

Seroprevalence of *Leptospira* in *Rattus Norvegicus* in Grenada, West Indies

J Keenan¹, R Sharma², R Dicker¹, J Rayner³, D Stone²

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Objective: To determine the seroprevalence of *Leptospira* and the serovars responsible for *Leptospira* exposure in rats in Grenada in order to assess rats as a reservoir host for human infection.

Design and Methods: *Rattus norvegicus* rodents were collected representing each of the six parishes on the island of Grenada. Serum from 237 rats was tested by the microscopic agglutination test (MAT) and an Immunoglobulin G (IgG) Enzyme-Linked Immunosorbent Assay (ELISA). Seroprevalence rates among parishes were compared using a chi-squared test of homogeneity.

Results: Of the 237 serum samples tested, 64 were positive by either MAT or ELISA for an overall seroprevalence of 27%. The ELISA identified 24.5 % (57/233) of the rats positive at a titer of $\geq 1:160$. The MAT identified 7.1% (13/183) of the rats positive at a titer of $\geq 1:100$. Six of the 13 MAT positive samples had antibodies to multiple serovars. The serovars identified by the MAT with the greatest frequency were from the *Icterohaemorrhagiae* serogroup. Two rats had antibodies for serogroup *Cynopteri*, the first time this serogroup has been identified on Grenada.

Conclusions: Our results for *Leptospira* exposure in rats in Grenada support *R. norvegicus* as an important reservoir host for *Leptospira*, particularly those from the *Icterohaemorrhagiae* serogroup. Because this serogroup is the primary serogroup responsible for documented human exposure in Grenada, exposed rats represent a public health threat.

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INTRODUCTION

Leptospirosis, the disease caused by an infection with pathogenic *Leptospira* species, is considered the most common zoonotic disease in the world (1, 2). Although it is found worldwide, it is significantly more common in tropical regions (3). *Leptospira* can infect numerous species of animals, including humans. *Rattus norvegicus*, the brown rat, is the most important reservoir for those *Leptospira* serovars contributing to human leptospirosis (4).

Serology is the preferred method for human leptospirosis diagnosis, with the microscopic agglutination test (MAT) as the gold standard (3). The MAT can determine serogroups, test sera from any animal and has a high specificity of up to $\geq 97\%$ (5). Although the Enzyme-Linked Immunosorbent Assay (ELISA) is more sensitive than the MAT, especially in the acute disease phase (6), the MAT is currently the serologic test used in reference laboratories because of its combined high degree of sensitivity and specificity (7).

Leptospirosis is considered the most common zoonotic disease in the world (1, 2, 6) and rodents, particularly those of the *Rattus* species, are the prominent source of *Leptospira* infection to both humans and domestic animals (6). Exposure to *Leptospira sp* and leptospirosis in humans and other species has been recognized and well documented in the Caribbean (8). However, there are few published reports on *Leptospira* exposure in rats in the Caribbean. In order to appropriately address the public health concerns of leptospirosis, the specifics of its epidemiology in particular geographic areas are needed. Until now, no study has focussed on the most likely primary reservoir of human infection in Grenada, the rat. This study determined the seroprevalence of *Leptospira* and the serovars responsible for *Leptospira* exposure in *R norvegicus* in Grenada.

MATERIALS AND METHOD

The serological classification system was chosen as it is best suited for epidemiological studies. Two hundred and sixty-one rats were trapped from both urban and rural areas, representing each of the six parishes on the island of Grenada. All of the rats trapped were adult *R norvegicus* (wild brown rats). Wire traps were used that were designed to capture one rat alive. The majority of rats were collected near the individuals' primary residence. Rodent collection began April 23, 2005 and continued to October 2006. There were 14 collection periods, each representing one week; eight of the 14 collection weeks gathered rats from multiple parishes at the same time. Burnt coconut, which had been shown previously to be the most effective bait, was used in each of the traps.

Rats were collected Monday to Friday of each sampling week, placed in temporary cages and provided food and water. Each Saturday, rats were collectively placed into 140 gallon drums and transported to St George's University, School of Veterinary Medicine (SGU SVM). Upon arrival at SGU SVM necropsy laboratory, each rat was assigned a specimen number and information regarding species, gender and parish of capture was recorded. Following humane euthanasia and necropsy, three to seven milliliters (mL) of blood was collected via an intra-cardiac venipuncture. Collected rat blood were allowed to clot for 10 minutes then centrifuged for 15 minutes at 2000 revolutions per minute (rpm). Resultant serum was stored at -20°C in individually labelled tubes in the Microbiology Laboratory at the SVM. Sera were sent to the *Leptospira* Laboratory in Barbados, West Indies, for anti-*Leptospira* antibody detection. The *Leptospira* Laboratory conducted both MAT and IgG ELISA tests on the rat sera. The required secondary antibody needed to test rat sera by ELISA, a goat anti-rat IgG conjugate, was provided to the *Leptospira* Laboratory by St George's University, School of Veterinary Medicine.

Modifications of the MAT and ELISA procedures as described by the WHO were utilized at *Leptospira* Laboratory (WHO Human Leptospirosis, 2003).

The *Leptospira* Laboratory interprets a MAT titre of ≥ 100 and an IgG ELISA titre of ≥ 160 as positive. We considered a positive sample to be one that was positive by MAT, ELISA or both. A chi-squared test of homogeneity was performed on the seroprevalence rates for the six parishes to determine if the rates were statistically different. Published prevalence rates from other Caribbean islands on *Leptospira* exposure were compared to our seroprevalence rates using a z test. All statistical analysis was conducted via EpiInfo and Microsoft Excel Data Analysis software.

Approval of protocols for trapping, transport and humane euthanasia of rats was obtained by the St George's University Institutional Animal Care and Use Committee.

RESULTS

Two hundred and sixty-one *R norvegicus* rats were trapped in Grenada. Twenty-four rats were unable to be tested due to insufficient serum sample. Of the remaining 237 samples, 179 were tested by both MAT and IgG ELISA; four were tested by only the MAT and 54 were tested by only ELISA. Equal numbers of sera were not tested by both the MAT and ELISA for two reasons. Firstly, for some rats, insufficient serum was collected to run both tests. Secondly, equivocal results obtained on a few samples were not able to be repeated due to insufficient remaining blood samples.

Of the 237 serum samples tested, 64 were positive by either MAT or ELISA for an overall seroprevalence rate of 27%. The ELISA test identified 24.5% (57/233) rats positive at dilutions of 1:160 or greater.

The MAT identified 7.1% (13/183) of the rats positive at a dilution of 1:100 or greater. Of the 179 rats tested by both methods, six were positive by both, six by MAT only and 34 by ELISA only. The seroprevalence rates for each parish are shown in Table 1. A statistically significant differ-

Table: *Leptospira* seroprevalence in *R. norvegicus* rats in Grenada by parish, as determined by MAT titres of 1:100 or greater or IgG ELISA titres of 1:160 or greater.

| | Positive | Negative | Total Tested | Per cent Positive | Per cent Positive 95% CI |
|---------------|-----------|------------|--------------|-------------------|--------------------------|
| St Patrick* | 12 | 5 | 17 | 70.59 | 46.52 – 86.66 |
| St John | 14 | 17 | 31 | 45.16 | 29.09 – 62.34 |
| St Mark | 11 | 24 | 35 | 31.43 | 18.56 – 48.11 |
| St Andrew | 10 | 24 | 34 | 29.41 | 16.85 – 46.30 |
| St George | 7 | 32 | 39 | 17.95 | 9.05 – 32.78 |
| St David* | 10 | 71 | 81 | 12.35 | 6.89 – 21.29 |
| Totals | 64 | 173 | 237 | 27.00 | 21.76 – 33.00 |

* Seroprevalence rate differs statistically from the rest of the parishes as a group.

ence among the parishes ($\chi^2 = 32.47$; $p = 4.8 \times 10^{-6}$, 5 d.f.) was observed. To determine which parish or parishes accounted for this difference, each parish was compared by χ^2 to the rest of the parishes as a group. A Bonferroni correction was used to adjust α for multiple comparisons to $\chi^2 = 0.0083$. The seroprevalence rate in St David was significantly lower and the rate in St Patrick was significantly higher than the rest of the parishes as a group. Of the 13 MAT-positive rats in our study, six showed evidence of exposure to more than one serovar. Most serovars identified belonged to the Icterohaemorrhagiae serogroup. Six rats were seropositive for the serovar Mankarso, six rats were seropositive for the serovar copenhageni and five rats were seropositive for the serovar icterohaemorrhagiae strain RGA. Two rats were seropositive for the serovar cynopteri in the Cynopteri serogroup and one rat was seropositive for the serovar ballum in the Ballum serogroup. Fig 1 shows the per cent of MAT-tested rats (n = 183) that were MAT-positive for each serovar encountered.

Three published reports were found that evaluated *Leptospira* seroprevalence in rats in other Caribbean islands (11–13). Two of these studies investigating *Leptospira* exposure in *Rattus* species in the Caribbean were from Barbados (11,12). The earlier study, published in 1991, analyzed samples by both MAT and culturing of tissue samples (12). A rat was considered positive if it was positive by either of these measures. This study tested both *R. norvegicus* (48/138) and *R. rattus* (32/98) rodents and found an overall prevalence rate of 33.9% (80/236) (12). The results of the chi-squared test comparing this prevalence rate to the overall

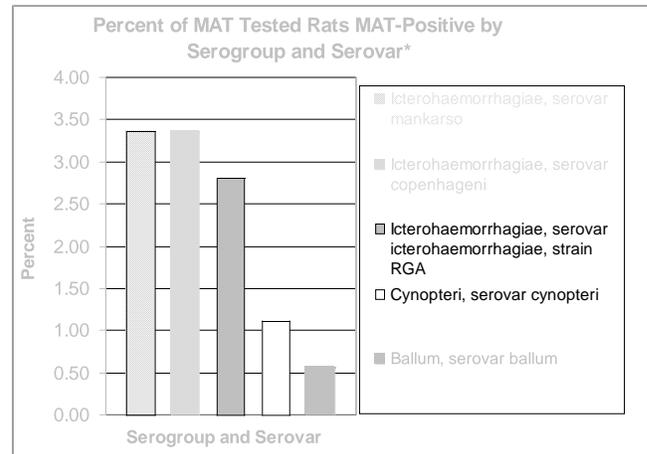


Figure: Per cent of *R. norvegicus* rats in Grenada positive for *Leptospira* serogroup- and serovar-specific antibodies as determined by a MAT titre of 1:100 or greater (n = 183).

* 6 of the 13 MAT-positive rats were positive for antibodies to multiple serovars.

seroprevalence rate in our study found no significant differences ($\chi^2 = 2.69$, $p = 0.10$).

The more recent study from Barbados, published in 1998, trapped rats (30.7% *R. rattus*, 69.3% *R. norvegicus*) over two different time periods eight years apart and found an overall seroprevalence rate of 19.1% (31/162) by MAT (11). When compared to our seroprevalence results, no significant differences were found ($\chi^2 = 3.28$, $p = 0.07$). One study from Trinidad tested serum from 46 rats (32 *R. rattus*, 7 *R. norvegicus* and 7 unknown *Rattus* species) by MAT. Eight rats were MAT-positive for anti-*Leptospira* antibodies at a titre of 1:100 or greater for a seroprevalence rate of 17.4% [8/46] (13). There was no significant difference in this seroprevalence rate with that of our results ($\chi^2 = 1.88$, $p = 0.17$).

DISCUSSION

Rats are an important reservoir host for pathogenic serovars of *Leptospira* and the most common source for human leptospirosis (6, 9–11). We found an overall seroprevalence rate of 27% for exposure to *Leptospira* in our sample of rats. There were no statistical differences in the seroprevalence rate of exposure in *R. norvegicus* in Grenada to other published prevalence rates of *Leptospira* exposure in *Rattus* species from Barbados (11, 12) or from Trinidad (9). Prior to our study, very little had been published on *Leptospira* serogroups in either rats or humans from the island of Grenada. One study identified a total of six rats seropositive for *Leptospira* exposure in Grenada, five of which were positive for serovars in the Icterohaemorrhagiae serogroup (13). Our study also identified Icterohaemorrhagiae as the dominant serogroup responsible for rat exposure in Grenada (76.9% of the 13 MAT-positive rats). Thus, it is probable that the majority of serovars contributing to rat exposure in Grenada have been from the Icterohaemorrhagiae serogroup over the last 26-plus years. In the sole study on human leptospirosis

in Grenada, published in 1979, the largest serogroup contributor to the 45 seropositive human cases was Icterohaemorrhagiae (38%), followed by Panama (24%) and Canicola [11%] (14). Thus, at least in the late 1970s and early 1980s Icterohaemorrhagiae was the main serogroup responsible for *Leptospira* exposure in rats and the most common serogroup responsible for human leptospirosis on the island. Although it is likely that rats continue to be an important Icterohaemorrhagiae reservoir host for human exposure, current data on human leptospirosis serogroups needs to be obtained to clearly document a correlation for this serogroup. Based on our results, rats in Grenada do not appear to serve as reservoir hosts for serogroups Panama and Canicola. Of interest is the possible role dogs play in the link between Icterohaemorrhagiae reservoir hosts, environment and humans. In an unpublished study in 2006 on dogs in Grenada, 19 of the 20 MAT-positive dogs were positive for serovars from the Icterohaemorrhagiae serogroup (personal communication). Rats may be the reservoir for the Icterohaemorrhagiae serogroup for both dog and human exposure but the close association between people and dogs in Grenada, may contribute to canine-to-human transmission.

A few interesting and unexpected observations from our study were the differences in seroprevalence rates among parishes, the failure of each MAT-positive rat to also be ELISA-positive, and the identification of two rats seropositive for serogroup Cynopteri. Environmental or precipitation variations as well as trap location placement in each parish may have contributed to these differences. As the ELISA is more sensitive than the MAT, we expected all MAT-positive rats would also be ELISA-positive. Notably, none of the three rats identified as MAT-positive to serogroup Cynopteri or Ballum were ELISA-positive. It is possible that the ELISA did not detect serovars from these two serogroups. Another plausible explanation for rats being MAT-positive and ELISA-negative is that a laboratory error was made during some of the ELISA tests yielding negative results.

This study is the first report of exposure to serogroup Cynopteri in any animal in Grenada. It was identified in two rats both trapped in the parish of St David at different times, thus ruling out the possibility that one rat exposed the other during confinement. This serogroup, however, is not rare and has been documented in the Caribbean in livestock (15), wild animals, (9) and humans (16). The significance of this *Leptospira* serogroup with regard to human or domestic animals in Grenada is unknown. Our results for *Leptospira* exposure in rats in Grenada support *R norvegicus* as an important reservoir host for *Leptospira*, particularly those from the Icterohaemorrhagiae serogroup. Because this serogroup is the primary serogroup responsible for documented human and canine exposure in Grenada, exposed rats represent a

public health threat. However, human *Leptospira* exposure in Grenada has not been investigated since 1979. Documenting the seroprevalence in this important reservoir host and Identifying the serogroups responsible for current exposure provides a better understanding of the role *R norvegicus* plays in the epidemiology of *Leptospira* in Grenada.

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