Detection of serum antibodies against *Leptospira* spp. in brown rats (*Rattus norvegicus*) from Grenada, West Indies

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Received: 18-12-2018, Accepted: 25-03-2019, Published online: 23-05-2019

**doi:** 10.14202/vetworld.2019.696-699 How to cite this article: Sharma RN, Thille K, Piechowski B, Tiwari K (2019) Detection of serum antibodies against *Leptospira* spp. in brown rats (*Rattus norvegicus*) from Grenada, West Indies, *Veterinary World, 12*(5): 696-699.

**Abstract**

**Background and Aim:** Leptospirosis is an emerging disease of animals and humans. Among rodents brown rats (*Rattus norvegicus*) are an important reservoir of bacteria *Leptospira*. There is a paucity of information on reservoirs of *Leptospira* spp. in Grenada. This study was conducted to estimate the prevalence of antibodies against *Leptospira* spp. in brown rats in a densely human populated area of Grenada.

**Materials and Methods:** Blood samples from 169 brown rats were collected and sera screened for antibodies against *Leptospira* spp. using enzyme-linked immunosorbent assay.

**Results:** Among a total of 169 brown rats trapped in two parishes in Grenada, 77/169 (45.5%) were positive for *Leptospira* spp. antibodies. A significant difference in seropositive population of brown rats between two collection sites was observed. No differences were found between sex and age of seropositive rats.

**Conclusion:** Due to the close contact of brown rats with humans in Grenada, rats should be considered a high-risk factor in transmission of *Leptospira* to humans. Appropriate preventive measures should be instituted to prevent the transmission of *Leptospira* infection to humans.

**Keywords:** brown rats, enzyme-linked immunosorbent assay, Grenada, *Leptospira* spp.

**Introduction**

Leptospirosis is an emerging zoonosis in the world. Leptospirosis is caused by spirochetes of the genus *Leptospira* [1]. *Leptospira* spp. include pathogenic and non-pathogenic bacteria. Pathogenic *Leptospira* include eight species divided into 250 serotypes and 24 serogroups [2]. Leptospirosis is a disease of humans and animals. Humans can get infected from animal reservoirs. Among animals, rodents are important reservoirs of *Leptospira* [3,4].

Brown rats (*Rattus norvegicus*) are considered major natural reservoir of leptospirosis [5,6]. The most usual route of transmission is close contact with urine, within animal groups as well as humans. Mucosae (nose and eyes) and skin abrasions in contact with infected urine are the usual routes of infection. Occasional route of transmission is by ingestion of water and food contaminated with urine or tissues of infected animals. Bacteria entering the body circulate and finally localize in the kidneys. *Leptospira* colonizes in the proximal convoluted tubules of the kidney [7]. Monahan et al. [8] reported that persistent colonization in kidneys is due to immune privileged tissue of the kidneys. Chronically infected rats are asymptomatic, but they excrete *Leptospira* in the urine [9].

Leptospirosis has been reported from countries of South America and the Caribbean [10]. There are limited studies on leptospirosis in Grenada. Leptospirosis has been reported in cattle, pigs, sheep, goat, dogs, and chickens in Grenada [11-13]. With the exception of Keenan et al. [14] report, no published research is available on leptospirosis in brown rats from Grenada.

The aim of the current research was to estimate the serum antibodies against *Leptospira* spp. in brown rats from Grenada.

**Materials and Methods**

**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.

**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 rats. The country is comprised of six parishes: St. Patrick, St. Mark, St. Andrew, St. John, St. George, and St. David. St. David and St. George parishes, which have a higher human population compared to the other four parishes, were selected for the study.
Collection of rats

A total of 169 rats were collected live from May 1 to July 14, 2017, using traps (45 cm l×15 cm w×15 cm h) with cheese and various local fruits as bait. Attempts were made to trap the rats from and near the residential buildings. Traps were placed 2 days/week in the evening and visited the morning of the next day. Traps with rats were covered with black cloth and transported to the necropsy laboratory of St. George’s University, School of Veterinary Medicine. Rats were anesthetized using 1-2% isoﬂurane in oxygen through portable vet anesthesia machine isoﬂurane vaporizer VET CE, manufacturer DRE (Avante Health Solutions Company, USA)

Collection of samples and testing

The anesthetized rats were examined for their physical health and weighed. Gender was also recorded. Rats below 100 g were grouped as young and those over 100 g as adult, following the methodology used by Panti-May et al. [15]. Blood was collected from the heart through the thoracic wall and rats were exsanguinated this way. Sera were separated from the blood by centrifugation at 1500 g for 15 min at room temperature and stored at −80°C until tested. Enzyme-linked immunosorbent assay (ELISA) test for Leptospira antibodies on sera was performed using rat Leptospira IgG ELISA kit from MyBioSource, Inc., San Diego, CA 92195-3308 USA. The antigen in the ELISA kit was whole Leptospira biflexa organisms.

Statistical analysis

Data were analyzed using a Chi-squared analysis and stratified by gender, age, and parish of rats in Microsoft Excel 2017 software. Statistical significance was set at p=0.05.

Results

Antibodies to Leptospira spp. were found in 77/169 (45.5%) rats of 169 tested. St. George parish had a higher number of positive rats 43/76 (56.5%) compared to St. David, where 34/93 (36.5%) rats were seropositive. Male and female and young and adult rats had equal prevalence of antibodies (male 45.9%; female 43.9%; young 42.1% and adults 46.0%). There was no statistical significance between gender and age. The serological results of ELISA according to parish, gender, and age are presented in Table 1.

Discussion

Diagnosis for leptospirosis is usually performed by serology: ELISA or microscopic agglutination tests (MAT) [4]. Serology is not useful in acute disease as antibodies develop in later stages of the disease. In recent years, PCR techniques have been used for the diagnosis of leptospirosis [4]. The detection of leptospires in tissue samples is based on culture, staining, and immunofluorescence techniques [7].

Previous researchers have reported variation in seropositivity of Leptospira in rats in different countries. Koma et al. [16] reported a 22% prevalence of anti-Leptospira antibodies by ELISA in R. norvegicus in Northern Vietnam. In 2010, a survey anti-Leptospira antibodies in R. norvegicus in Brazil revealed 63% of rats seropositive [17]. They used immunofluorescence on kidney smears to identify Leptospira. An earlier study in Brazil [5] reported 68.1% positive R. norvegicus for Leptospira antibodies diagnosed by MAT. Sharon et al. [18] found 92% of rat serum samples positive for anti-Leptospira antibodies in the Philippines by MAT. Norway rats in Maryland, USA, were found positive (65.3%) for anti-Leptospira antibodies [19] diagnosed by ELISA. In Caribbean nations, including Grenada, studies on Leptospira were confined to livestock and humans; however, in Grenada, only one study made by Keenan et al. [14] reported the sero-prevalence of Leptospira in R. norvegicus. They identified 24.5% of rats positive for Leptospira antibodies by ELISA and 7.1% positive by MAT. Compared to the previous study [14] in Grenada, this study found higher seropositivity (45.5%) in R. norvegicus for Leptospira antibodies by ELISA. The prevalence of antibodies for Leptospira spp. ranged from 22% to 92% in different countries. The variation in the prevalence of antibodies against Leptospira in different geographical location may be due to changes in climate and environmental conditions [20].

Comparison of the prevalence of antibodies in different studies is also complicated by sample size and laboratory methods [21]. The antibody estimation in serological diagnosis is dependent on the Leptospira spp. antigen present in ELISA kit. The antigen component of ELISA differs from one to another ELISA kit; however, in most products, the nature of antigen is not described. Most kits use crude whole cell lysate as antigens [22]. Same authors [22] evaluated the diagnostic utility of recombinant antigens, which contain portions of genes encoding the lepto- spiral outer membrane protein (LipL32, Omp11, LipL36, LipL41, and Hsp58) in serodiagnosis of leptospirosis. Researchers recommended LipL32 had the highest sensitivities in the acute and convalescent phases of the leptospirosis. Other recombinant antigens gave insignificant reaction. In the present study, we used ELISA which contained whole L. biflexa organism. Since the details of L. biflexa organism are not available, it would be inappropriate to compare our serological results with previous researchers using different antigens.

In the present study, statistically significant differences in the prevalence of antibodies for Leptospira in brown rats were found between St. George and St. David parish. The climatic and environmental conditions being the same in both parishes, the difference is not fully understood. Future research involving a higher number of rats from all six parishes of the country may be more insightful regarding the distribution of antibodies of Leptospira in rat population of Grenada.

In the present study, no significant differences were found in the prevalence of antibodies
between young and adult rats which are in accordance with Suepaul et al. [23] for many species of animals, except rats where juvenile were more susceptible. Contrary to our finding; the previous researchers [24,25,26] observed higher prevalence in older rats.

There was no difference in antibody prevalence between male and female rats. This finding is in accordance with the previous researcher [27], whereas Easterbrook et al. [19] reported a higher prevalence of antibodies in female rats in the USA. In human patients from 14 Caribbean countries, males had a higher frequency of seropositivity compared to females. [28]. The difference between genders is not well explained since both sexes are equally exposed to *Leptospira* infection.

**Conclusion**

The high prevalence of antibodies found in brown rats for *Leptospira* spp. in Grenada should be of great concern. In Grenada, cases of *Leptospira* infections in humans have been reported [28]. Due to the close contact of brown rats with humans in Grenada, rats should be considered a high-risk factor in transmission of *Leptospira* to humans. Preventive measures should be directed toward minimizing the exposure of rats with humans and possible reduction in Grenada’s rat population.

**Authors’ Contributions**

RNS planned and supervised the research and manuscript writing; KeT trapping of rats, collection of blood, helping in ELISA, statistical analysis of results, and review of the manuscript; KaT performed ELISA and edited manuscript; BP collection of blood, and performance of ELISA. All authors read and approved the final manuscript.

**Acknowledgments**

The authors appreciate the financial support from the St. George University, Grenada, through a grant “One Health research Initiative” (OHRI 06-14-10).

**Competing Interests**

The authors declare that they have no competing interests.

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