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Short communication

Evidence of *Leptospira* spp. in blood of dogs in a rural community in Yucatan, Mexico

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ABSTRACT

Objective. To evidence the frequency of *Leptospira* spp. in blood of dogs in a rural community in the state of Yucatan, Mexico. **Materials and methods.** Blood samples were collected from 120 dogs from Maxcanu, Yucatan. Characteristics such as sex, age, and breed were recorded, and owners were asked about the vaccination history. The samples were transported to the laboratory and a polymerase chain reaction diagnostic test was conducted to amplify two fragments of the 16S ribosomal gene belonging to *Leptospira* spp. **Results.** The frequency of *Leptospira* spp. was 1.7% (2/120; 95%CI= 0.2–5.9%). Both positive dogs were male mongrel puppies (mix of breeds) with no vaccination history. **Conclusions.** There was a low frequency of *Leptospira* spp. in the blood of the studied dogs. More epidemiological research is needed to identify the *Leptospira* species involved in the infection and the risk of transmission to the inhabitants or other domestic animals at the study site.

Keywords: Bacteria; epidemiology; leptospirosis; mammals; pets; zoonoses (*Source: DeCS*).

RESUMEN

Objetivo. Evidenciar la circulación de *Leptospira* spp. en sangre de perros de una comunidad rural del estado de Yucatán, México. **Materiales y métodos.** Se recolectaron muestras sanguíneas en 120 perros de Maxcanu, Yucatán. Además, se registraron características como sexo, edad, raza y se preguntó a los dueños sobre el historial de vacunas. Las muestras se transportaron al laboratorio y mediante la prueba diagnóstica de reacción en cadena de la polimerasa (PCR), se identificó la amplificación de dos fragmentos del gen 16S ribosomal perteneciente a *Leptospira* spp. **Resultados.** La frecuencia de *Leptospira* spp. encontrada fue de 1.7% (2/120; IC95%= 0.2 – 5.9%). Ambos

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perros positivos fueron machos, cachorros, mestizos (mezcla de razas) y sin historial de vacunación. **Conclusiones.** Se describe una frecuencia baja de *Leptospira* spp. en sangre de los perros estudiados. Es necesaria más investigación epidemiológica para conocer las especies de *Leptospira* involucradas en la infección e identificar el riesgo de transmisión a los habitantes u otros animales domésticos del sitio de estudio.

Palabras clave: Bacterias; epidemiología; leptospirosis; mascotas; mamíferos; zoonosis (*Fuente: DeCS*).

INTRODUCTION

Leptospirosis is a neglected and reemerging zoonosis that has highly variable morbidity and mortality rates in endemic regions. It is caused by spirochete bacteria that belong to the pathogenic subgroup of the genus *Leptospira* (1,2). In Mexico it is a public health problem; however, only a few cases have been recorded, associated with environmental factors such as temperature, humidity and high rainfall (1).

Humans contract infection with *Leptospira* spp. mainly through direct contact with urine from natural reservoir animals or accidental hosts (domestic or wild) such as cattle, pigs, horses, opossums, bats and rodents, or indirectly by contact with urine-contaminated media such as food, natural or artificial sources of water, and soil (2).

In 2015, more than a million cases were estimated worldwide, with approximately 60 thousand deaths, most of them in areas with tropical and subtropical climates in developing countries (2). In the state of Yucatan (southeast of Mexico), leptospirosis is endemic with reports of seroprevalence in humans of up to 88.2% (3).

Although rodents are the most important natural reservoirs of *Leptospira* spp. and the main route of transmission in environments where they circulate (1,2), dogs are chronic carriers of numerous pathogenic species and serovars. In fact, they are considered the natural reservoirs of *L. interrogans* serovar canicola, due to the adaptation of the bacteria to the renal environment of these animals (4). In the second stage of infection (known as immunological), pathogenic leptospires invade the kidney tissue of dogs; therefore, the excretion of infecting bacteria through the urine can last up to four years, generating transmission to other domestic animals and susceptible people, mainly those who live with them (4,5).

Information on canine leptospirosis in Mexico is limited. Although vaccination produces false positives due to the generation of antibodies, most of the studies on dogs have been carried out using serological tests, mainly microagglutination (MAT), reporting seroreaction against numerous serovars belonging to pathogenic species, especially *L. interrogans* (4). In this context, the use of tools such as the polymerase chain reaction (PCR) allows the detection of DNA of *Leptospira* spp. in fluids (blood) and tissues with high sensitivity and specificity, even when the hosts do not show clinical signs (6).

The objective of this work was to assess the presence of *Leptospira* spp. (leptospiremia) in the blood of dogs domiciled in a rural community in the state of Yucatan, Mexico.

MATERIALS AND METHODS

Study site. The study site was the rural community of Maxcanu, Yucatan, Mexico, located in the municipality of the same name (20°33”-20°46”N and 89°53”-90°24”W). The climate of the municipality is warm subhumid with rains in summer with an average temperature of 29°C and an annual rainfall of up to 1.100 mm (7). Its vegetation is tropical deciduous forest with fragments of secondary vegetation intercalated with grass for cutting and forage for ruminants.

Study population. This research was approved by the research ethics committee of the *Centro de Investigaciones Regionales “Dr. Hideyo Noguchi”* (CIR by its acronym in Spanish) of the *Universidad Autónoma de Yucatán* (UADY by its acronym in Spanish), Merida, Mexico (registration code: CEI-007-2018).

Samples from Maxcanu were collected from January to April 2019. Only dogs whose owners (upon invitation) participated in the study and granted their authorization by signing the informed consent were included.

Records of each dog's sex (male or female), age (puppy: less than one year old, adult: older than one year to less than six years old, and geriatric: older than six years) and breed (pure or mixed breeds, known in the region as *mestizos*) were collected. Additionally, the owners reported on the dog's vaccination history (number and type of vaccine).

Biological samples and total DNA extraction. After observation of each dog, a whole blood sample (maximum 7 ml) was collected from the saphenous vein with the help of a syringe and placed in a sterile centrifuge tube (BD Vacutainer®; United States [USA]). During the field work, the samples were stored in portable coolers with refrigerants (approximately 4°C), before being transferred to the laboratory and centrifuged at 3.500 rpm (2.000 x g) at room temperature (24°C) for 10 min. A fraction of the buffy coat and plasma were removed and collected in a sterile 1.8 ml microcentrifuge vial (Eppendorf®; Germany) for storage at -80°C, until use in the extraction of total DNA.

Total DNA extraction was performed with the commercial QIAamp DNA Mini Kit® (QIAGEN; Germany), protocol "DNA Purification from liquids and fluids", following the manufacturer's specifications, and the extracted DNA was stored at 4 °C. The quantification and measurement of the purity of the extracted product was determined with a NanoDrop-2000® spectrophotometer (Thermo Scientific®; USA). All extractions had concentrations within the range of 50–100 ng/ml.

Detection of *Leptospira* spp. in blood. This was performed through end-point PCR amplification of two different fragments of the 16S ribosomal gene (*16S-rRNA*), as described in Torres-Castro et al (8).

The primers, reagent concentrations, conditions used in the thermal cycler and the positive (*Leptospira* DNA typified as *L. interrogans*) and negative controls (all the reagents of the master mix, but without template DNA) for both reactions were the same as those previously described (8).

The electrophoresis of the products was carried out in 8% polyacrylamide gels, stained with silver nitrate. The results were recorded on a transilluminator (Hoefer Inc®; USA) and by photographs.

RESULTS

One hundred and twenty dogs from Maxcanu were studied. Of these, 66 were males (55%) and 54 females (45%). Forty-eight were puppies (40%), 49 were adults (40.8%), and 23 were geriatric (19.2%). Three of them were purebred (2.5%), and the rest (98.5%) were mongrels (mixed races). Forty-eight dogs had received some previous vaccination (40%), generally against the rabies virus.

The presence of *Leptospira* spp. in blood was identified in two dogs (1.7%; 95%CI = 0.2–5.9%) (Figure 1). Both were male mongrel puppies with no previous vaccinations.

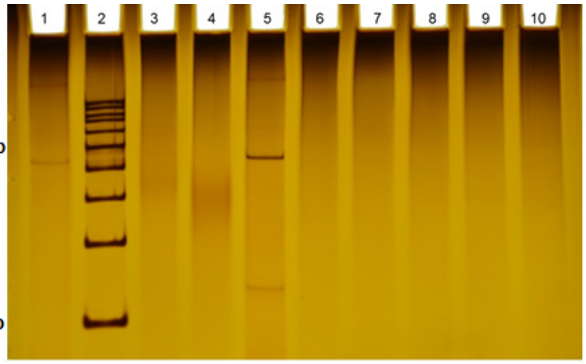

Figure 1. Polyacrylamide gel (8%), stained with silver nitrate, showing a positive product (440 base pairs [bp]) from the 16S ribosomal gene of *Leptospira* spp. in domesticated dogs from Maxcanu, Yucatan, Mexico. Lane 1: positive control; 2: 100 bp molecular weight marker; 3, 4: negative products; 5: positive product; 6–9: negative products; 10: negative control.

DISCUSSION

Infection with *Leptospira* spp. in Yucatan dogs has been demonstrated with the detection of antibodies against serovars of different pathogenic species (9,10), findings that, together with the evidence generated in this study, indicate that infection with these bacteria in dogs in the region has an endemic pattern. The circulation of dogs infected with *Leptospira* spp. has been defined as a noticeable factor for environmental contamination with the bacteria, and increases the risk of transmission to susceptible people who usually live with these animals (11).

Although the infecting species of *Leptospira* was not identified in the Maxcanu PCR-positive dogs, it is likely that it belongs to the pathogenic subgroup, because in previous studies carried out in Yucatan (9,10), reactions were found against the serovars grippotyphosa, canicola, icterohaemorrhagiae, panama, australis, pyrogenes, and bratislava, which correspond to serogroups of pathogenic species (9,10). Likewise, studies carried out with rodents captured in Yucatan such as *Mus musculus* (synanthropic), *Rattus rattus* (synanthropic), and *Heteromys gaumeri* (wild), have reported, with the use of molecular tests and bioinformatic analysis, the pathogenic species *L. kirschneri* and *L. interrogans* (12,13). The latter includes the serovar canicola (5). These rodents (especially *M. musculus* and *R. rattus*) can transmit pathogenic leptospires to dogs indirectly through accidental exposure to urine-contaminated media such as the surrounding soil and water, and also through the consumption of poorly stored food (dog croquettes) that is accessible to and contaminated by rodents (14).

During the observation and study of the dogs, it was noted that most of them lived in neglected conditions, similar to those described by Cortez-Aguirre et al. (15) for dogs in a city in Southeastern Mexico. Likewise, few owners mentioned that their animals had been given additional vaccines other than the one administered against the rabies virus by the Yucatan public health services, indicating that the vaccine against infection with *Leptospira* serovares canicola and icterohaemorrhagiae is rarely administered. It was also observed that many dogs did not have a place to rest or sleep within the premises where they lived, so it is common for them to roam or spend the night in neighboring properties and even on public roads, parks and markets. This behavior generates direct contact with accidental *Leptospira* hosts, as well as with sewage, water tanks or other artificial sources of water, which can act as common sources of infection, since they are frequently contaminated with viable leptospires (16). Similarly, this roaming behavior contributes to direct contact with other infected dogs from the same region (11).

Despite the low frequency of infection found in the studied group of dogs, it has been reported that urbanized environments are important scenarios for the transmission of *Leptospira* to the canine population, not only because of the high number of rodents (natural reservoirs) that usually circulate, but also due to the presence of other small mammals (accidental hosts) that eventually come into contact with dogs (9,11,17).

On the other hand, a predisposition to infection with *Leptospira* has been identified in male dogs, probably due to their greater mobility and roaming over larger areas compared to females (18). This predisposition has also been described in mongrel dogs (18); however, although the only animals positive for infection found in the present study shared these characteristics, an epidemiological approach is necessary to understand the risk of infection associated with these and other traits of the Maxcanu and Yucatan dogs.

The detection of DNA from *Leptospira* spp. in dogs, even when they are asymptomatic (as was the case in the dogs identified by PCR in the studied group) or the disease is subclinical, is enough evidence to consider them as possible disseminators of infective bacteria (5). In this sense, it is important to note that the severity of canine leptospirosis depends on characteristics of both the affected individual (age and immune response) and the pathogen (infecting species and virulence) (5,18), and therefore, it is relevant to carry out new sampling campaigns and molecular analyses in order to identify the circulating *Leptospira* species in the canine population of Yucatan.

Conflict of interests

All the authors state no conflict of interest.

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