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Salmonella Serovars Isolated from Brown Rats (*Rattus norvegicus*) from Grenada, West Indies: Prevalence and Antimicrobial Susceptibility

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Authors' contributions

This work was carried out in collaboration between all authors. Author RS designed the study, oversaw the research and wrote the first draft of the manuscript. Author HH supervised the laboratory work and reviewed the manuscript. Author GA conducted serotyping of isolates. Author KT performed trapping of rats with record keeping. Authors DB, NM and TSH performed collection of specimen and assisted in bacteriological culture. Author RNT performed culture work, antimicrobial testing. Author VA supervised laboratory work and analyzed the data.

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ABSTRACT

Aim: The purpose of this study was to estimate the prevalence of *Salmonella* species and to determine the antimicrobial susceptibility of isolates in brown rats (*R. norvegicus*) from two parishes (St. George and St David) of Grenada, West Indies.
Study Design: *Salmonella* spp. was investigated in brown rats from Grenada, West Indies.
Place and Duration of Study: Rats were trapped from two parishes: St David and St. George of Grenada, West Indies. Duration of study was from May to July 2017.

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Methodology: One hundred and seventy rats were trapped from 2 parishes (St. George and St David) of Grenada which have dense human population. The trapping was performed near the human dwellings. After necropsy, intestinal contents were collected and cultured for *Salmonella* bacteria using enrichment and selective culture techniques. Serotyping of *Salmonella* isolates was performed at the OIE Salmonella reference laboratory Guelph, Ontario, Canada. Antimicrobial susceptibility of the serovars was tested.
Results: Fifteen rats (8.8%) were found positive for *Salmonella* spp. Five serovars of *Salmonella* were identified: *S. javiana* (36.8%); *S. panama* (26.3%); *S. oranienburg* and *S. montevideo* (15.7%) each; and L: Rough (5.2%). *S. oranienburg* has been isolated for the first time in Grenada. All serovars were found susceptible to 10 antimicrobial drugs; amoxycillin clavulanic acid, ampicillin, Choramphenicol, cephalothin, ciprofloxacin, ceftazidime, acefotaxime, imipenem, gentamycin and neomycin. Resistance of serovars to two antimicrobial drugs (tetracyclin and sulfamethoxazole-trimethaprim) was observed.
Conclusion: All serovars identified in brown rats in Grenada are known pathogens causing serious disease in humans. Presence of *Salmonella* spp. in rats in a densely human populated area of Grenada may play a role in transmission of *Salmonella* to humans.

Keywords: Antimicrobials; brown rats; Grenada; prevalence; Salmonella; serovars.

1. INTRODUCTION

Food borne diseases are a serious health problem worldwide. *Salmonella* spp*., Shigella* spp. and *Campylobacter* spp*.* are mostly implicated in food borne diseases. The association of *Salmonella* spp. with food borne diseases in the Caribbean region is scarce [1]. Recently Maria et al*.* [2] while reviewing the incidence of food borne diseases in Caribbean region found seven Caribbean nations experiencing food borne diseases. Out of seven Caribbean nations, *Salmonella* spp*.* was the main cause of the disease in Trinidad and Tobago [3], Dominica [4], St. Lucia [5], Grenada [6], and Barbados [7]. Foodborne diseases in mammals, birds and humans are caused be ingestion of contaminated food with zoonotic pathogens including *Salmonella* spp*.*

Wild rodents including various species of rats have been shown to carry zoonotic pathogens including *Salmonella*. Brown rat (*R. norvegicus*) of Muridae family has been studied most for the presence of *Salmonella* spp. because of their living habit in the proximity of the human dwellings. They pose a great risk of transmission of *Salmonella* bacteria through contamination of food. Brown rats carrying *Salmonella* in their intestine were reported from many countries including Wet markets in Thailand [8], West midlands, UK [9], Argentina [10], Canada [11], and California, USA [12,13]. Antimicrobial resistance in zoonotic bacteria of enteric origin is a growing concern. Studies on drug resistance in *Salmonella* from rats in the Caribbean region are minimal and need to be addressed.

In Caribbean, there is only report of Nkogwe et al. [14] for isolation of *Salmonella* spp. from brown rats in Trinidad and Tobago. In Grenada, *Salmonella* spp. was isolated from Cane Toads [15], Blue Land Crabs [16], Small Indian Mongoose [17], Domestic green iguanas [18] and non-diarrheic dogs [19]. As far as authors are aware, there is no report of isolation of *Salmonella* spp*.* from the brown rats in Grenada. The first objective of this study was to estimate the prevalence of *Salmonella* spp. and to determine their serovars in brown rats in Grenada. The second objective was to determine the antimicrobial resistance profile of Salmonella isolates against 12 antimicrobial drugs.

2. MATERIALS AND METHODS

2.1 Ethical Approval

The project (Detection of zoonotic pathogens in brown rats *(R. norvegicus*) in Grenada) was approved by the Institutional Animal Care and Use Committee (IACUC # 16009-R) of the St. George's University Grenada.

2.2 Study Area

Grenada is the southernmost country in the Caribbean sea with an area of 348.5 Km^2. The country with low hills, small trees and shrubs and tropical climate is most suitable for brown rats. The country is divided in six parishes. The trapping of rats was performed near the human dwellings, in two parishes (St. David and St. George), which have a dense human population compared to other 4 parishes.

2.3 Collection of Rats

One hundred seventy rats were collected live from 1st May to 14th July, 2017, using live traps (45 cml x 15 cm w x 15 cm h) with cheese or local fruits as bait. Attempts were made to trap the rats approximately 10 meters away from the residential buildings. Traps were placed randomoly in residential areas. Traps were placed two days a week in the evening and visited next day morning. Traps with rats were covered with black cloth and transported to the necropsy laboratory of the school of veterinary medicine and were anaesthetised using 1-2% isoflurane in oxygen via anesthesia machine (portable vet anaesthesia machine isoflurane vaporiser VET CE), manufacturer DRE (Avante Health Solution Company, USA).

2.4 Collection of Samples and Bacterial Culture

In anaesthetised rats, blood was collected from the heart through the thoracic wall and rats were exsanguinated this way. The abdominal cavity of rats was opened using a surgical blade and a pair of forceps. Intestinal contents especially from the middle and posterior end of the intestine were collected in sterile bottles. Recommended culture method for the isolation of *salmonella* by Gorski et al. [12] was followed with the the slight modification described by Drake et al. [15] and Sylvester et al. [18]. 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 lysin deoxycholate 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 5 individual colonies with typical *Salmonella* morphology (Red translucent colonies with black centers). were sub cultured on Trypti-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. A *Salmonella typhimurium* ATCC 14028 was used as reference culture. Pure *Salmonella* cultures were stored in TSA slants and shipped in cold packed containers for serotyping to OIE *Salmonella* reference laboratory, Guelph, Ontario, Canada. Serotyping of the isolates was carried out using either the traditional phenotypic serotyping method or DNA microarray- based alternative method called the Salmonella Geno-Serotyping Array (SGSA) [20]. The phenotypic serotyping method detects (O) antigens of the Salmonella isolates via slide agglutination [21]. The flagellar (H antigens) were identified with a microtiter plate well precipitation [22]. The antigenic formulae and serovars of *Salmonella* isolates were identified as per White- Kauffmann-Le Minor (WKL) scheme [23]. The SGSA detects the genes encoding surface O and H antigens and reports the corresponding *Salmonella* serovar in accordance with the existing WKL serotyping scheme.

Antimicrobial susceptibility testing of *Salmonella* isolates was performed using the disc diffusion method in Mueller -Hinton agar, as recommended by the Clinical and Laboratory Standards Institute (CLSI) [24]. The antibiotic discs used in this research were: amoxicillin clauvulanic 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, gentamycin (GM) 10 µg, neomycin (NM) 30 µg, tetracyclin (TE) 30 µg, trimethoprim/sulfamethoxazole (SXT) 25 µg. Antibiotics were obtained from BD, Frankin Lakes, NJ. The inhibition zone for all antimicrobials except neomycin were interpreted based on the CLSI guidelines (2015). For neomycin guideline from manufacturer were used. Escherichia coli ATCC 25922 was used as quality control strain [25].

2.5 Statistical Analysis

The data was analysed by the statistical analysis: Fisher's exact test, using graphical statistical software (http://www.graphpad.com/quickcales/contingency 2).

3. RESULTS

On bacteriological culture 15 from 170 rats (8.8%) were found positive for *Salmonella* spp. Eleven rats were positive each for single serotype and four rats had mixed infection with two serotypes, making a total of 19 serotypes. In one rat mixed infection with *S. panama* and *S. oranienburg*, in two rats mixed infection with

S. javiana and *S. montevideo* and in one rat mixed infection with *S. panama* and rough strain *(L:rough-0:g,m,s:-)* was found. Results of isolation with serovars are presented in Table 1.

Upon testing the salmonella serovars for antimicrobial susceptibility, all five serovars *(S. javiana, S. panama, S. montevideo, S. Oranienburg and S. rough type)* were found susceptible to ten antimicrobial drugs: amoxycillin-clavulanic acid, ampicillin, chloramphenicol, cephalothin, cifrofloxacin, ceftazidime, cefotaxime, imipenem, gentamycin and neomycin.

Resistance of serovars to two antimicrobial drugs (Tetracyclin and Sulfamethoxole- trimethoprim) was found. The results of resistance of serovars to 2 antimicrobial drugs are presented in Table 2.

4. DISCUSSION

Present study revealed 8.8% prevalence of *Salmonella* spp. in pooled traps of brown rats from St George and St David parishes of Grenada. Comparable level of prevalence found in our study was reported in West Midland UK (10%) and 6.0% in Lyon, France [26]. Considerably low level prevalence (0.5%) has been found in Vancouver, Canada [11]; 2. 0% in Trinidad and Tobago [14] and 0.8% in Argentina [10]. Higher prevalence compared to our results was reported from Kelantan, Malaysia (15.6%) [27]; from Netherlands 20.% [28], from Japan (28.7%) [29], and from Thailand (50.4%) [8]. The variability of the prevalence of *Salmonella* spp. from *R. norvegicus* in various countries of the world is not well explained. Variation could be because of samples collected from rats of highly variable sanitary conditions (Hilton et al. 2002; Alexis et al. 2016). Unfortunately, Salmonella isolates from *R. norvegicus* was not typed for serovars by many previous researchers.

Salmonella javiana was most common serovar (36.8%) in our study. *S. javiana* is known to cause serious *salmonella* outbreaks in humans [30]. *S. javiana* is reported as fourth most common *Salmonella* serovar identified in humans [31]. In Grenada prevalence *S. javiana* has been reported in Indian mongoose [17] who reported 8% *Salmonella javiana* serovar in 42% *Salmonella* positive mongoose. Serovar *Salmonell javiana* was found in 8(33.3%) out of 24 Salmonella positive cane toads [15], one dog (5.7%) out of 8 was found positive for *Salmonella javiana* [19]. Our results show that *Salmonella javiana* was most common isolate from brown rats, similar to mongoose from Grenada.

Second most common serovar found in our study was *Salmonella panama* (26.3%). From Grenada only report of *S. panama* is from mongoose [17]. *S. panama* is isolated from many foods, animals and water [32]. It generally causes gastroenteritis in humans [33]. *S. panama* has been reported more invasive than other serotypes in children causing meningitis [34]. Infected mothers causing meningitis in infants have been indicated as symptomless carriers of *S. panama* [35].

Third most common serovar *Salmonella oranienburg* in the present study was (15.7%). Multistate (Minnesota, Michigan and Wisconsin) outbreak in the USA in humans associated with *S. javiana* and *S. oranienburg* were reported due to consumption of contaminated cheese [36]. A serious gastrointestinal disease in humans involving *S. oranienburg* was reported from many countries (Denmark, Austria, Belgium, Finland, Sweden, the Netherlands and Canada) due to contamination of Chocolate produced by a company in Germany [37]. In animals infection with *S. Oranienburg* has been found in horses and turkeys in California [38]. In Grenada, *S. orenienburg* has not been isolated previously.

Fourth serovar isolated from brown rats in the present study was *S. montevideo* (15.7%). In Latin America and the Caribbean Galanis et al. [39] reported S. Montevidio as the 4th most common serovar in humans; whereas CDC [31] records *S. montevideo* as the 7th most common serovar in human illness in North America.

Table 1. Salmonella serovars from brown rats in Grenada

Serovars	Antigens	Number (percent) of serotypes
S. javiana	9,12;1,z28:1,5	7 (36.8)
S. panama	9,12:l,v:1,5	5 (26.3)
S. *montevideo*	6,7:g,m,s:-	3 (15.7)
S. oranienburg	6,7:m,t:-	3 (15.7)
L:ROUGH-O:g,m,s:-	-:g,m,s:-	1 (5.2)

Table 2. Antimicrobial resistance of Salmonella serovars

Anti-microbial drugs	Number (%) of resistant isolates				
	S. javiana	***S. panama***	***S. montevideo***	***S. oranienburg***	***S. L:RoughO:g,m,s:-***
Sulfamethoxazole-Trimethoprim (SXT)	2/7 (28.5)	5/5 (100)	2/3 (66.6)	3/3 (100)	1/1 (100)
Tetracycline (TE)	3/7 (42.8)	5/5 (100)	3/3 (100)	3/3 (100)	1/1 (100)

In Grenada, S. *montevidio* was isolated from 4 of 11 (36.3%) *Salmonella* positive Blue Land crabs [16]; 42% from *Salmonella* positive mongoose [17] and 25% from *Salmonella* positive cane toads [15] and 11.4% from *Salmonella* positive dogs [19].

In the present study, all five serovars (*S. javiana, S. monteviseo, S. panama, S. oranienburg* and rough type L:RoughO:g,m,s:-) were found susceptible to 10 antimicrobial drugs; amoxycillin clavulanic acid, ampicillin, chloramphenicol, ciprofloxacin, cephalothin, ceftazidime, cefotaxime, imipenem, gentamycin and neomycin. However, the resistance of Salmonella serovars to 2 antimicrobial drugs was found significant. Two of seven and 3 of 7 isolates of *S javiana*. were found resistant to sulphamethoxazole -trimethaprim (SXT) and tetracycline (TX) respectively. Two isolates out of 3 of *S. montevideo* were resistant to SXT while all remaining isolates of *Salmonella* in the present study were resistant to both SXT and TX. One isolate of *Salmonella* from green iguana [18] and one isolate of *S. Montevidio* from mongoose [17] in Grenada were found resistant to tetracycline. Many studies in Grenada have shown that tetracycline resistance is high in variety of bacterial isolates [40]. However, antimicrobial resistance is not common feature of *Salmonella* isolates in wildlife [41]. It may be because of lack of exposure of wild life to antimicrobial drugs. The explanation of variability of antimicrobial resistance among bacterial isolates from wild animals is lacking. Long term studies on the subject might elucidate the drug resistance in wild life species.

5. CONCLUSION

In the present study prevalence of 8.8% *Salmonella* spp. in brown rats (*R. norvegicus*) from Grenada was found. Three *Salmonella* serovars namely *S. javiana, S. montevedio,* and *S. panama* isolated from rats in the present study were reported earlier in various wildlife in Grenada. Isolation of *S. oranienburg* is being reported for the first time in Grenada. These serovars are known pathogens causing severe disease in humans. Because of constant contact between humans and rats, latter may play an important role as reservoir and transmission of *Salmonella* to humans. Rats are recognised as a source of contamination of grains, food and other edible material. There is need to educate Grenadian community regarding proper maintenance of hygienic conditions in and around their dwellings to prevent proliferation of rat population. Control program should include the measures to prevent contamination of food material from rat feces.

This is the first report of isolation of *Salmonella* spp. from brown rats (*R. norvegicus*) from Grenada.

ETHIC

The project (Detection of zoonotic pathogens in brown rats *(R. norvegicus*) in Grenada) was approved by the Institutional Animal Care and Use Committee (IACUC # 16009-R) of the St. George's University Grenada.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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