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REGULAR ARTICLES

Leptospirosis in pigs, dogs, rodents, humans, and water in an area of the Colombian tropics

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Abstract Leptospirosis is a reemerging zoonosis of global distribution and is one of the causes of hemorrhagic fevers in the tropics. We sought to determine seroprevalence in humans and animals and isolate *Leptospira interrogans* sensu lato in domestic animals, rodents, and water sources. The study was conducted in a tropical area of the middle Sinú in Cordoba, Colombia. In a prospective descriptive study, we collected blood and urine from pigs and dogs, sera from rural human workers, sera and kidney macerates of rodents, and water samples from environmental sources. We used microagglutination to screen for antibodies to 13 serovars. Strains were cultured on the Ellinghausen–McCullough–Johnson–Harris medium and confirmed by PCR amplifying *lipL*32 gene. Seroprevalence was 55.9 % in pigs, 35.2 % in dogs, and 75.8 % in humans; no antibody was detected, and no *Leptospira* were isolated from kidney macerates of rodents. Seven *L. interrogans* sensu lato strains were isolated: three from pigs, two from dogs, and two from water. High seroprevalence in pigs, dogs, and humans, concomitant to isolation of strains, demonstrates that in Cordoba, transmission exists among animals, the environment, and humans, which warrants the implementation of public health intervention measures to reduce the epidemiological impact of leptospirosis in the region.

Keywords Epidemiological surveillance · Transmission · Zoonoses · Public health · Disease reservoirs · Social environment

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Introduction

Leptospirosis is a zoonosis of global distribution; it is considered a reemerging disease, endemic in tropical countries, which, because of their geoclimatic and social conditions, favor its transmission (Guerra 2013). In domestic and wild animals, chronic renal carriers maintain viable leptospires which may be excreted via urine becoming a source of infection for humans and other animals (Monahan et al. 2009). Humans can be infected with any serovar of *Leptospira* from any animal or environment, basically, through contact with fluids from infected animals (Lim 2009; Marga et al. 2013).

The organisms may enter the body via lacerations in the skin, contact with mucosa or conjunctiva, inhalation of aerosols, or ingestion of contaminated food or beverages (Monahan et al. 2009; Musso and La Scola 2013). Leptospirosis is considered an occupational disease, primarily affecting farmers, fishermen, veterinarians, and workers in sewers and slaughterhouses (Brown et al. 2011). A risk of infection for humans is the exposure during water-related recreational activities (Monahan et al. 2009; Marga et al. 2013).

The tropical environment favors survival of *Leptospira* outside reservoirs in stagnant or slowly flowing warm water. Water and soil contaminated by excreta of infected animals are also infection sources (Ganoza et al. 2006; Adler and Peña 2010). In recent years, this ecological aspect has gained importance, because of the increased incidence of exposure to contaminated environments (Vijayachary et al. 2008). Our aim was to measure the seroprevalence of *Leptospira* infection in humans and animals and isolate *Leptospira interrogans* sensu lato from animals and water sources.

Materials and methods

Type of study and geographic location We conducted a prospective, descriptive study between 2009 and 2011. The farms

studied were dedicated to pig farming and located in Monteria (8°46′15″N, 75°51′31″O), Cerete (8°52′22″N, 75°46′47″O), and Cienaga de Oro (8°52′44″N, 75°42′8″O). The three municipalities (Fig. 1) are located according to the departmental territorial geographic plan in a middle region of the Sinú River in the department of Cordoba, Colombia.

Study of pigs, dogs, rodents, and humans The total population of pigs in reproductive age in the three municipalities studied was 19.328. Considering a seroprevalence maximum (50 %), 95 % reliability, and 5 % standard error, the sample size for the microagglutination test (MAT) was 377, but blood samples were taken from 383 pigs in 18 farms. Bacterial cultures were performed according to the MAT results, and we took at each farm ten urine samples, such as four pigs with titres from 1:100 to 1:400, four with titres ≥1:400, and two with negative serologic results. This study included 54 dogs living on the study farms, and 39 rodents were captured with Sherman traps randomly distributed in different areas of the farms such as domestic areas, stables, and package store; the distribution of rodent species was *Mus musculus*, 49 % ($n=19$); *Rattus norvegicus*, 31 % ($n=12$); and *Rattus rattus*, 21 % ($n=8$). There were no rodents captured in five farms. The study included 62 workers who inhabited and worked on the farms and volunteered to participate.

Study of water We analyzed 54 water sources that were proportionally distributed among the farms selected. These sources included animal drinking water ($n=18$), water from wells ($n=18$), and wastewater from pig stables ($n=18$).

Serology There were 383 sera of pigs, 54 of dogs, 39 of rodents, and 62 of humans analyzed by MAT. We screened for antibodies against *L. interrogans* sensu lato using the MAT according to specifications of the International Office of Epizootics; 11 serovars were used: serogroup Australis (serovar Bratislava), serogroup Autumnalis (serovar Autumnalis), serogroup Batavie (serovar Batavie), serogroup Serjoe (serovar Hardjo), serogroup Grippotyphosa (serovar Grippotyphosa), serogroup Icterohaemorrhagiae, (serovar Icterohaemorrhagiae), serogroup Mini (serovar Mini), serogroup Pomona (serovar Pomona), serogroup Pyrogenes (serovar Zanoni), serogroup Tarassovi (serovar Tarassovi),

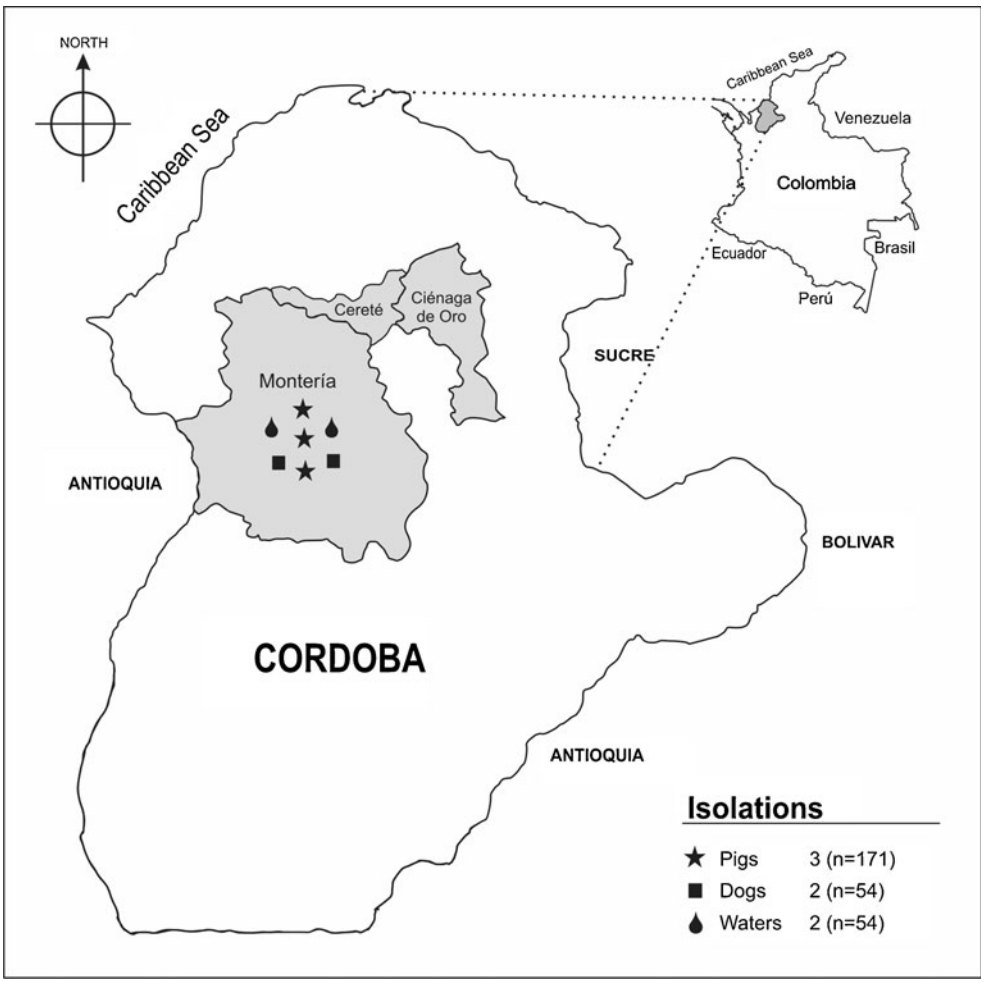

Fig. 1 Geographical distribution of *L. interrogans* sensu lato in Cordoba, Colombia

and serogroup Canicola (serovar Canicola). Titres ≥1:100 were considered positive.

L. interrogans culture in urine and water Following MAT, 183 pig samples were selected for bacteriological cultures, but 12 urine samples were lost, and only 171 were cultured: 20 with negative serology, 75 with titres between 1:100 and 1:400, and 76 with titres >1:400. The urine samples of the 54 dogs and 54 water samples were also cultured. Samples were cultured in Ellinghausen–McCullough–Johnson–Harris (EMJH) medium (Difco, Detroit, MI, USA), enriched with 1 % rabbit serum with and without supplementing nalidixic acid and 5-fluorouracil. Approximately 0.5 ml of the water and urine samples was seeded directly onto the EMJH medium; 2 ml was filtered through a 0.45-μm nitrocellulose membrane, and 0.5 ml was inoculated onto the EMJH medium. The cultures were incubated at 29–30 °C and examined weekly under dark-field microscopy for 4 months.

L. interrogans from cultures of kidney macerates of rodents Upon necropsies, one kidney was collected from each rodent, sectioned in half, and macerated in 2 ml PBS at pH 7.2; 0.5 ml of the suspension was inoculated directly in the EMJH medium. The rest was deposited in 5 ml of PBS at pH 7.2 and allowed to stand at 30 °C for 30 min. After this incubation, 0.5 ml of the supernatant medium was inoculated in EMJH medium.

Molecular identification The DNA was extracted from field isolates, meaning *Leptospira* isolates (strains) obtained in the field study and maintained in the laboratory. Cultures were used between 6 and 10 days of growth; 5 ml was taken and centrifuged at 13,000 rpm. The pellet was resuspended in 1 ml of TE. The DNA extraction was done with the Qiagen kit (QIAamp® DNA Mini Kit). Confirmation of pathogenic strains was conducted by using 270F and 692R-specific primers that amplify a 423-bp fragment, which encodes for the *Lip*32 protein (Levett et al. 2005). The conditions of the PCR reaction mixture were as follows: 1× PCR buffer, 1.25 nM dNTPs, 2.5 mM $MgCl_2$, 1 mM 270F initiator, 1 mM 692 R initiator, and Taq DNA pol 0.4 U. Each PCR cycle was 94 °C×1 min, 55 °C×1 min, and 72 °C×2 min; 34 cycles were performed.

Ethical aspects The Ethics Committee at the Institute of Investigations Biologics of Tropic (IIBT) at the University of Cordoba reviewed and approved the study protocol, considering articles 5 and 87 from decree 309 of 25 February 2000 from the Colombian Ministry of the Environment (2000). For the human population, farm workers were invited to participate on a volunteer basis through signed consent; the work was classified as minimum risk according to the Colombian Ministry of Health (008430/1993).

Results

Serological results The seroprevalence found were as follows: pigs, 55.9 % (214/383); dogs, 35.2 % (19/54); humans, 75.8 % (47/62); and rodents, 0 % (0/39). The percentage distribution by serogroups in the species studied is shown in Table 1.

Pig urine culture Of 171 pig urine samples cultured, three *Leptospira* strains were isolated in females in Montería and two of them were obtained in the same farm. Two females showed titres ≥1:800 for serovars Pomona, Tarassovi, Canicola, Grippotyphosa, and Icterohaemorrhagiae, while in the third female, the higher reactivity was 1.400 for serovar Pomona.

Dog urine culture Two isolates were obtained. One was from a seronegative dog; the other dog had titres of 1:100 for Serjoe and Batavie serogroups (1:200 for Grippotyphosa, Canicola, Tarassovi, and Mini serogroups; and 1:400 for Australis, Icterohaemorrhagiae, Pyrogenes, and Autumnalis serogroups).

Rodent culture From 39 cultures of kidney macerates, no *Leptospira* strains were isolated.

Water culture From 54 water samples, nine strains were isolated, but only two were identified as *L. interrogans* sensu lato in Monteria. The first strain was obtained from animal drinking water and the second from wastewater.

Rodents No *Leptospira* were isolated from the kidney of rodents.

Molecular identification The PCR identified seven isolates (three in pigs, two in dogs, and two water samples) like pathogenic strains, which amplified a product of about 423 bp of the gene *lipL*32 of pathogenic *Leptospira*.

Table 1 Percentage distribution of serogroups of *L. interrogans* sensu lato among different species in the middle Sinú area

Species/serogroup	Pigs %	Dogs %	Humans %
Mini	28.7	7.4	32.2
Batavie	34.7	9.2	37.1
Serjoe	40.2	9.2	43.5
Tarassovi	40.4	14.8	35.4
Autumnalis	42.8	11.1	50.0
Australis	43.6	14.8	38.7
Grippotyphosa	47.7	18.5	53.2
Pyrogenes	50.3	11.1	38.7
Icterohaemorrhagiae	56.4	14.1	54.8
Pomona	61.6	18.5	56.4
Canicola	62.4	14.1	64.5

Discussion

Isolates of *Leptospira* from the urine of pigs and dogs and from water sources demonstrate high endemicity and a potential public health problem in this area of the Colombian Caribbean. The infection was confirmed with high seroprevalence in pigs and dogs, which helps to explain the very high prevalence of antibody in the human population at risk.

In contrast with our results from the Caribbean, lower seroprevalences have been reported in the Colombian Andes (Table 2). The higher seroprevalence in pigs in the Caribbean region may be due to geoclimatic conditions such as high precipitation and relative humidity, sanitary deficiencies, and a higher number of nontechnical pig farms in the Caribbean.

Our negative findings for *Leptospira* infection in domestic rodents could indicate the existence of an unknown wild or domestic reservoir that is important for the maintenance of infection in the pig population in our study area. The highest seroprevalence in pigs in our study was for serogroups Canicola, Pomona, and Icterohaemorrhagiae (Table 1), suggesting transfer of specific serovars between pigs and dogs.

Given the lack of vaccination records against leptospirosis in dogs, the 35.2 %, seroprevalence in dogs probably represents past infections. In our study, the Pomona and Grippotyphosa serogroups presented the highest frequency, followed by Canicola, Icterohaemorrhagiae, Australis, and Sejroe (Table 1). The Pomona serogroup has been classified as emergent in dog's leptospirosis, and these titres may be due to contact with other dogs and with reservoir skunks or raccoons for these serogroups (Ribotta et al. 2000), which are common animals in these tropical areas of Colombia. In Brazil, only 7.1 % seroprevalence was found in dogs, and it was concluded that the presence of *Leptospira* in the environment was the source of infection (Table 2).

The very high seroprevalence in humans may be due to the population evaluated (occupational risk), to the endemicity of leptospirosis in humid tropical regions where environmental conditions favor transmission of *Leptospira*, and to permanent contact with pigs and dogs. The highest seroreactivity of the exposed human population was to serogroups Canicola, Pomona, and Icterohaemorrhagiae (Table 1). Close contact of these individuals with dogs was noted in the farms. Ours is the first study on a human population in Colombia that reports on antibodies against the Mini, Tarassovi, and Batavie serogroups (Table 1). Antibody to these serovars was also found in dogs and pigs from these same farms. Forty percent (25/62) of the seropositive humans had no compatible symptomatology, and 35 % (22/62) manifested compatible symptoms during the previous 9 months. The most frequent symptoms were cephalalgia, myalgia, fever, and malaise. The lack of symptomatology compatible with a clinical picture of leptospirosis (jaundice, kidney failure, liver failure) confirms the

Table 2 Seroprevalence of *Leptospira interrogans* sensu lato in different countries

Country	Area of influence	Seroprevalence (%)	Serovars	References
Pigs				
Colombia	Caribbean	55.9	13	Present study
Dogs				
Brazil	Paraná	7.1	24	Oliveira et al. 2012
Brazil	Paraná	12.2	30	Fonzar and Langoni 2012
USA	Michigan	24.9	6	Stokes et al. 2007
USA	Washington	17.1	6	Davis et al. 2008
Thailand	Bangkok	83.5	ND	Jittapalapong et al. 2009
Mozambique	Mayotte	93.1	8	Desvars et al. 2012
		87.5		
Colombia	Tolima	20.2	5	Romero et al. 2010
Colombia	Tolima	21.4	5	Romero et al. 2010
Colombia	Caribbean	35.2	13	Present study
Humans				
Brazil	Paraná	8.0	30	Fonzar and Langoni 2012
Peru	Lima	1.2	22	Platts et al. 2011
USA		2.5	6	Whitney et al. 2009
Portugal	Azores Islands	22.0	26	Vieira et al. 2006
Mexico	Veracruz	4.0	13	Navarrete et al. 2006.
Italy	Bari	0.0	19	Monno et al. 2009
New Zealand		9.5	2	Benschop et al. 2009
Colombia	Tolima	6.0	5	Romero et al. 2010
Colombia	Caribbean	75.8	13	Present study

ND not determined

high endemicity in the study area associated with asymptomatic infections or anicteric forms characterized by general nonspecific signs and symptoms; however, those patients also did not remember clinical manifestations, suggesting that leptospirosis reveals the possibility of the disease being asymptomatic or underreported, or associated with other hemorrhagic diseases such as dengue, malaria, hepatitis A and E, fever, and other hemorrhagic fevers (Musso and La Scola 2013; Chaudhry et al. 2013).

The serological and bacteriological negative results in rodents may be due to serial rodent control on the farms. Domestic cats were also observed; cats could have prevented a permanent population of rodents on the farms. In contrast to our results, Bunnell et al. (2000) found 20 % seroprevalence in wild rodents in the Peruvian Amazon. Agudelo et al. (2010) found 25.2 % seroprevalence in *R. norvegicus* in a food market in Medellin, Colombia, and concluded that serovar Icterohaemorrhagiae has been established in rodents, and these act as carriers of pathogenic leptospires for susceptible humans.

Presence of two pathogenic *Leptospira* was demonstrated in water sources, which constitutes a risk to human and animal public health. Studies on the role of water in transmitting leptospirosis such as those of Lesem et al. (2010) in Israel reported that most human cases were acquired from activities related to water sources. Viau and Boehm (2011) in Hawaii evidenced the presence of pathogenic *Leptospira* genomes in coastal waters during the Hawaiian rainy season. Chusri et al. (2012) demonstrated pathogenic leptospires in water rafting practices. Lagi et al. (2013) in Italy reported on the transmission of leptospirosis from water sources. Garvey et al. (2013) in Ireland concluded that the increased incidence of leptospirosis is associated with contact with animals or water sports, and Agampodi et al. (2013) in Sri Lanka reported on an outbreak of leptospirosis in clerks participating in a water sport event.

The need for rapid diagnosis of leptospirosis has led to the development of numerous PCR assays, which offers great advantages compared with isolation. This method is rapid, sensitive, specific, and robust. There have been developments on PCR assays for detection of universal genes as *Gryb*, *rrs*, and *secY*, or genes restricted to pathogenic leptospires as *lipL32*, *lfb1*, *ligA*, and ligB2 (Musso and La Scola 2013). The use of PCR for detecting the *lipL32* gene fragment was useful to distinguish between pathogenic isolates and saprophytes.

Conventional PCR assays are being replaced by real-time PCR (qPCR) for the detection of pathogenic leptospires in urine, kidney, fetuses, and reproductive downloads. Detection and quantification of DNA from pathogenic *Leptospira* was performed using specific primers for the gene *Lip32*. In southwestern Iran, it has been allowed to detect pathogenic leptospires in the kidney, urine, and blood of cattle samples (Azizi et al. 2012) and in Dublin in urine canine samples (Rojas et al. 2010), highlighting the increased risk of zoonotic diseases in these regions.

Furthermore, from the environmental view, it allows investigating whether or not the water sources (rivers, ponds, troughs) are contaminated with pathogenic leptospires. In France, qPCR was performed using the detection and quantification of *Lip32* gene in water samples. The method represents a tool that could be integrated into future programs of public health surveillance in recreational waters (Vein et al. 2012). The detection and monitoring of pathogenic leptospires in water allow intervention measures to help reduce the occurrence of leptospirosis in humans and animals.

Conclusion

The high seroprevalence that we found in pigs, dogs, and humans and the isolation of seven strains of *L. interrogans* sensu lato in pigs, dogs, and water demonstrate that transmission exists among animals, the environment, and humans, which originates from endemic zones demarcated for this zoonoses in Cordoba. The high prevalence of *L. interrogans* sensu lato in Cordoba warrants the implementation of public health intervention measures to reduce the epidemiological impact of leptospirosis in the region.

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