecal samples were obtained from 114 tortoises from five parishes of Grenada, and cultured for *Salmonella* using enrichment and selective culture techniques. The serotyping of isolated *Salmonella* was performed at the Office International des Epizooties *Salmonella* Reference Laboratory, Guelph, Ontario, Canada. The isolates were tested for antimicrobial susceptibility in the Microbiology Laboratory of the School of Veterinary Medicine, St. George's University, Grenada.

Results: Fifteen tortoises (13.2%) out of 114 were positive for *Salmonella* spp. Five serovars were identified: *Salmonella* Javiana (6.3%), *Salmonella* Rubislaw (6.3%), *Salmonella* Saintpaul (18.75%), *Salmonella* Glostrup (6.3%), and *S. I: Rough.O;e,h:1,2* (62.5%). All isolates were sensitive to the 12 antimicrobial drugs.

Conclusion: Three serovars (*S. Javiana*, *S. Saintpaul*, and *S. Rubislaw*) are known pathogens causing disease in humans. Since tortoises included in the study were pets, the tortoises may be a possible source of transmission of *Salmonella* to their owners. The tortoise owners should be educated on the importance of hygienic raising of their pets. This is the first report of isolation of *Salmonella* spp. from pet red-footed tortoises in Grenada.

Keywords: antimicrobial susceptibility, Grenada, red-footed tortoises, *Salmonella* spp.

Introduction

Foodborne diseases in mammals and birds are a serious problem worldwide. Ingestion of contaminated food and water with zoonotic pathogens including *Salmonella* spp. can cause serious diseases in humans and animals.

Recently, Maria *et al.* [1] while reviewing foodborne diseases in the Caribbean reported *Salmonella* spp. as the main cause of the disease in Trinidad and Tobago [2], Dominica [3], St. Lucia [4], Grenada [5], and Barbados [6]. Reptiles including various species of tortoises have been shown to carry zoonotic pathogens including *Salmonella* spp. from different parts of the world including pet turtles in Korea [7], Russian tortoises in Spain [8], Russian tortoises in Poland [9],

gopher tortoises in Georgia [10] and in Florida [11], desert tortoises in Arizona [12], European tortoises [13], red-foot tortoises in Brazil [14], captive reptiles from Croatia [15], and from reptiles in Taiwan [16].

In Grenada, *Salmonella* spp. has been reported from wild animal species, including cane toads [17], blue land crabs [18], small Indian mongoose [19], green iguana [20], and brown rats [21]. There is no published report on the isolation of *Salmonella* spp. from tortoises in Grenada.

This study was undertaken with two objectives: (1) To estimate the prevalence of *Salmonella* spp. and their serovars in red-footed tortoises, and (2) to determine the antimicrobial resistance of the *Salmonella* isolates.

Materials and Methods

Ethical approval and informed consent

The research project was approved by the Institutional Animal Care and Use Committee (IACUC) of St. George's University (IACUC 18009. 23rd August 2018). Tortoise owners were randomly selected from five out of the six parishes of the Island of Grenada based on their availability and the acceptance of the owners to include their tortoise in this

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study. The research plan was explained to the owners in detail, and written consent was obtained from those who agreed to participate in the research before the collection of samples from the randomly selected tortoises.

Study area

Grenada is the southernmost country in the Caribbean sea with an area of 348.5 km². The country with low hills, small trees, shrubs, and tropical climate is most suitable for the habitat of tortoises. The environmental temperature remains within a range of 20°C-30°C, preferred climate for red-footed tortoises. The country is divided into six parishes; St. Patrick, St. Mark, St. Andrew, St. John, St. George, and St. David. The terrain throughout the country is similar.

Collection of fecal samples

A cross-sectional study was performed to meet the aim of the research on the population of pet tortoises in Grenada. Demographic information on the location of tortoises in different parishes, gender, and age were taken at the time of sample collection. Fecal samples from 114 tortoises were collected using fecal swabs in the Cary-Blair Transport Medium (Becton, Dickinson and Co., Sparks, MD, USA) during a period of 5 months, November 2018-March 2019. The fecal swabs were transported to the Microbiology Laboratory of School of Veterinary Medicine and cultured the same day. For the collection of fecal swabs, the tested tortoise was put on its back on a hard surface and cloaca was stimulated by gentle finger touch.

Bacterial culture and serotyping

Recommended culture method for the isolation of *Salmonella* by Gorski *et al.* [22] was used with slight modification, as described by Drake *et al.* [17] and Sylvester *et al.* [20]. Briefly, 0.1 ml intestinal content was inoculated into 10 ml of tryptic soy broth (TSB; Remel Lenexa, KS) a non-selective medium and incubated at 37°C for 24 h. After incubation, an aliquot (0.1 ml) of the TSB was inoculated into 10 ml of Rappaport-Vassiliadis (RV) broth (Difco, BD, Sparks, MD) which is selective enrichment medium for *Salmonella*. The culture was incubated at 42°C for 48 h. Subcultures of the RV broth culture were made on xylose lysine desoxycholate selective indicator agar (Difco) and incubated at 37°C for 18-24 h. To increase the chances of isolation of multiple serovars of *Salmonella*, up to five individual colonies with typical *Salmonella* morphology (red translucent colonies with black centers) were subcultured on tryptic soy agar (TSA) plates. Bacterial colonies from TSA plates were tested for agglutination using *Salmonella* O Antiserum Poly A-1 and Vi (Difco BD). All agglutination positive cultures were inoculated into API 20E (Analytical Profile Index, BioMerieux Inc. Durham, NC) strips and incubated at 37°C for 18-24 h for confirmation of *Salmonella* spp. *Salmonella* Typhimurium ATCC 14028 was used as a reference culture. Pure *Salmonella* cultures were

stored in TSA slants and shipped in cold packed containers for serotyping to the World Organization for Animal Health (Office International des Epizooties; OIE) *Salmonella* Reference Laboratory of the Public Health Agency of Canada's National Microbiology Laboratory at Guelph Ontario, Canada, using established methods [23,24]. The serovars were named as per the antigenic formulae listed by Grimont [25].

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed as previously described [26] as recommended by the Clinical and Laboratory Standards Institute (CLSI 2015) [27]. The antibiotic disks used in this research were: Amoxicillin clavulanic acid (AmC) 30 µg, ampicillin (AM) 10 µg, chloramphenicol (C) 30 µg, ceftazidime (CAZ) 30 µg, cefotaxime (CTX) 30 µg, cephalothin (CF) 30 µg, ciprofloxacin (CIP) 5 µg, imipenem (IPM) 10 µg, gentamicin (GM) 10 µg, neomycin (NM) 30 µg, tetracycline (TE) 30 µg, and trimethoprim/sulfamethoxazole 25 µg. Antibiotics disks were obtained from BD, Franklin Lakes, NJ. The CLSI guidelines were used for the interpretation of the inhibition zone size for all antibiotics except for neomycin which as interpreted based on the guideline from manufacturer zone [26,27]. *Escherichia coli* ATCC 25922 was used as a quality control strain [26,28].

Statistical analysis

The data were analyzed by the statistical analysis: Fisher's exact test using graphical statistical software (<http://www.graphpad.com/quickcalcs/contingency2>).

Results

Salmonella spp. was isolated from 15 (13.2%) out of 114 tortoises tested. Single serotype was isolated from 14 tortoises and one tortoise had two serotypes, making a total of 16 serotypes. In one tortoise, mixed infection with *Salmonella* Saintpaul and *S. I: Rough.O;e,h:1,2* was found. *Salmonella* serovars were *Salmonella* Javiana, *Salmonella* Rubislaw, *S. Saintpaul*, *Salmonella* Glostrup, and *S. I: Rough.O;e,h:1,2*. Results of *Salmonella* isolation from different parishes of Grenada are included in Tables-1 and 2.

All *Salmonella* serovars isolated in the present study were found susceptible to all 12 antimicrobial drugs.

Discussion

The present study revealed a 13.2% prevalence of *Salmonella* spp. in red-footed tortoises from Grenada. Five serovars *S. Javiana*, *S. Glostrup*, *S. Rubislaw*, *S. Saintpaul*, and *S. I: Rough.O;e,h:1,2* were identified from the isolates. There is a paucity of *Salmonella* research on red-footed tortoises. However, our findings were comparable to Oberdan *et al.* [14] who reported a 12.3% prevalence of *Salmonella* spp. in red-footed tortoise from Brazil. The prevalence of *Salmonella* spp. in gopher tortoises (*Gopherus polyphemus*) has been reported in South Georgia 2.6% [10] and Florida

Table-1: Isolation of *Salmonella* Spp. from red-footed tortoises in Grenada.

Parish	Male tested	Positive	Female tested	Positive	Unknown sex tested	Positive	Total	Positive
St. Andrew	8	0	8	0	6	0	22	0
St. George	20	1	24	1	8	0	52	2 (3.9%)
St. David	2	1	8	1	0	0	10	2 (20%)
St. John	6	0	7	0	0	0	13	0
St. Patrick	14	9	2	1	1	1	17	11 (64.7%)
Total	50	11 (22%)	49	3 (6.1%)	15	1 (6.7%)	114	15 (13.2%)

Table-2: *Salmonella* serovars from red-footed tortoises in Grenada.

Serovar	Number (%)*	Parishes
<i>Salmonella</i> Javiana	1 (6.3)	St. Patrick
<i>Salmonella</i> Glostrup	1 (6.3)	St. George
<i>Salmonella</i> Rubislaw	1 (6.3)	St. George
<i>Salmonella</i> Saintpaul	3 (18.75)	St. David (1) St. Patrick (2)
S. I: Rough.O;e,h:1,2	10 (62.5)	St. David (1) St. Patrick (9)

**Salmonella* Saintpaul and S. I: Rough.O;e,h:1,2 were isolated from one positive tortoise

10% [11], and in Russian tortoises in Poland, 18.5% [9]. The variation in the prevalence of *Salmonella* spp. among different breeds and places could be because of their different living environments [29].

In the present study, S. I: Rough.O;e,h:1,2 (62.5%) was the most common serovar isolated. Approximately 90% of S. I: Rough.O;e,h:1,2 were confined to one parish, St. Patrick. S. I: Rough.O;e,h:1,2 lack O antigen and are untypable. To know the pathogenicity and clinical significance of untypable isolates, they need to be further studied.

S. Saintpaul was isolated from 18.7% tortoises, in the present study. In Grenada, S. Saintpaul was isolated from blue land crab [18]. This serovar is known as the common cause of human salmonellosis in Europe, Australia, and the USA [30].

In the present investigation, S. Javiana serovar was isolated from one tortoise (6.7%). S. Javiana has been reported as the most common cause of *Salmonella* outbreaks in humans [31]. S. Javiana has been associated with other wildlife species such as amphibians [32,33] and marsupial [34]. S. Javiana has been previously isolated from gopher tortoises [10], and red-footed tortoises [14]. In Grenada, S. Javiana has been reported in mongoose [19] and cane toads [17]. Ravindra *et al.* [21] reported S. Javiana as the most common serovar in brown rats from Grenada.

One tortoise was positive for S. Rubislaw (6.7%) in the present investigation. This serovar has been reported from humans in Georgia [35] and in Florida [36]. Human cases have been reported as a result of exposure to reptile and amphibians [37]. In Grenada, S. Rubislaw has been isolated from wild species in cane toads [17] and mongoose [19].

A new serovar, S. Glostrup was isolated from one tortoise. Published literature is not available on its pathogenicity and the relationship to human cases.

Further study is needed to elucidate the characteristics of S. Glostrup serovar.

In the present study, the isolation of *Salmonella* spp. was significant ($P = 0.02$) higher in male tortoises (22%) compared to females (6.1%) (Table-1). Contrary to our findings, Lockhart *et al.* [10] and Charles *et al.* [11] reported no difference in the prevalence of *Salmonella* spp. infection in gopher tortoises in relation to sex. A further study involving a large population of red-footed tortoises is suggested to highlight the effect of gender on the prevalence of *Salmonella* spp. infection.

All isolates including S. I: Rough.O;e,h:1,2 were subjected to antibiotic sensitivity test in the study. All strains were sensitive to 12 antimicrobial drugs used in the test, including third-generation cephalosporins, and ciprofloxacin, the most commonly used drugs for human salmonellosis. Similar to our findings, Charles *et al.* [11] reported all *Salmonella* isolates from gopher tortoises from Florida susceptible to ten commonly used antimicrobial drugs. Chun-Yu *et al.* [16] found S. Typhimurium isolated from turtles in Taiwan, showing a wide range of resistance to ampicillin, chloramphenicol, gentamicin, streptomycin, trimethoprim-sulfamethoxazole, and tetracycline. Variable results for antimicrobial drugs for *Salmonella* spp. isolates in wildlife have been observed by the previous researchers [38]. Lack of exposure of wildlife to antimicrobial drugs may be the reason for the variability of the result in different studies. Long-term studies on the antimicrobial resistance in wildlife may elucidate this complex phenomenon.

Conclusion

In the present study, three pathogenic *Salmonella* serovars, namely, S. Javiana, S. Rubislaw, and S. Saintpaul were isolated from red-footed tortoises in Grenada. These serovars have previously been isolated from wildlife in Grenada. Since tortoises included in the present study were pets, *Salmonella* positive tortoises may be a possible source of infection to their owners. The tortoise owners should be educated on the importance of hygiene after raising and handling of their pets. This is the first report of isolation of *Salmonella* spp. from pet red-footed tortoises in Grenada.

Authors' Contributions

This research was carried out in collaboration between all authors. RNS designed the study, oversaw the research, and wrote the first draft of the

manuscript. HH supervised the bacteriological work and finalized the manuscript. VAA organized the collection of samples and supervised the laboratory work, RN performed bacteriology of samples, VM helped in bacterial culture, performed antimicrobial sensitivity test and kept records, GA, and BH performed serotyping and identification of serovars. All authors revised, read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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