Seroepidemiology of leptospirosis in dogs and rats in Trinidad

Suepaul, S.M.1, Carrington, C.V.2, Campbell, M.1, Borde, G.1 and Adesiyun, A.A.1*

1School of Veterinary Medicine
2School of Medicine, Faculty of Medical Sciences, The University of the West Indies, Trinidad and Tobago
*Corresponding author email: Abiodun.Adesiyun@sta.uwi.edu
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Abstract. Stray dogs (n=207), suspected canine cases of leptospirosis (n=50) and rats (n=200) from the Caribbean island of Trinidad were subjected to the Microscopic Agglutination Test (MAT) for leptospirosis. The seroprevalence in stray dogs was 15.5% (n=32), the predominant serogroup was Icterohaemorrhagiae (14.5%; n=30) with agglutinations to serovars Copenhageni at 5.8%, Icterohaemorrhagiae at 4.8%, Mankarso at 3.9%. The seroprevalence among suspected canine cases was 72% (n=36) with Icterohaemorrhagiae again being the predominant serogroup at 60% inclusive of serovars: Copenhageni, 44%; Mankarso, 14%; and Icterohaemorrhagiae 2%. A seroprevalence of 16.5% was determined in rats, all agglutinations were to the Icterohaemorrhagiae serogroup (inclusive of serovars Copenhageni, 9.5%; Icterohaemorrhagiae, 5.5%; and Mankarso, 1.5%). Overall serovar Copenhageni was the most common serovar as 11.6% of all the animal species tested by the MAT were positive and may be an important zoonotic serovar in Trinidad. The titres of infecting serovars of *Leptospira* in suspected canine cases of leptospirosis were considerably higher than that found in stray dogs and in rats where the lowest titres were found. Age and sex were not significant risk factors except in the case of rats where age was significant, indicating that juvenile rats were at a significantly higher risk. There was no definite pattern of the distribution of positive animals or the serovars when using the MAT. Data obtained in the current study indicate that dogs and rats in Trinidad have the potential to be sources of leptospiral infections for humans. This potential has public health implications making it imperative to control rat and stray dog populations in the island to reduce the risk of human leptospirosis.

INTRODUCTION

Leptospirosis is a re-emerging bacterial zoonosis with worldwide distribution (Langston & Heuter, 2003). It occurs more frequently in tropical regions and is perpetuated in the environment by maintenance hosts such as rodents. These rodents can shed the organism in the environment. Leptospirosis has been serologically identified in stray dogs (Weekes et al., 1997; Adesiyun et al., 2006; Jimenez-Coello et al., 2008) and rodents worldwide (Levett et al., 1998; Vanasco et al., 2003; De Faria et al., 2008).
A previous study conducted between February to July of 2005 (Adesiyun et al., 2006) reported an overall seroprevalence of 14.6% for leptospiral infections in dogs of Trinidad. In that study, a seroprevalence of 48.0% was detected in clinically ill dogs suspected of having leptospirosis, with the predominant serovars detected being Mankarso, Icterohaemorrhagiae, Autumnalis and Copenhageni (Adesiyun et al., 2006). In that study a seroprevalence of 4.4% was also detected in stray dogs.

The need to conduct adequate surveillance of leptospirosis in tropical countries, in areas such as Trinidad and Tobago where disease incidences tend to be higher than in temperate countries, primarily because the tropical climates (rainfall and temperature) support the survival of leptospires in the environment, has been emphasized (Levett, 2001). Additionally, in a given locale, regular surveillance can provide insights into spatio-temporal changes in disease prevalence that may result from changes in environmental conditions.

The primary objective of this study was to use the MAT to determine the seroprevalence of leptospirosis and the infecting Leptospira serovars in dogs and rats in Trinidad. The risk factors associated with infection in study animals was also determined. We also investigated the possibility of changes in the prevalence of leptospirosis and the causative serovars over time in Trinidad by comparing the findings in the current study with those of an earlier study (Adesiyun et al., 2006) on canine leptospirosis.

MATERIALS AND METHODS

Samples were collected on the island of Trinidad, 61½° west longitude and 10½° north latitude. The minimum sample size was determined using the equation n = \( \frac{1.96^2 \times P_{\text{exp}} \times (1-P_{\text{exp}})}{d^2} \) (Thrusfield, 2007). A precision rate (d) of 5% was used for dogs and 6% for wild rodents with a reported prevalence rate (P_{\text{exp}}) of 20% for dogs (Venkataraman & Nedunchelliyun, 1992) and 21% (Damude and Jones, 1979) for wild rodents. A confidence interval of 95% was used yielding estimated sample sizes of 246 and 177 for dogs and rats respectively. For the investigation a total of 257 dogs and 200 rats were sampled.

All samples were collected over a two-year period (September 2005 to September 2007). Suspected canine cases of leptospirosis (n=50) were animals presented to veterinary clinics across the island with clinical signs suggestive of leptospirosis based on the clinician's judgement including signs such as jaundice, anorexia and vomiting. Stray dog samples (n=207) were acquired through weekly visits to the Trinidad and Tobago Society for the Prevention of Cruelty to Animals (TTSPCA).

Free-living rats (n=200) were trapped at several locations (around houses, mostly of the Rattus spp.) with the use of metal cages. Once trapped, the cages were placed in ventilated black bags to reduce excitement and stress to the animals. The Regional Health Authorities (RHAs) and rodent control groups across the island assisted in trapping rats around homes, public buildings and markets. Rats were euthanized by initially being rendered unconscious using carbon dioxide gas. Thereafter a minimal dosage of 85 mg ketamine per kg mixed with 15 mg xylazine per kg (Wixson et al., 1987) was injected intramuscularly into the thigh of each captured rat. If the desired response (which was: no response to pain and the loss of righting reflex), was not achieved more of the mixture was given to effect.

The rats were weighed and data collected on sex and sexual maturity. In males the position of the testes whether scrotal or abdominal was observed while in females, the vagina (closed or perforated) and nipples (enlarged or small, lactating or not) were determined as earlier described (Vanasco et al., 2003). The body condition of each rat was also noted. Blood was collected via cardiac puncture with a 21G 1½ inch needle attached to a sterile 3 ml syringe, it was then placed into a 4-ml sterile red top vacutainer. This approach was taken because in addition to collecting clotted blood for sera for the current study, heparinized whole blood (unclotted)
and the kidneys were collected for another investigation (Suepaul et al., 2010a).

The Ethics Committee of the Faculty of Medical Sciences, University of the West Indies, approved the protocol.

The MAT was performed using a panel of 23 serovars as antigens (Suepaul et al., 2010a), which were kindly provided by Royal Tropical Institute, Koninklijk Instituut voor Tropen, (KIT), Amsterdam, The Netherlands. The MAT procedure followed standard protocol as described by Royal Tropical Institute in the manual for the International Course on Laboratory Methods for the Diagnosis of Leptospirosis (KIT Biomedical Research, 2006; Suepaul et al., 2010a).

SPSS (Statistical Package for Social Sciences) version 15.0 was used to perform Chi-square analyses of the data on the seroprevalence of leptospiral infections as they relate to by selected risk factors (animal species/type: stray dogs, owned dogs and wild rats, age, sex, geographical location; and for owned dogs: vaccination status, exposure to rodents and use for hunting. Geographical Information System (GIS) data were collected by the use of an eTrex® GPS (global positioning system) navigator. GPS readings were however not available for the stray dogs because they were collected by the RHAs which did not have the facilities to document the exact locations from which the stray dogs were collected. For suspected canine cases, the addresses of the owners were obtained, after which these locations were visited and GPS readings were taken. For the rats, GPS readings were collected at the sites at which they were trapped.

RESULTS

Table 1 provides a summary of the frequency of detecting serovars of _Leptospira_ in dogs and rodents using the MAT based on 23 serovars. By using MAT, the seroprevalence for leptospirosis in stray dogs was 15.5% (32 of 207), the individual titres of agglutinations and serovars are shown in Table 2. The highest seroprevalence was for the Icterohaemorrhagiae serogroup at 14.5% (30 of 207) and the agglutinations to the individual serovars were as follows: 12 (5.8%) were Copenhageni, 10 (4.8%) were Icterohaemorrhagiae, and 8 (3.9%) were Mankarso. In addition, agglutinations also occurred to serovars Bim 1 (0.5%) and Hebdomadis 1 (0.5%). Of the 207 serum samples tested 22 (10.6%) showed agglutinations to more than one serovar in

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>Serovar</th>
<th>Stray Dogs (n=207)</th>
<th>Suspected Canine Cases (n=50)</th>
<th>Rats (n=200)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Icterohaemorrhagiae</td>
<td>Copenhageni</td>
<td>12 (5.8)</td>
<td>22 (44.0)</td>
<td>19 (9.5)</td>
</tr>
<tr>
<td></td>
<td>Mankarso</td>
<td>8 (3.9)</td>
<td>7 (14.0)</td>
<td>3 (1.5)</td>
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<tr>
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<td>10 (4.8)</td>
<td>1 (2.0)</td>
<td>11 (5.5)</td>
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<tr>
<td>Autumnalis</td>
<td>Bim</td>
<td>1 (0.5)</td>
<td>2 (4.0)</td>
<td>–</td>
</tr>
<tr>
<td>Australis</td>
<td>Bratislava</td>
<td>–</td>
<td>1 (2.0)</td>
<td>–</td>
</tr>
<tr>
<td>Ballum</td>
<td>Arborea</td>
<td>–</td>
<td>2 (4.0)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Ballum</td>
<td>–</td>
<td>1 (2.0)</td>
<td>–</td>
</tr>
<tr>
<td>Hebdomadis</td>
<td>Hebdomadis</td>
<td>1 (0.5)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>32 (15.5)</strong></td>
<td><strong>36 (72.0)</strong></td>
<td><strong>33 (16.5)</strong></td>
</tr>
<tr>
<td>Type of animal</td>
<td>Serovar</td>
<td>No. (%) of animals seropositive with titre:</td>
<td></td>
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<td>---------------</td>
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<td>------------------------------------------</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>20</td>
<td>40</td>
<td>80</td>
</tr>
<tr>
<td>Stray dog</td>
<td>Bim</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Hebdomadis</td>
<td>1 (0.48)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Copenhagheni</td>
<td>1 (0.48)</td>
<td>1 (0.48)</td>
<td>5 (2.4)</td>
</tr>
<tr>
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<td>1 (0.48)</td>
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<tr>
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<td>Mankarso</td>
<td>3 (1.45)</td>
<td>2 (0.97)</td>
<td>1 (0.48)</td>
</tr>
<tr>
<td>Suspected canine cases of leptospirosis</td>
<td>Arborea</td>
<td>1 (2.0)</td>
<td>1 (2.0)</td>
<td></td>
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<tr>
<td></td>
<td>Ballum</td>
<td>1 (2.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bim</td>
<td>1 (2.0)</td>
<td>1 (2.0)</td>
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<tr>
<td></td>
<td>Bratislava</td>
<td>1 (2.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Copenhagheni</td>
<td>2 (4.0)</td>
<td>1 (2.0)</td>
<td>3 (6.0)</td>
</tr>
<tr>
<td></td>
<td>Icterohaemorrhagiae</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mankarso</td>
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<td>2 (4.0)</td>
<td>1 (2.0)</td>
</tr>
<tr>
<td>Rat</td>
<td>Copenhagheni</td>
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<td>1 (0.5)</td>
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<tr>
<td></td>
<td>Icterohaemorrhagiae</td>
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<td>1 (0.5)</td>
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<tr>
<td></td>
<td>Mankarso</td>
<td>1 (0.5)</td>
<td>1 (0.5)</td>
<td>1 (0.5)</td>
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</tbody>
</table>
the panel. Age (p=0.151) and sex (p=0.66) of stray dogs were not statistically significantly associated with leptospiral infections.

Of the 50-suspected canine cases of leptospirosis tested, 72% were seropositive. Overall, predominant agglutinations were detected to the Icterohaemorrhagiae serogroup with 60% (30 of 50) of the dogs being seropositive, comprising agglutinations to serovars Copenhageni (44.0%), Mankarso (14.0%) and Icterohaemorrhagiae (2.0%). Agglutinations were also detected to three other serovars. Overall, sera from 22 (44%) dogs agglutinated more than one serovar. The p-values obtained by Chi-square analyses demonstrated that seroprevalence was not statistically significant association of clinical signs such as jaundice (p=0.763), anorexia (0.629) and vomiting (0.736) nor with the following risk factors: age (p=0.373), sex (p=0.733), use for hunting (p=0.550), exposure to rodents (p=0.524) and vaccination status (p=0.213).

Of the 200 rats tested, 33 (16.5%) were tested positive by MAT. All of the agglutinations occurred to the Icterohaemorrhagiae serogroup inclusive of 19 (9.5%) to serovar Copenhageni, 11 (5.5%) to serovar Icterohaemorrhagiae and 3 (1.5%) to serovar Mankarso as seen in Table 1. It was found that agglutinations to more than one serovar occurred in 15 (7.5%) of 200 sera tested. Statistical analysis revealed that age was statistically significantly (p=0.000) associated with infection but not sex (p=0.247).

Table 2 shows the titres and serovars agglutinated by the sera of stray dogs, suspected cases of canine leptospirosis and rats. For the stray dogs, both of dogs seropositive to non- Icterohaemorrhagiae serogroup had titres of 1:320 to serovars Bim and Hebdomadis compared to serovars Arborea, Ballum, Bim and Bratislavia recovered from suspect cases of canine leptospirosis where the titres of seropositive dogs were mostly (4 of 6) at 1:640.

The three serovars (Copenhageni, Icterohaemorrhagiae and Mankarso) in the Icterohaemorrhagiae serogroup agglutinated by sera obtained from the dogs and rats. The titres were very high for sera collected from suspect canine cases of leptospirosis where 19 (63.3%) of 30 agglutinations were at titres of 1:1280 and higher compared with only 10 (33.3%) of 30 and 8 (32.0%) of 25 agglutinations detected for stray dogs and rats respectively.

**DISCUSSION**

The seroprevalence of leptospirosis in the stray dogs tested is 15.5% with the majority of agglutinations was occurring to the Icterohaemorrhagiae serogroup. The range of seroprevalences reported previously varies from 6.4% for a study in Italy where the majority of agglutinations was to serogroup Icterohaemorrhagiae (Cerri et al., 2003) to 62% reported in Barbados where most aglutinations were to serogroups Australis, Icterohaemorrhagiae and Autumnalis (Weekes et al., 1997). Other reported seroprevalences include 20% in Brazil (predominant serovars being Autumnalis, Pomona, Grippotyphosa and Patoc) (Batista et al., 2004) and 35% in the Yucatan, Mexico with Canicola, Icterohaemorrhagiae and Panama being the major serogroups (Jimenez-Coello et al., 2008).

In an earlier study in Trinidad, a seroprevalence of 4.4% was reported for leptospiral infections in stray dogs sampled between the months of February and May of 2005 (Adesiyun et al., 2006). In the current study based on samples collected from September 2005-2007, with the majority of samples being taken in 2006, we report a higher seroprevalence at 15.5%. Furthermore, in the earlier study the predominant serovar detected was Mankarso while in the current study serovar Copenhageni was prevalent. Factors which may have been responsible for the overall increase in the seroprevalence include, changes in environmental factors such as temperature. Of relevance is the fact that the Meteorological Office of Trinidad and Tobago recorded above the 30 year long term average temperature (>26.02°C) for 2005 to 2007 and higher than average rainfall for the
years 2005 and 2006, 1998.8 mm and 2121.1 mm respectively and the 30 year long term average is 1885.6 mm. Humidity remained at its normal high of about 82% for the period 2005 to 2007. High year round temperature, rainfall and humidity, such as experienced in tropical countries have been reported to favour the occurrence of leptospirosis (Sehgal, 2006). Additionally, high rainfall has been associated with increased flooding and surface water which favour the contamination and survival of leptospires. In general, a contributing factor to leptospiral infection in Trinidad during the sample period of this study and that by Adesiyun et al. (2006) may be attributed to the rapid urbanization occurring in Trinidad (Earth Trends, 2003). During the last five to eight years the government of Trinidad and Tobago embarked on an aggressive housing scheme utilizing construction in formerly uninhabited parts of the country. This practice may have brought both humans and dogs closer to rats which are the reported to be reservoirs of the serovar Copenhageni (De Faria et al., 2008).

In the current study, age and sex of stray dogs were not significantly associated with seroprevalence, an indication that other risk factors may have been responsible for infection. It has been previously reported that male dogs were at a significantly greater risk than female dogs and that older dogs were at a significantly greater risk that dogs less than 1 year old (Ward et al., 2002). However, another study found that sex and age were not significantly associated with leptospirosis infection (Meeyam et al., 2006). Our findings may therefore be a reflection of the fact that dogs in the environment in Trinidad, regardless of the sex and age, are repeatedly exposed leptospires shed into the environment by rodents and other wildlife which are recognized as important reservoirs for leptospires (Levett et al., 1998; Vanasco et al., 2003; De Faria et al., 2008).

A seroprevalence of 72% found in suspected canine cases of leptospirosis in the current study, with serogroup Copenhageni being the most prevalent, is comparable to published reports including a study in the USA where a seroprevalence 59% (predominant serovar Grippotyphosa) was documented (Ward et al., 2004) and 75% in Barbados (predominant serogroups Icterohaemorrhagiae and Australis) (Weekes et al., 1997). Our findings (72%) is however higher than the 48% reported in an earlier study in the island (Adesiyun et al., 2006). The differences between both local studies could have been due to a true increase in the prevalence of leptospirosis in the country. It could also be due to differences in the panel of antigens used. The panel used in the current study has a wider range of antigens (23 serovars) than the one used in the previous study in the island which contained only 17 serovars which may have increased the sensitivity in detecting seropositive dogs in the current study.

Of significance was our finding that properly vaccinated dogs were just as likely to display the clinical signs of leptospirosis as unvaccinated dogs suggesting that the commercial vaccines used in Trinidad, which are expected to prevent clinical leptospirosis, failed to offer adequate protection to the dogs studied. Recent work by Suepaul et al. (2010b), in a hamster model, suggested that the 2 most commonly used brands of vaccines (one brand containing serovars Icterohaemorrhagiae and Copenhageni and the other containing serovars Canicola, Icterohaemorrhagiae, Grippotyphosa and Pomona) do not adequately protect against the predominant circulating serovars in Trinidad (Copenhageni and Mankarso). Our findings also provide evidence of the lack of cross-protection across serovars in vaccinated dogs (Suepaul et al., 2010b).

The seroprevalence of 16.5% (Icterohaemorrhagiae serogroup) reported for rats sampled in the current study is higher than the 4.8% reported in Thailand (predominant serovars Sejroe, Pyrogenes, Copenhageni and Pomona) (Wangroonsarb et al., 2002) but lower than the 21.7% found in Iran rats (predominant serogroups: Icterohaemorrhagiae, Grippotyphosa, and Hardjo) (Garoussi et al., 2006), 42% in Barbados (predominant serovar was Copenhageni) (Levett et al., 1998) and 90.9% Terceira Island, Azores (predominant serovar was Arborea).
These variable seroprevalence of leptospiral infections across countries therefore reflect the level of infections in local rat populations in these countries which could be influenced by the environmental conditions.

Of interest was the finding in the current study that juvenile rats were at a significantly higher risk of being seropositive for anti-leptospiral antibodies than older rodents. This finding is at variance with that reported in Brazil and Argentina where older rats were reported to be more at risk than juvenile rats (Vanasco et al., 2003; De Faria et al., 2008). This difference cannot be readily explained but may reflect differences in the types and practices of rats studied, as well as environmental differences.

Dogs are considered to be good indicators of the distribution of different leptospiral serovars in its environment (Bakoss et al., 1992) and rodents are important reservoirs of infection (Faine, 1994; Vanasco et al., 2003; Priya et al., 2007; Koizumi et al., 2008), continuously shedding leptospires in their urine. The detection of similar serovars of leptospires in both dogs and rats in our study is therefore not surprising and is in agreement with a previous report, describing them as common maintenance hosts (Zacarias et al., 2008). The considerably lower titres of the same infecting serovars detected in rats compared with either stray dogs or suspected cases of canine leptospirosis further confirms the reservoir status of rats for leptospral infections. The high titres detected to the serovars in suspected cases of canine leptospirosis in our study are also an indication that they are responsible for the clinical signs observed. Our study highlights the epidemiological significance, of rat-dog transmission. Rats may excrete the organism in their urine which may contaminate the food and water consumed by dogs and humans, resulting in infection. It has been recommended that rodent control is one of the most important measures used to control or prevent leptospirosis in dogs, livestock and humans (Sambasiva et al., 2003; Meeyam et al., 2006). Similar serologic epidemiologic study on human leptospirosis would be required to determine the significance of rats and dogs in zoonotic transmission in Trinidad.

In conclusion, our study has highlighted the significance of serogroup Icterohaemorrhagiae, serovar Copenhageni, based on its high frequency of detection in rats, stray dogs and suspected cases of canine leptospirosis coupled with the comparatively high titres in infected dogs but low titres in rats, being reservoirs, in Trinidad. Our findings also suggest that this serovar may be of zoonotic and public health importance in Trinidad. The significance of this serovar in the country can however not be fully elucidated until the important infecting serovars of *Leptospira* in human population have been determined.

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