*Am. J. Trop. Med. Hyg.*, 88(1), 2013, pp. 178–183
doi:10.4269/ajtmh.2012.12-0232


# Cross-Sectional Study of *Leptospira* Seroprevalence in Humans, Rats, Mice, and Dogs in a Main Tropical Sea-Port City

Claudia M. E. Romero-Vivas,* Margarett Cuello-Pérez, Piedad Agudelo-Flórez, Dorothy Thiry, Paul N. Levett, and Andrew K. I. Falconar

*Grupo de Investigaciones en Enfermedades Tropicales, Departamento de Medicina, División Ciencias de la Salud, Universidad del Norte, Barranquilla, Colombia; Instituto Colombiano de Medicina Tropical, Universidad CES, Medellín, Colombia; Saskatchewan Disease Control Laboratory, Regina, Saskatchewan, Canada*

*Abstract.* Samples were collected from 128 symptomatic humans, 83 dogs, 49 mice, and 20 rats (*Rattus rattus*: 16; *Rattus norvegicus*: 4) in neighborhoods where human leptospirosis have been reported within the principal sea-port city of Colombia. Seroprevalences were assessed against 19 pathogenic, 1 intermediate pathogenic, and 1 saprophytic *Leptospira* serogroups. Pathogenic *Leptospira* were confirmed using conventional *Leptospira*-specific polymerase chain-reaction and pulsed-field gel electrophoresis analysis was used for serovar identification. Seroprevalences of 20.4%, 12.5%, 25.0%, 22.9%, and 12.4% were obtained against one to seven different serogroups in mice, *R. rattus*, *R. norvegicus*, dogs, and humans, respectively. The DNA was confirmed to be from pathogenic *Leptospira* by detecting the *lipL32* gene in 12.5%, 3.7%, and 0.03% of the *R. rattus*, dog, and human samples, respectively. The first genetically typed Colombian isolate was obtained from a rat and identified as *Leptospira interrogans* serovar Icterohaemorrhagiae/Copenhageni.

## INTRODUCTION

Leptospirosis is a neglected zoonotic disease that has emerged as a public health problem throughout the world, however leptospirosis is more prevalent in tropical areas.[1,2] Its urban transmission is associated with high rainfall, poor sanitation, high rat infestations, and the presence of stray dogs.[3–5]

The first urban outbreak of leptospirosis in Colombia occurred in 1995 after flooding in Barranquilla, its principal sea-port, and resulted in four deaths from infections with the *Leptospira interrogans* Icterohaemorrhagiae serogroup.[6,7] However, few studies have been performed in *Leptospira* seroprevalences of urban-dwelling animals in this country. In one study, stray dogs (Cali, Valle) had a 41.1% *Leptospira* seroprevalence,[8] whereas brown rats (*Rattus norvegicus*) trapped in a grocery center (Medellín, Antioquia) had a 25.0% seroprevalence.[9]

Barranquilla is located on the Caribbean coast, has a population of ~1.2 million, lies at 12 feet above sea level, and is the principal sea-port of Colombia. The average annual temperature is 28°C, with little seasonal variation, and two wet seasons occur from April to July and September to December, during which many streets become flooded, despite having a network of open concrete drainage channels. Three neighborhoods in this city (Las Américas, Rebolo, Por Fin), have historically been considered as "hot spot" areas for human leptospirosis by the local health authorities (Secretaria Distrital de Salud de Barranquilla). We performed a descriptive, cross-sectional study to assess the pathogenic *Leptospira* seroprevalences of humans, rats, mice, and dogs against 19 different pathogenic, one intermediately pathogenic, and one non-pathogenic *Leptospira* serogroups in these neighborhoods. Active infections were further confirmed by the detection of the pathogenic leptospira gene (*lipL32*) by a conventional polymerase chain-reaction (PCR) assay.[10] *Leptospira* serovar-level characterization of the isolate was performed using pulsed-field gel electrophoresis (PFGE) analyses.[11,12]

* Address correspondence to Claudia M. E. Romero-Vivas, Grupo de Investigaciones en Enfermedades Tropicales, Departamento de Medicina, Universidad del Norte, Km 5 antigua via a Puerto Colombia, Barranquilla, Atlantico, Colombia 1. E-mail: clromero@uninorte.edu.co

## MATERIALS AND METHODS

**Study population and sample collection.** A descriptive, cross-sectional study was performed at different times from June 2007 to November 2009 in humans, dogs, and rodents in these three neighborhoods (Las Américas, Rebolo, Por Fin). Streets within each neighborhood where human leptospirosis cases/deaths were reported were selected for the dog and rodent collections.

*Humans.* After written consent was obtained, full clinical information for leptospirosis,[13] and first (S1) blood samples (10 mL without heparin) were collected from humans who presented with leptospirosis-like symptoms at outpatient clinics (one in each neighborhood) and four hospitals located on the study areas. Four to 30 days later, the second (S2) blood samples were obtained from each of them, and when possible, 10 mL mid-stream urine samples were collected.

These patients were asked whether they 1) owned a dog and, if so, whether the dog wandered the streets or entered their houses or backyards; 2) had recently seen or had contact with rodents in their houses or backyards; 3) observed flooding of the roads or drainage channels near their houses, or in their backyards; or 4) walked through such flooded areas barefoot.

*Dogs.* The sample size calculation was obtained by StatCal for descriptive studies using EpiInfo 6.0 (CDC, Atlanta, GA). The total dog population in these study areas was obtained from records from the anti-rabies virus dog vaccination program performed in each neighborhood (Las Américas: $N$ = 1,425; Por Fin: $N$ = 1,097; Rebolo: 1,367) during 2007. Based on a 41.1% dog *Leptospira* seroprevalence, as reported in Cali (Colombia),[8] with a 95% confidence level, at least 27 dogs were required to be studied in these neighborhoods. Written consent was obtained from dog owners, including owners whose dogs were found wandering freely on the streets. Free roaming dogs were captured using dog catching poles and

muzzled. Physical examinations for leptospirosis-like signs and symptoms were conducted and recorded by qualified veterinarians. Blood samples (4 mL) were obtained from their cephalic veins. The diuretic furosemide (Diurivet, Provet, Bogotá, Colombia) (3 mg/pound body weight) was administered 15 minutes before 10 mL urine samples were collected by bladder puncture (cystocentesis).

*Rodents.* The rodent populations in these neighborhoods included mice (*Mus musculus*) and rats (*R. norvegicus* and *Rattus rattus*). The sample size was calculated according to the published table by Faine and others[14] assuming an infinite rodent population and a 27.0% average seroprevalence for synanthropic rodents obtained in Brazil,[15] United States (Hawaii and Baltimore),[16,17] United Kingdom,[18] and India,[19] with a 95% confidence level. From this calculation, at least 45 rodents were required to be sampled in these study neighborhoods.

For live rodent captures, traps were placed inside houses where signs of rodent activity were observed from May to September 2009. Both commercial ("Humane" mouse trap, Catchmaster, Brooklyn, NY) and locally made cage traps baited with peanut butter and oats or cooked meat were used. The captured rodents were anesthetized using 100 mg/kg tiletamine/zolazepam (Zoletil 50, Laboratorios Virbac S.A., Colombia) and transferred to a biological safety level-3 (BSL-3) facility where blood (1–2 mL) was obtained by cardiac puncture, and kidneys were aseptically extracted.

**Blood, urine, and kidney sample processing.** One kidney from each rodent was subjected to aseptic maceration in 2 mL of phosphate buffered saline using a sterile mortar and pestle. The human, dog, and rodent (clotted blood) and rodent (macerated kidney) samples were centrifuged at 2,000 × *g* for 10 min at 25°C to ensure that the *Leptospira* were not pelleted, and the sera and kidney supernatants were collected. The human and dog urine samples were centrifuged at 7,000 × *g* for 20 min at 25°C, their supernatants were discarded, and the pellets were suspended in 1 mL of phosphate buffered saline. All the samples, including the kidneys pellets and supernatants were immediately stored at −85°C.

***Leptospira* culture.** Resuspended urine pellets (humans and dogs) and macerated kidney supernatants (rodents) were cultured immediately after centrifugation from 10 clinically suspected *Leptospira* patients, 54 dogs, and 69 rodents. Triplicate 200 μL volumes (600 μL total) were added to Ellinghausen, McCullough, Johnson, Harris-Difco medium (Sparks, MD) containing 0.4 g/mL 5-fluorouracil (Ebewe, Pharma, Unterach, Austria), and incubated at 30°C for up to 4 months. Weekly inspections were performed using dark field microscopy.

**Pathogenic *Leptospira* detection in samples and isolates.** The detection of active pathogenic *Leptospira* infections in the sera (humans, dogs, and rodents), urine pellets (humans, dogs), macerated kidney supernatants (rodents), and the human, dog, and rodent sample cultures were performed targeting the *lipL32* gene using the primers *lipL32*/270F (CGCTGAAATGGGAGTTCGTATGATT) and *lipL32*/692R CCAACAGATGCAACGAAAGATCCTTT) and the conventional PCR protocol already described.[9,10] Briefly, genomic DNA was extracted using Qiamp DNA mini kits (Qiagen, Hilden, Germany), and the PCRs were performed in 25 μL volumes containing 2–5 μL of DNA, 0.25 mM dNTPs (Bioline, Taunton, MA), 1.0 IU of Taq DNA polymerase (Thermo Scientific Fermentas, Waltham, MA), 3.0 mM $MgCl_2$, and 0.1 μM of LipL32/270F and LipL32/692R primers. The PCR program consisted of an initial cycle at 95°C for 5 minutes, followed by 35 cycles at 94°C for 1 minute and 55°C for 1 minute, and a final extension step at 72°C for 5 minutes. A band of 423 bp was considered positive.

***Leptospira* isolate typing.** The PFGE was performed as described previously.[11,12] Briefly, *Leptospira* DNA samples were digested with *Not I* (Roche, Mannheim, Germany), and *Salmonella* Braenderup H9812 strain DNA was used as standard size markers. The PFGE was performed in a 1% agarose gel using a CHEF DR III system (Bio-Rad, Hercules, CA) for 18 hr at 14°C, 0.5× Tris-Borate/EDTA buffer recirculation, with 2.16 and 35.07 second switching times at 120°, a gradient of 6V/cm, and a linear ramping factor. The resultant gel was stained with GelRed (Biotium) for 30 minutes and analyses were performed using BioNumerics version 4.0 (Applied Maths, Inc., Austin, TX). The PFGE profiles were then compared with a library of more than 200 reference strains and clinical isolates of *Leptospira* species[12] and DNA profiles with up to three fragment differences were considered to be the same serovar (Dice correlation: 73.7–100%).

**Microscopic agglutination test (MAT).** The MAT was performed as described previously[13] using 19 pathogenic, 1 intermediate pathogenic, and 1 saprophytic *Leptospira* serogroups, provided by the *Leptospira* Reference Laboratory (Hereford, UK). The following serogroups (serovars) were used: Australis (Bratislava), Autumnalis (Bangkinang), Ballum (Ballum), Bataviae (Bataviae), Canicola (Canicola), Celledoni (Anhoa), Djasiman (Djasiman), Grippotyphosa (Grippotyphosa), Fainei (Hurstbridge), Hebdomadis (Hebdomadis), Icterohaemorrhagiae (Icterohaemorrhagiae), Louisiana (Lousiana), Manhao (Lincang), Panama (Panama), Pomona (Pomona), Pyrogenes (Pyrogenes), Sarmin (Sarmin), Sejroe (Hardjo and Jin), Semaranga (Patoc), Shermani (Shermani), and Tarassovi (Vughia). The human, dog, and rodent serum samples were initially screened by MAT with *Leptospira biflexa* serovar Patoc at 1:100, 1:25, and 1:5 dilutions, respectively, and all samples that agglutinated ≥ 50% of them were further titrated using the MAT against each of the pathogenic and intermediately pathogenic serogroups. The MAT cut-off titers were those dilutions that caused ≥ 50% agglutination at ≥ 1:100 (humans and dogs) and ≥ 1:40 (rodents), and the highest titers obtained against one or more serogroups were recorded. Acute human leptospirosis was identified when MAT titers of their S1 versus S2 samples were increased from negative to ≥ 1:100 or by ≥ 4-fold.

**Statistical analyses.** Epidemiological and laboratory information for humans, dogs, and rodents were entered into an EpiInfo version 6.04 software system (CDC) database. Statistical $P$ values of $< 0.05$ in two-sided tests were reported as statistically significant differences.

**Ethics statement.** The Universidad del Norte Ethics Committee, following Colombian national guidelines, approved this study involving human patients and animals. Written consent was obtained from all of the patients, or in the case of children, from their parents before any samples or details were collected. Written consent was also obtained from each dog owner and house owners where rodent traps were placed.

## RESULTS

**Human patients.** Although there were 132 eligible patients who agreed to participate in the study, paired serum samples

could only be collected from 128 of these patients who displayed leptospirosis-like symptoms. A single serum sample was also included in this study that was obtained from one fatal case (patient #15). Their ages ranged from 3 months to 70 years, the majority (58.8%) occurring in young (0–14 years of age) patients, most of whom (52.8%) were school students. Seventy percent of these people reported to have seen rodents in their houses and 29.6% owned dogs.

The MAT results identified reactions with pathogenic *Leptospira* in 16 of 128 (seroprevalence: 12.5%; 95% confidence interval [CI]: 7.7–21.1%) of these patients (Table 1). The most prevalent presumptive serogroups were Australis (31.3%: 5 of 16), followed by two cases each of the Sarmin and Icterohaemorrhagiae (12.5%: 2 of 16) serogroups. One fatal case (patient #15), for which only an S1 sample was obtained, could not therefore be serologically confirmed. Acute serologically confirmed leptospirosis cases were more frequently observed in the residents of Por Fin (9 of 7,630: 0.12% than Las Américas [6 of 9,740: 0.06%] or Rebolo [2 of 19,610: 0.01%]) during June 2007. The strongest reactions of patients' serum samples were obtained against seven *Leptospira* serogroups in Por Fin, three in Las Américas (Australis, Serjoe and Sarmin), and two in Rebolo (Australis and Canicola), although at least one patient from each of these three neighborhoods had the strongest reactions against only the Australis serogroup.

Most of the cases were anicteric leptospirosis (87.5%: 14 of 16), did not require hospitalization, and were not treated. The most common symptoms in these patients were fever (100%: 16 of 16), headache (70.6%: 11 of 16), eye pain (50.0%: 8 of 16), and nausea/vomiting (43.8%: 7 of 16). A serologically confirmed leptospirosis case (patient #14) (6.3%: 1 of 16) developed hepatic damage and this patient's S1 and S2 serum samples mostly reacted with the *Leptospira* serogroup Ballum (Table 1). A probable leptospirosis case (patient #15) who displayed classical Weil's disease symptoms died 24 hr after arrival at the hospital. This patient's S1 serum sample had the highest (1:3,200 titer) reaction against the *Leptospira* serogroup Tarassovi.

Most of the patients involved in the study (81.3%: 13 of 16) reported to have seen or had contact with rodents inside their houses. However, there was no significant association between the presence of rodents and the occurrence of the disease in these people ($P = 0.96$; odds ratio [OR] = 1.2; 95% CI [0.29–3.15]). Despite 30% (45 of 128) of the population owning a dog and all of them having contact with dogs, there was no significant association with the occurrence of the disease ($P = 0.73$; OR = 1.2; 95% CI [0.42–3.36]).

The *lipL32* gene was detected by PCR in only one S1 sample obtained from a 29 year old man (patient #6) who lived in Las Américas. This patient's S1 and S2 samples strongly reacted against the *Leptospira* Sarmin serogroup (Table 1). No isolations could be obtained from the 10 urine cultures, including those from patients who were MAT positive (patient #4, #6, and #8).

**Dogs.** From November to December 2008, blood samples were collected from 83 dogs and urine samples were obtained from 54 (65%) of them. The seroprevalence of these dogs against pathogenic *Leptospira* serogroups was 22.9% (19 of 83) (95% CI: 13.8–35.8%). Their highest MAT titers were generated against the Lousiana (1:25,000 and 1:6,400), Autumnalis (1:1,600), and the intermediate pathogenic Fainei (1:3,200 and 1:1,600) serogroups (Table 2). All four dogs (21.1%: 4 of 19) that had the highest MAT titers (1:100 to 1:800) against the Icterohaemorrhagiae serogroup, among the serogroups tested, were collected in Rebolo. In contrast, most of the dogs (75%: 3 of 4) that had the highest MAT titers against serogroup Tarassovi were collected in Las Américas. Uniquely, two dogs collected in Por Fin had the highest MAT against the *Leptospira* Autumnalis serogroup, or equal highest against this serogroup and the Fainei serogroup.

TABLE 1

Details of 16 human serologically confirmed leptospirosis cases with the presumptive serogroups from three "hot spot" human leptospirosis neighborhoods of Barranquilla*

| Neighborhood | P # | Age (Sex) | S1 Day | Occupation† (Animal contact) | S1 serogroup (Titer) | S2‡ Day | S2 serogroup (Titer) |
|---|---|---|---|---|---|---|---|
| Las Américas | 1 | 39 (F) | 2 | H (H/S) | Australis (1:100) | 6 | Australis (1:6,400) |
| | 2 | 17 (F) | 5 | S (H/S) | no titer | 8 | Australis (1:400) |
| | 3 | 19 (M) | 4 | U (H/S) | Australis (1:100) | 12 | Australis (1:400) |
| | 4 | 24 (M) | 9 | U (H/S) | no titer | 11 | Serjoe (1:400) |
| | 5 | 7 (M) | 3 | S (H/S) | no titer | 5 | Sarmin (1:400) |
| | 6 | 29 (M) | 0 | R (H/S) | no titer$^{PCR}$ | 6 | Sarmin (1:400) |
| Por Fin | 7 | 59 (F) | 1 | H (H/S) | no titer | 7 | Pomona (1:3,200) |
| | 8 | 17 (F) | 2 | S (H/S) | no titer | 7 | Tarassovi (1:1,600) |
| | 9 | 16 (M) | 0 | S (B/S) | no titer | 6 | Icterohaemorrhagiae (1:400) |
| | 10 | 11 (F) | 2 | S (B/S) | no titer | 8 | Australis (1:400) |
| | 11 | 6 (M) | 2 | S (H/S) | no titer | 4 | Icterohaemorrhagiae (1:400) |
| | 12 | 14 (F) | 2 | S (H/S) | no titer | 5 | Fainei (1:400) |
| | 13 | 7 (M) | 4 | S (B/S) | no titer | 10 | Autumnalis (1:800) |
| | 14 | 20 (F) | 5 | H (H/S) | Ballum (1:100) | 10 | Ballum (1:1,600) |
| | 15 | 36 (M) | 15 | U (H/S) | Tarassovi (1:3,200) | ‡ | § |
| Rebolo | 16 | 23 (M) | 5 | U (H/S) | Australis (1:100) | 9 | Australis (1:800) |
| | 17 | 37 (F) | 3 | H (H/S) | Canicola (1:200) | 9 | Canicola (1:1,600) |

*Details (age, sex, day of first [S1] collection after the onset of symptoms, occupation, contact with animals, and their highest microscopic agglutination test [MAT] titers against particular *Leptospira* serogroups) of 16 leptospirosis patients (P #1–14 and P #16–17), and one presumptive leptospirosis patient (P #15), from three human leptospirosis high-risk neighborhoods (Las Américas, Por Fin, and Rebolo) in Barranquilla. The highest MAT titers obtained against a particular serogroup are shown.

†Occupation as a house-wife (H), school student (S), recreationist (R) (outdoor training instructor), or unused (U), with details of contact with mice, rats, and dogs inside their houses (H), their backyards (B), or in the streets (S) (in parentheses).$^{PCR}$ Sera PCR positive.

‡Day of collection of 16 second (S2) serum samples after the onset of fever.

§Patient #15 died before the S2 serum sample could be obtained.

TABLE 2

Seroprevalence and presumptive serogroups of symptomatic and asymptomatic dogs collected in three human leptospirosis high-risk neighborhoods of Barranquilla*

| Neighborhood | MAT ≥ 1:100 Serogroup-reactive/ total (%) | Serogroup (titer) Symptomatic = S |
|---|---|---|
| Las Américas | 4/26 (15.4%) | Fainei (1:1,600) **S**<br>Tarassovi (1:100) **S**<br>Tarassovi (1:200) **S**<br>Tarassovi (1:800) **S** |
| Por Fin | 3/30 (10.0%) | Louisiana (1:400) **S**<br>Autumnalis (1:1,600) **S**<br>Autumnalis/Fainei (1:1,400) |
| Rebolo | 12/27 (44.4%) | Icterohaemorrhagiae (1:100) **S**<br>Icterohaemorrhagiae (1:200) **S**<br>Icterohaemorrhagiae (1:400) **S**<br>Icterohaemorrhagiae (1:800) **S**$^{PCR}$<br>Louisiana (1:800)<br>Louisiana (1:6,400) **S**<br>Louisiana (1:25,000) **S**<br>Tarassovi (1:100) **S**<br>Canicola (1:800)<br>Fainei (1:3,200) **S**<br>Grippotyphosa (1:800)<br>Icterohaemorrhagiae/ Grippotyphosa/Sarmin (1:400) **S** |
| Positive/Total | 19/83 (22.9%) | Symptomatic/ asymptomatic: 15/19 (78.9%) |

*Eighty-three dogs collected from three human leptospirosis high-risk neighborhoods (Las Américas, Por Fin, and Rebolo) of Barranquilla were assessed for signs and symptoms of canine leptospirosis (**S** = symptomatic), and their highest microscopic agglutination test (MAT) titers were determined against a panel of 20 different pathogenic *Leptospira* serogroups. The highest MAT titers obtained against one or more serogroups are shown.**PCR** Urine PCR positive.

Three of the dogs from Rebolo (25.0%: 3 of 12) and one collected in Por Fin (33.3%: 1 of 3) had the highest MAT titers against the *Leptospira* Louisiana serogroup (Table 2). Two dogs collected in Rebolo had the highest or equal highest MAT titers against the Grippotyphosa serogroup or the Grippotyphosa/Icterohaemorrhagiae/Sarmin serogroups. However, one dog collected in Rebolo had the highest MAT titer against the Canicola serogroup.

Most (78.9%: 15 of 19) of *Leptospira* MAT-positive dogs, which generated the highest MAT titers against pathogenic *Leptospira* of seven different serogroups, showed common canine leptospirosis signs/symptoms.[14] These signs/symptoms were arched back (26.3%: 5 of 19), vomiting (15.8%: 3 of 19), swollen kidneys (10.5%: 2 of 19), depression (10.5%: 2 of 19), fever (26.3%: 5 of 19), jaundice (5.3%: 1 of 19), bloody urine (hematuria) (5.3%: 1 of 19), and bloody feces (melena) (5.3%: 1 of 19). However, there were no statistically significant differences between any of the signs/symptoms displayed by the MAT positive versus MAT negative dogs. Despite urine samples from 14 of 19 MAT-positive dogs being cultured, no *Leptospira* isolates were obtained. The pathogenic specific *lipL32* gene was however identified by PCR in the urine samples from 2 of the 4 dogs (3.7%), one of which was from Rebolo and was MAT positive (Icterohaemorrhagiae serogroup: 1:800 titer), whereas the other from Por Fin but was MAT negative.

**Rodents.** Four months (from May to September 2009) were taken to capture 69 rodents, but only low numbers of *R. norvegicus* (4 of 69: 5.8%) could be trapped. Most (71.0%: 49 of 69) were domestic mice (*M. musculus*) and black rats (*R. rattus*) (23.2%: 16 of 69). The majority were captured in the residents' kitchens (49.3%: 34 of 69), and backyards (43.5%: 30 of 69) (Table 3).

For these rodents, MAT-positive titers were defined by a ≥ 1:40 cut-off titer as described previously.[9] In this study, seroprevalences of 20.4% (10 of 49) (95% CI: 9.1–31.6%), 12.5% (2 of 16) (95% CI: 0.0–28.7%), and 25.0% (1 of 4) (95% CI: 0.0–67.0%) were obtained for mice, black rats, and brown rats, respectively (Table 3). Six MAT-positive mice had the highest titers against a single *Leptospira* serogroup (Grippotyphosa: $N = 3$; Icterohaemorrhagiae: $N = 1$; Canicola: $N = 1$; Mini: $N = 1$), whereas the other four MAT-positive mice had equal highest titers against two serogroups. Two mice from Las Américas and one from Por Fin had the highest MAT titers against serogroup Grippotyphosa. In contrast, the highest MAT titers of one mouse captured in Por Fin was against the Icterohaemorrhagiae serogroup (1:80), whereas one mouse captured in Rebolo had the highest MAT titers against the Icterohaemorrhagiae/Australis serogroups (1:80) (Table 3).

Serum samples from two black rats (*R. rattus*) from Las Américas and Por Fin were strongly positive against the Serjoe (20.0%: 1 of 16) or Grippotyphosa (11.10%: 1 of 9)

TABLE 3

Seroprevalences and highest microscopic agglutination test (MAT) titers against presumptive *Leptospira* serogroups of mice and rats collected in three human high-risk neighborhoods of Barranquilla*

| Neighborhood | MAT-positive/total (%): *Leptospira* serogroup (MAT titer) | | |
|---|---|---|---|
| | *Mus musculus* | *Rattus rattus* | *Rattus norvegicus* |
| Las Américas | 3/14 (21.4%):<br>Grippotyphosa (1:40)<br>Panama/Pyrogenes (1:40)<br>Grippotyphosa (1:80) | 1/5 (20.0%):<br>Serjoe (1:40) | 0/2 (0.0%) |
| Por Fin | 2/16 (12.5%):<br>Icterohaemorrhagiae (1:80)<br>Grippotyphosa (1:40) | 1/9 (11.1%):<br>Grippotyphosa (1:160) | 0/1 (0.0%) |
| Rebolo | 5/19 (26.3%):<br>Canicola (1:40)<br>Mini (1:40)<br>Canicola/Serjoe (1:40)<br>Icterohaemorrhagiae/Ballum (1:40)<br>Icterohaemorrhagiae/Australis (1:80) | 0/2 (0.0%) | 1/1 (100.0%):<br>Australis (1:40) |
| Positive/Total | 10/49 (20.4%) | 2/16 (12.5%) | 1/4 (25.0%) |

*The MAT titers (≥ 1:40) of house mice (*Mus musculus*) ($N = 49$), black rats (*Rattus rattus*) ($N = 16$), and brown rats (*Rattus norvegicus*) ($N = 4$) collected in three human leptospirosis high-risk neighborhoods (Las Américas, Por Fin, and Rebolo) of Barranquilla were determined against 20 different pathogenic *Leptospira* serogroups, and the highest MAT titers against one or more serogroups are shown.

serogroups, respectively, although one brown rat (*R. norvegicus*) (25%: 1 of 4) captured in Rebolo strongly reacted with the Australis serogroup.

The pathogenic specific *lipL32* gene was identified by PCR in two kidney samples from *R. rattus* (12.5%: 2 of 16) collected in Por Fin and Rebolo and both of them were MAT negative.

Of the 69 kidney supernatant cultures, only one *Leptospira* strain was isolated from a MAT-negative *R. rattus* collected in Rebolo. This isolate was characterized to its serovar level using PFGE analysis. In this analysis, identical DNA restriction fragment patterns (DICE correlation: 100%) were identified between this isolate (Colombia I15) and two *Leptospira interrogans* Icterohaemorrhagiae strains isolated from the United States, which were in the database.[12] Although there was a fragment difference with *L. interrogans* serovar Copenhageni M20, it could not be discriminated from the serovar Icterohaemorrhagiae (data not shown).

## DISCUSSION

The study attempted to assess *Leptospira* occurrence in human and animal populations in the neighborhoods of Las Americas, Por Fin, and Rebolo, which have been consistently identified as "hot spots" for human leptospirosis in the principal sea-port city of Colombia, where the first case of urban Colombian leptospirosis was documented in 1995.[6,7] These three neighborhoods were composed of the two lowest economic strata in this country, being dissected by large numbers of open concrete drainage channels that overflowed onto the surrounding dirt roads during the wet seasons. Their house design, with open eaves and hollow-brick ventilation sites in their walls, allowed rodents to readily enter these residents' houses, as a result, these patients reported to have seen or had contact with rodents inside their houses. Most of the patients owned dogs, but none of these dogs had received leptospirosis vaccination, and they roamed the streets during the day and entered their owners' houses and backyards at night. During this study, we noted that most of the confirmed *Leptospira* patients were housewives and unemployed men, as well as ≤ 17-year-old students, who spend most of their time at home thereby suggesting household rather than occupational transmission. Most of the houses (81.2%: 13 of 16) of patients were also located within 65.6 yards of a main open concrete drainage channel where children also played barefoot, and which often overflowed onto the dirt streets during the wet seasons. However, the other non-*Leptospira* patients who were assumed to be equally at risk of *Leptospira* infections did not show antibody responses to *Leptospira*.

Acute serologically confirmed leptospirosis cases were more frequently observed during June 2007, when around half of these cases occurred and when high rainfall was recorded in both May (8.47 inches) and June (3.98 inches), that resulted in increased risk of flooding and human exposure to animal urine, as shown in other urban areas.[3] All but two of these patients were anicteric. One of the two icteric patients had a fatal outcome. These latter cases were presumptively associated with the *Leptospira* Tarassovi and Ballum serogroups, respectively. The former serogroup has mainly been associated with pigs,[20] and the latter mainly with domestic mice.[21] Because the *Leptospira* serogroup Tarassovi has been associated with Weil's disease,[22] it may account for the severe and fatal Weil's disease-like symptoms displayed by patient #15. Although these results suggested that this serogroup could have been circulating between the human and dog populations, transmission from domestic pigs could not be excluded. Although we obtained antibody reactions against pathogenic serogroups that were associated with severe disease using the gold standard MAT, cross-reactions between serogroups have been shown to occur, and this can make it impossible to definitively determine the infective serogroup/serovar, unless isolations were obtained and characterized.[23]

Overall, therefore, the presumptive pathogenic *Leptospira* serogroups common to humans, dogs, and mice were Icterohaemorrhagiae and Canicola, especially in Rebolo. Half of the human leptospirosis patients from Las Américas and Rebolo generated the highest MAT titers against Australis and antibody reactions were also observed in *R. norvegicus*. This serogroup is mainly associated with equines.[20,24] Although there were serological reactions against serogroup Grippotyphosa in the dog and rodent (*M. musculus* and *R. rattus*) populations, none of the sera from the patients in this study reacted against this serogroup, despite human serum samples most strongly reacting with this serogroup in healthy people in Barranquilla,[25] and elsewhere in Colombia (Uraba, Antioquia).[26] When performing our field work, we observed that many of the residents bred or maintained domestic pigs, horses, and donkeys in their backyards. We are presently attempting to isolate *Leptospira* strains from these domestic animals to assess their roles as potential sources of infections in these neighborhoods.

Interestingly, serum samples collected from several dogs had very high MAT titers against the Louisiana serogroup. Although we were unable to isolate these leptospires, we believe this to be the first serological evidence for the circulation of this serogroup in Latin America. A strain of the Louisiana serogroup was previously isolated from an armadillo (LSU 1945) in the United States, and a human patient (R/740 lanka) from Sri Lanka,[20] but its normal maintenance host is unknown.

The neighborhood with the most diversity in presumptively identified *Leptospira* serogroups in dogs and rodents was Rebolo, which is located immediately adjacent to the principal international dock area. This is also the principal port of Colombia and therefore the importation of new *Leptospira* serogroups by rodents transported on these visiting commercial ships is likely.

Evidence of current infections with pathogenic *Leptospira* was obtained by PCR from human, dog, and black rat populations. The intermediate pathogenic leptospires cannot be detected targeting the *lipL32* gene, which is detectable only in pathogenic leptospires.[27] Thus, if the humans or animals were infected with the intermediately pathogenic Fainei serogroup, which presumptively circulated in the human and dog populations, it would not be detected by this PCR.

Isolation rates of *Leptospira* spp. from rats and mice have varied.[28–30] Our *Leptospira* isolation rates from *R. rattus*, *R. norvegicus*, and *M. musculus* kidney samples of 6.3% (1 of 16), 0.0% (0 of 4), and 0.0% (0 of 49) were therefore similar to the 3.2% (3 of 94), 6.3% (6 of 86), and 0.0% (0 of 55) obtained from these three species in Madagascar, respectively.[31] We also confirmed that the infection of the black rat with *L. interrogans* serovar Icterohaemorrhagiae/Copenhageni could not be differentiated using PFGE as previously shown.[32] This I15 isolate is now the first isolate to be included in a Colombian MAT diagnostic panel.

Received April 10, 2012. Accepted for publication October 7, 2012.

Published online November 13, 2012.

Acknowledgments: We thank the health authorities of Barranquilla for their logistical support and technical help through their local outpatients' clinics and their hospitals (Hospital Universidad del Norte, Hospital Pediátrico, and Clínica General del Norte), Patricia Mendez and her team for performing the veterinary assessments and procedures, Yimmis De la Hoz, Pablo Maldonado, and Pedro Arango (Secretaria Distrital de Salud de Barranquilla) for help with the dog and rodent collections. We are also greatly indebted to the two anonymous reviewers for the thorough revisions and suggestions for improving the contents of this article.

Financial support: This study was supported by the Departamento Administrativo de Ciencia Tecnología e Innovación, Colciencias ID Project Number 1215-408-20551.

Authors' addresses: Claudia M. E. Romero-Vivas, Margarett Cuello-Pérez, and Andrew K. I. Falconar, Grupo de Investigaciones en Enfermedades Tropicales, Departamento de Medicina, Universidad del Norte, Barranquilla, Colombia. E-mails: clromero@uninorte.edu.co, margarethc@uninorte.edu.co, and afalconar@uninorte.edu.co. Piedad Agudelo-Flórez, Instituto Colombiano de Medicina Tropical, Universidad CES, Medellín, Colombia, E-mail: pagudelo@ces.edu.co. Dorothy Thiry and Paul N. Levett, Saskatchewan Disease Control Laboratory, Regina, Saskatchewan, Canada, E-mails: dthiry@health.gov.sk.ca and plevett@health.gov.sk.ca.

## REFERENCES

1. Desvars A, Cardinale E, Michault A, 2011. Leptospirosis in small tropical areas. *Epidemiol Infect 139:* 167–188.
2. World Health Organization, 2011. *Weekly Epidemiological Record*. Available at: http://www.who.int/wer/2011/wer8606.pdf. Accessed January 19, 2012.
3. Reis RB, Ribeiro GS, Felzemburgh RD, Santana FS, Mohr S, Melendez AX, Queiroz A, Santos AC, Ravines RR, Tassinari WS, Carvalho MS, Reis MG, Ko AI, 2008. Impact of environment and social gradient on *Leptospira* infection in urban slums. *PLoS Negl Trop Dis 23:* e228.
4. Villanueva SY, Ezoe H, Baterna RA, Yanagihara Y, Muto M, Koizumi N, Fukui T, Okamoto Y, Masuzawa T, Cavinta LL, Gloriani NG, Yoshida S, 2010. Serologic and molecular studies of *Leptospira* and leptospirosis among rats in the Philippines. *Am J Trop Med Hyg 82:* 889–898.
5. Maaciel EA, de Carvalho AL, Nascimento SF, de Matos RB, Gouveia EL, Reis MG, Ko AI, 2008. Household transmission of *Leptospira* infection in urban slum communities. *PLoS Negl Trop Dis 2:* e154.
6. Epstein PR, Calix Pena O, Blanco-Racedo J, 1995. Climate and disease in Colombia. *Lancet 346:* 1243–1244.
7. Perez-Garcia J, 1997. Histopathological findings in necropsies with leptospirosis [in Spanish]. *Colomb Med 28:* 4–9.
8. Rodríguez AL, Ferro BE, Varona MX, Santafé M, 2004. Exposure to *Leptospira* in stray dogs in the city of Cali. *Biomedica 24:* 291–295.
9. Agudelo-Flórez P, Londoño AF, Quiroz VH, Angel JC, Moreno N, Loaiza ET, Muñoz LF, Rodas JD, 2009. Prevalence of *Leptospira* spp. in urban rodents from a groceries trade center of Medellin, Colombia. *Am J Trop Med Hyg 1:* 906–910.
10. Levett PN, Morey R, Galloway RL, Turner D, Steigerwalt AG, Mayer LW, 2005. Detection of pathogenic leptospires by real-time quantitative PCR. *J Med Microbiol 54:* 45–49.
11. Herrmann JL, Bellenger E, Perolat P, Baranton G, Saint Girons I, 1992. Pulsed-field gel electrophoresis of *Not* I digests of leptospiral DNA: a new rapid method of serovar identification. *J Clin Microbiol 30:* 1696–1702.
12. Galloway RL, Levett PN, 2010. Application and validation of PFGE for serovar identification of *Leptospira* clinical isolates. *PLoS Negl Trop Dis 14:* e824.
13. World Health Organization, 2003. *Human Leptospirosis: Guidance for Diagnosis, Surveillance and Control*. Geneva: World Health Organization and International Leptospirosis Society.
14. Faine S, Adler B, Bolin C, Perolat P, 1999. Leptospira *and Leptospirosis*. Melbourne: MediSci.
15. De Faria M, Calderwood M, Athanazio DA, McBride JA, Hartskeer RA, Pereira MM, Ko AI, Reis MG, 2008. Carriage of *Leptospira interrogans* among domestic rats from an urban setting highly endemic for leptospirosis in Brazil. *Acta Trop 108:* 1–5.
16. Higa HH, Fujinaka IT, 1976. Prevalence of rodent and mongoose leptospirosis on the island of Oahu. *Public Health Rep 91:* 171–177.
17. Vinetz JM, Glass GE, Flexner CE, Mueller P, Kaslow DC, 1996. Sporadic urban leptospirosis. *Ann Intern Med 125:* 794–798.
18. Webster JP, Ellis WA, McDonald DW, 1995. Prevalence of *Leptospira* spp. in wildbrown rats (*Rattus norvegicus*) on UK farms. *Epidemiol Infect 114:* 195–201.
19. Priya CG, Hoogendijk KT, Berg M, Rathinam SR, Ahmed A, Muthukkaruppan VR, Hartskeerl RA, 2007. Field rats form a major infection source of leptospirosis in and around Madurai, India. *J Postgrad Med 53:* 236–240.
20. Brenner DJ, Kaufmann AF, Sulzer KR, Steigerwalt AG, Rogers FC, Weyant RS, 1999. Further determination of DNA relatedness between serogroups and serovars in the family Leptospiraceae with a proposal for *Leptospira alexanderi* sp. nov. and four new *Leptospira* genomospecies. *Int J Syst Bacteriol 49:* 839–858.
21. da Silva EF, Félix SR, Cerqueira GM, Fagundes MQ, Neto AC, Grassmann AA, Amaral MG, Gallina T, Dellagostin OA, 2010. Preliminary characterization of *Mus musculus*-derived pathogenic strains of *Leptospira borgpetersenii* serogroup Ballum in a hamster model. *Am J Trop Med Hyg 83:* 336–337.
22. Yang CW, Pan MJ, Wu MS, Chen YM, Tsen YT, Lin CL, Wu CH, Huang CC, 1997. Leptospirosis: an ignored cause of acute renal failure in Taiwan. *Am J Kidney Dis 30:* 840–845.
23. Levett PN, 2003. Usefulness of serologic analysis as a predictor of the infecting serovar in patients with severe leptospirosis. *Clin Infect Dis 36:* 447–452.
24. Perolat P, Lecuyer I, Postic D, Baranton G, 1993. Diversity of ribosomal DNA fingerprints of *Leptospira* serovars provides a database for subtyping and species assignation. *Res Microbiol 144:* 5–15.
25. Macías-Herrera JC, Vergara C, Romero-Vivas C, Falconar A, 2005. Presentation of leptospirosis in Atlántico state (Colombia) from January 1999 to March 2004 [in Spanish]. *Salud Uninorte 20:* 18–29.
26. Agudelo-Florez P, Restrepo-Jaramillo BN, Arboleda-Naranjo M, 2007. Leptospirosis in Uraba, Antioquia, Colombia: a seroepidemiological and risk factor survey in the urban population [in Spanish]. *Cad Saude Publica 23:* 2094–2102.
27. Matthias MA, Ricaldi JN, Cespedes M, Diaz MM, Galloway RL, Saito M, Steigerwalt AG, Patra KP, Ore CV, Gotuzzo E, Gilman RH, Levett PN, Vinetz JM, 2008. Human leptospirosis caused by a new, antigenically unique *Leptospira* associated with a *Rattus* species reservoir in the Peruvian Amazon. *PLoS Negl Trop Dis 2:* e213.
28. Levett PN, Walton D, Waterman LD, Whittington CU, Mathison GE, Everard CO, 1998. Surveillance of leptospiral carriage by feral rats in Barbados. *West Indian Med J 47:* 15–17.
29. Matthias MA, Levett PN, 2002. Leptospiral carriage by mice and mongooses on the island of Barbados. *West Indian Med J 51:* 10–13.
30. Songer JG, Chilelli CJ, Reed RE, Trautman RJ, 1983. Leptospirosis in rodents from an arid environment. *Am J Vet Res 44:* 1973–1976.
31. Rahelinirina S, Léon A, Harstskeerl RA, Sertour N, Ahmed A, Raharimanana C, Ferquel E, Garnier M, Chartier L, Duplantier JM, Rahalison L, Cornet M, 2010. First isolation and direct evidence for the existence of large small-mammal reservoirs of *Leptospira* spp. in Madagascar. *PLoS ONE 5:* e14111.
32. Romero EC, Blanco RM, Galloway RL, 2009. Application of pulsed-field gel electrophoresis for the discrimination of leptospiral isolates in Brazil. *Lett Appl Microbiol 48:* 623–627.