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Serological and Molecular evidence of pathogenic *Leptospira* species in cattle from slaughterhouses in Veracruz State, Mexico

Evidencia serológica y molecular de especies patógenas de *Leptospira* en rastros de bovinos del estado de Veracruz, México

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ABSTRACT

Cattle raising is the most important livestock activity in Mexico, highlighting the fact that the Country is eight place worldwide in the production of bovine meat. However, cattle can be affected by leptospirosis (a bacterial disease caused by 17 species of spirochetes of the genus *Leptospira*), which cause reproductive problems that translate into great economic losses. Additionally, these zoonotic agents can cause a rapidly evolving febrile condition in humans, which can be solved after the first week of symptoms or could progress to develop a severe late-phase manifestations. Despite the great impact of these agents on the economy and Public Health, epidemiological surveillance against the infectious disease that cause is not present in municipal slaughterhouses (MS). Therefore, the aim of this study was to identify, using serological and molecular methods, the circulating *Leptospira* species in three MS in Veracruz State. The frequency of anti-*Leptospira* antibodies was 67.5%. Additionally, five sequences were recovered that were 99% similar to *L. interrogans*. This work represents the first national effort for the evaluation of MS as sentinel units, that allow establishing the diversity of species of the genus *Leptospira* that circulate in cattle and establishing intervention measures for workers risk mitigation, who come into contact with the fluids and organs of infected animals.

Key words: Leptospirosis; cattle; sentinel units; *Leptospira interrogans*

RESUMEN

La ganadería bovina es la actividad pecuaria más importante de México, destacándose el hecho de que el país ocupa el octavo lugar a nivel mundial en la producción de carne bovina. Sin embargo, el ganado puede verse afectado por la leptospirosis (enfermedad bacteriana causada por 17 especies de espiroquetas del género *Leptospira*), que ocasionan problemas reproductivos que se traducen en grandes pérdidas económicas. Además, estos agentes zoonóticos pueden causar una condición febril de evolución rápida en humanos, la cual puede resolverse después de la primera semana de presentación de síntomas o puede progresar y desarrollar manifestaciones severas en una fase tardía. A pesar del gran impacto de estos agentes en la economía y la salud pública, la vigilancia epidemiológica frente a la enfermedad infecciosa que los provocan no está presente en los mataderos municipales (MM). Por tanto, el objetivo de este estudio fue identificar, mediante métodos serológicos y moleculares, las especies de *Leptospira* circulantes en tres MM de Veracruz. La frecuencia de anticuerpos anti-*Leptospira* fue del 67,5%. Además, se recuperaron cinco secuencias con un 99% de similitud con *L. interrogans*. Este trabajo representa el primer esfuerzo nacional para la evaluación de los MM como unidades centinela, que permitan establecer la diversidad de especies del género *Leptospira* que circulan en los bovinos y establecer medidas de intervención para mitigar los riesgos para los trabajadores que entren en contacto con los fluidos y órganos de animales infectados.

Palabras clave: Leptospirosis; ganado; unidades centinelas; *Leptospira interrogans*

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INTRODUCTION

The impact of leptospirosis in domestic animals and those intended for consumption, as well as in humans, is not fully understood [9, 24, 39]. The severity of the disease varies according to the serovar and infecting species of *Leptospira*, as well as the species of animal host that is affected [11, 24, 51]. Such is the case with bovine leptospirosis, which occurs worldwide and is the result of infection by several species and serovars that affect cattle (*Bos taurus*). The acute phase of the disease is mainly subclinical, while the greatest economic losses are caused as a result of low fertility, abortions and low milk production, which occur during the chronic phase of the disease [24, 51]. The genus *Leptospira* encompasses at least 68 species of spirochetes that are classified into two main groups: 1) the saprophytic or free-living group, which is divided in two subgroups: the S1 with 23 species and S2 with five species; and 2) the pathogenic group, which is subdivided into two subgroups: P1 (pathogenic) with 19 species and P2 (intermediate) with 21 species (which may or may not cause disease, depending on the species of host it infects) [28, 55]. It is currently recognized that pathogenic species are the cause of infections, both in humans and animals, causing lung, kidney and/or reproductive disorders [2, 3, 11, 24, 55]. It should be noted that the infection caused by these bacteria is known as leptospirosis, which is a zoonosis that has a wide distribution worldwide, mainly in Countries or areas with tropical or subtropical climates. *Leptospira* pathogenic species can have direct or indirect transmission from contact with contaminated water, soil, tissue, or fluids [24].

Bovines are the animals most studied with regard to the detection and manifestations caused by infection with *Leptospira* spp. [11]. In these studies, different diagnostic methods (bacteriological, serological, and molecular) have been used; however, few studies have been carried out on slaughtered cattle destined for human consumption. In the period from 1975 to 2020, 44 studies have been recorded in 12 Countries, demonstrating the presence of six pathogenic species and 27 serovars [4, 7, 13, 14, 17, 18, 19, 20, 23, 26, 29] (TABLE I). The prevalence fluctuate between 3.8-85% with an average of 31.1% in 9 Countries of the Nearctic Region where *L. interrogans*, *L. borgpetersenii* and *L. kirschneri* were reported; in contrast, in the Neotropical Region the prevalence fluctuate between 2.7-79.8% with an average of 36.4% in 10 Countries with six species detected (*L. interrogans, L. borgpetersenii, L. santarosai, L. kirschneri, L. noguchii, and L. alstonii*), Likewise, *L. interrogans* and *L. borgpetersenii* and *L. kirschneri* are circulate in both Zoogeographic Regions [30, 31, 33, 34, 41, 42, 43, 44, 45, 46, 47, 48, 50] (TABLE I). It is necessary to highlight that, during the aforementioned period, in Mexico, only a single study has been carried out in this type of animal, in the State of Guerrero, in which 380 animals were sampled, detecting anti-*Leptospira* antibodies against two *Leptospira* species (*L. interrogans* and *L. borgspetersenii*) and four serovars (Australis, Ballum, Bataviae, and Grippotyphosa) (TABLE I) [8].

Although the municipally slaughterhouses (MS) are sentinel units in the epidemiological surveillance of other zoonotic pathogens (e.g., *Brucella* and *Mycobacterium tuberculosis*), in the case of *Leptospira*, there is no active surveillance. Therefore, the aim of the present

TABLE I
Studies carried out worldwide in cattle sent to slaughterhouses for the detection of *Leptospira* spp.

Leptospira species / Serovars	Sample	Result	Country [Ref]
L. interrogans: Hardjo	Kidney	Isolates 3.7%	Argentina [34]
L. interrogans: Pomona	Kidney / Blood	Isolates 16%	Australia [3]
L. interrogans: Hebdomadis	Blood	44.3% serological evidence	Australia [33]
L. interrogans: Hardjo	Foetus	AFD en 4.6%	UK [12]
L.interrogans: Hardjo/Grippotyphosa/Pomona	Blood / Kidney	MAT 14.5%, ELISA 39.5%, Isolates 6.4%	USA [52]
L. interrogans: Hardjo	Blood/LCR	MAT 15.4%, Isolates 66.6%	Canada [26]
L. interrogans: Hardjo	Blood	Seroreactors 75.3%	Chile [21]
L. interrogans: Hardjo	Genital tract	Isolates 65%	UK [13]
L. interrogans: Kennewicki | *L. borgpetersenii*: Hardjo bovis	Kidney / Blood	Isolates 13.5%, IF 7.6%	Canada [42]
L. interrogans: Hardjo / Pomona	Kidney / Blood	Isolates 28.6%, MAT 34.4%	Canada [22]
L. interrogans: Hardjo	Kidney	Isolates 8.3%	Australia [48]
L.borgpetersenii: *Hardjobovis*	Isolates	DNA restriction endonuclease analysis 91%	Ireland [14]
L. interrogans: Hardjo / Pomona / Grippotyphosa	Kidney	Isolates 85%	USA [31]
L. interrogans: Grippotyphosa / Pomona | *L. borgpetersenii*: Hardjo	Kidney / Blood	Isolates 1.7%, Serology 48.7%	USA [32]
L. interrogans	Kidney	Isolates 10.4%	Zimbabwe [17]
L. interrogans: Bratislava / Bataviae / Grippotyphosa | *L. borgpetersenii*: Hardjo / Ballum	Blood	MAT 32.8%	Mexico [8]
L. interrogans: Grippotyphosa / Sejroe / Icterohaemorrhagiae / Hardjo	Blood	MAT 7.4%	Czech Republic [53]
L. interrogans: Mhou / Marondera	Isolates	Mhou (3/4); Marondera (1/4)	Zimbabwe [19]
L. santarosai: Gatuni	Kidney	Two isolates typified by CAAT	Zimbabwe [18]

***TABLE I* (cont...)**
Studies carried out worldwide in cattle sent to slaughterhouses for the detection of *Leptospira* spp.

L. borgpetersenii: Tarassovi / Hardjo	Blood / Kidney / Urine	MAT 5.7%, Isolates 1.4%	Australia [54]
L. interrogans: Pomona / Grippotyphosa / Canicola	Blood/Urine	MAT 37.7%, Isolates 1%	Iran [45]
L. interrogans: Hardjo / Canicola	Blood	10% by MAT	Jamaica [5]
Leptospira sp	Kidney / Blood / Urine	PCR: Kidney 79.2, Blood 29.2%, Urine 55.5%	Iran [4]
L. interrogans: Pomona / Hardjo / Bataviae | *L. borgpetersenii*: Tarassovi	Blood	MAT 51%	Tanzania [49]
L. interrogans: Hardjo	Blood	3.5% seropositivity	Nigeria [36]
L. interrogans: Bataviae | *L. borgpetersenii*: Ballum | *L. kirschneri*: Cynopteri	Blood	MAT 40%	Egyp [25]
Leptospira sp.	Urine	Nested PCR 43%	Iran [46]
L. borgpetersenii, L. kirschneri, *L. interrogans*	Kidney	Nested PCR 12.2%	Sri Lanka [20]
L. borgpetersenii: Hardjo bovis | *L. interrogans*: Pomona	Urine / Blood / Kidney	MAT and PCR	New Zealand [16]
L. borgpetersenii	Kidney	DF 59%, DFA 78%, PCR 29.7%, Isolates 8.1%	USA [44]
L. borgpetersenii: Hardjo bovis | *L. interrogans*: Pomona	Urine / Kidney / Blood	qPCR 21.2%, MAT 73%	New Zealand [15]
Leptospira sp.	Kidney	PCR 15.4%, PCR-RFLP 40.8%	Iran [50]
L. santarosai: Sejroe | *L. noguchii*: Australis	VF / Urine	Isolates 4.3%, PCR: Urine 31.0%, VF 50.4%.	Brazil [30]
L. santarosai, L. *alstonii*, *L. interrogans*	Urine and/or FV	Isolates 2.7%	Brazil [41]
L.borgpetersenii: Hardjo bovis | *L. interrogans*: Bratislava / Hardjo / Pomona / Icterohaemorrhagiae | *L. kirschneri*: Grippotyphosa	Blood / Urine	MAT 20%, Isolates 3.8%, FAT 5.5%, PCR 8.8%	USA [35]
L. borgpetersenii: Sejroe	Blood / Urine / Kidney / FV	MAT 37%, Isolates 5.0%, PCR 63.9%	Brazil [40]
L. interrogans	TU	PCR 18%	Brazil [6]
L. borgpetersenii, L. kirschneri	Kidney / Urine	qPCR: Kidney 7.2%, Urina 5.8%	Uganda [2]
L. borgpetersenii, L. interrogans: Mankarso	Blood / Kidney	Seroprevalence 79.8%, Positive by RT-PCR 18.2%	Saint Kitts [47]
Leptospira sp., *L. borgpetersenii*, *L. kirschneri*, *L. interrogans*, *L. santarosai*	Blood / Urine / Kidney	MAT 46.6%, PCR: Urina 14.9%, Kidney 5.8%	Brazil [23]
L. kirschneri, L. borgpetersenii: Hardjo bovis / Tarassovi	Kidney / Urine	Prevalece by real-time PCR 19%	Madagascar [43]
L. noguichii	Urine (isolates)	PCR and BLAST 98% identity with n=38	Brazil [29]
L. borgpetersenii: Hardjo	Blood	MAT 27.6%	South Africa [10]
Leptospira sp. Hardjo prajitno / Bratislava	Kidney	Isolates 76.4%, MAT 83.3%, WSs 41.6%, IH 35%	Nigeria [1]

Reference [Ref], Indirect Immunofluorescence (AFD), Direct Fluorescent Antibody (DFA), Dark field (DF), Enzyme-Linked ImmunoSorbent Assay (ELISA), Microscopic Agglutination Test (MAT), Quantitative Polymerase Chain Reaction (qPCR), Vaginal Fluid (VF), Uterine Tissue (UT), Restriction Fragment Length Polymorphism (RFLP), Basic Local Alignment SearchTool (BLAST), Fluorescent Antibody Test (FAT), Cross-Agglutinin Absorption Test (CAAT), Reverse Transcription Polymerase Chain Reaction (RT-PCR), Warthin Silver (WSs), immunohistochemical (IH)

study was to identify, using serological and molecular methods, the circulating *Leptospira* species in three MS in Veracruz State**.**

MATERIALS AND METHODS

Sample collection

The study was carried out in three MS in the Central zone of the State of Veracruz, Mexico. The exact number of livestock production units (UPP) of origin of the animals slaughtered in the three traces of study is unknown, however, these cattle destined for consumption come from UPP that belong to 17 Municipalities of the State of Veracruz. The MS 1 receives animals from eight Municipalities (Actopan, Alto Lucero, Emiliano Zapata, Ixhuatán, Paso de Ovejas, Tlalixcoyan, Teocelo and Úrsulo Galván); MS 2 from nine (Actopan, Alvarado, Cotaxtla, Cuitláhuac, Ignacio de la Llave, Jamapa, Manlio Fabio Altamirano, Medellín and Tlalixcoyan); and MS 3 froma single one (Tierra Blanca). A non-probabilistic convenience sampling was carried out between April and June 2019, in which samples were taken from all the cattle that arrived at the MS site on the day of the visit for sampling, considering only as inclusion criteria cattle that arrived at the MS.

in which, for the serological diagnosis, 80 blood samples were collected in vacuum tubes without anticoagulant (BD Vacutainer® red cap), obtaining a volume of 5 to 7 milliliters (mL) of blood from the jugular vein during the exsanguination of the animals, at the time of preparation of the carcass. Likewise, for the molecular identification of *Leptospira* spp., 55 samples of kidney lobes were taken after the evisceration of the animals was performed, which were fixed in containers with 96% ethanol.

Sample processing

The biological samples were transported to the laboratory of Infectious Diseases in the Diagnostic Unit of the Ranch "Torreón del Molino" of the Faculty of Veterinary Medicine and Zootechnics of the Universidad Veracruzana. The tubes with blood were centrifuged in a centrifuge (Hsiang Tai Model CN-3600, Taiwan, China) at 1,000 G for 10 minutes (min) to separate the serum from the clot. Once the serum was separated, it was deposited in 500 microliters (µL) polypropylene microtubes that were kept frozen at –20 °C in a Freezer (Thermo Scientific, 05LFEETSA, Massachusetts, United States –USA-) until later use.

Antibodies detection

The detection of anti-*Leptospira* antibodies was carried out using the microscopic agglutination technique (MAT) [38]. The analysed sera were prepared individually, 100 µL of each sample was deposited in polyethylene tubes with 2,400 µL of Phosphate Buffered Saline (PBS) to obtain an initial dilution of 1:25, and they were kept refrigerated at 4 °C until later use (LG, LT57BPSX, Busan, South Korea). The MAT was performed by placing 50 µL of PBS in each of the wells of a 96-well ELISA plate, adding 50 µL of the initial dilution (1:25); subsequently, 50 µL of the serovar to be evaluated was added to each well. This was repeated in total with 10 different serovars, which belong to three different *Leptospira* species**.**

The reaction was evaluated using dark field microscopy (Carl Zeiss, Axio Lab A1, Jena, Germany). Samples that did not react with 50, 75 or 100% agglutination were discarded. All the samples (n=54) that presented the aforementioned reaction were titrated until they did not present a minimum agglutination of 50% of *Leptospira*, and in this way, the degree of exposure to the different serovars and species of *Leptospira* was assessed, considering as seroreactors all those sera that reacted to titers ≥ 1:100 [8].

Leptospira genome detection and analysis

Total Deoxyribonucleic acid (DNA) extraction was carried out using Chelex-100 chelating resin (Bio-Rad®, United States of America (USA)) [37]. The extractions were carried out individually using 3-5 grams (g) of the central part (kidney lobe) of the kidney samples preserved in ethanol, which were macerated and placed in polypropylene microtubes. A total of 500 µL of a 10% Chelex-100 solution and 20 µL of proteinase K (SIGMA life sciences®, USA) were added to each tube. The samples were incubated for 24 hours (h) at 56 °C (IVYX Scientific, 0745556232573, Washington, USA). Subsequently, they were centrifuged at 25,000 G for 15 min, and the supernatant was collected and deposited in new polypropylene microtubes that were stored at –20 °C for later use.

For the detection of pathogenic *Leptospira* DNA, it was performed a conventional polymerase chain reaction probe (PCR) in a Veriti 96-Well Fast Thermal Cycler (ThermoFisher Scientific, 4375305, Massachusetts,USA) that amplified a fragment of 430 base pairs (bp) of the *LipL32* gene, by using the following pair of primers: Forward 5'-ATCTCCGTTGCACTCTTTGC-3' and Reverse 5'-ACCATCATCATCATCGTCCA-3 [56]. PCR amplification was performed using the following thermal conditions: an initial denaturation cycle at 95 °C for 5 min, 35 cycles at 95 °C for 1 min, 55 °C for 30 seconds (s), and 72 °C for 1 min, and a final extension at 72 °C for 7 min [56].

The PCR products were visualized on 2% agarose gels stained with fluorochrome iQ ™ SYBR® Green Supermix (Bio-Rad®, USA), using 1X TAE (40 miliMol (mM) Tris-acetate, pH 8.0, 1 mM Ethylenediaminetetraacetic acid (EDTA)) as a running buffer and a 100 bp Phi X174 DNA molecular weight marker, for 45 min at 85 volts (V). Subsequently, the gels were visualized with the aid of the Odyssey CLx Imaging System (LICOR Biosciences®) [56].

The positive PCR products were purified using the QIAquick kit (Quiagen®, Hilden, Germany) and sent for sequencing by the Sanger method (Life Technology® 3500 xl) to Macrogen, Korea. The recovered sequences were visualized using BioEdit, and once curated, local alignments were performed using the BLAST-n tool available from GenBank® (on the NCBI platform). Additionally, reference sequences of validated *Leptospira* species deposited in GenBank® were obtained and aligned with those recovered in the present study, and a phylogenetic reconstruction was performed using the maximum likelihood method with the general time reversible substitution model under 10,000 bootstrap iterations in Mega v.10.

The results were analysed with descriptive epidemiology. Likewise, the differences between the MS: one (n=30), two (n=30) and three (n=20), were evaluated calculating the 95% confidence intervals (CIs); the general frequency (of anti-*Leptospira* antibodies) and specificity (by serovar) were determined with the statistical program STATA V.14.

RESULTS AND DISCUSSION

Serological assays identified anti-*Leptospira* antibody titres (from positive animals) that ranged from 1:100 to 1:3,200, obtaining a general antibody frequency of 67.5% (54/80; 95% CI 56.1-77.5). The higher frequency of seroreacting animals was 71.8%, obtained in the first slaughterhouse (23/30; 95% CI 53.2-86.2) (TABLE II). The species with the highest frequency of positive hosts were *L. interrogans* serovar Hardjo with 18.7% (15/80; 95% CI 10.9-29.0), followed by *L. borgpetersenii* serovar Mini with 25.0% (20/80; 95% CI 15.9-35.9), and *L. santarosai* serovar Tarassovi with 37.5% (30/80; 95% CI 26.9-49.0) (TABLES II AND III).

TABLE II
Frequency of anti-*Leptospira* antibodies by slaughterhouse

Slaughterhouse	n	Positive	F% ($CI_{95\%}$)
1	30	23	71.8 (53.2-86.2)
2	30	15	50.0 (31.2-68.7)
3	20	16	84.2 (60.4-96.6)
Total	80	54	67.5 (56.1-77.5)

F: frequency, Confidence Interval at 95%: ($CI_{95\%}$)

From the collected kidneys (n=55), 30 of them corresponded to the first MS, five of them to the second MS, and 20 of them to the third MS, from which six samples amplified positive for the 430 bp of the LipL32 fragment, which represent a frequency of 10.9% (6/55; 95% CI 4.1-22.2). The six PCR positive products were sequenced, and five full sequences were retrieved for the phylogenetic reconstruction. Sequences were deposited in GenBank under the following Accession numbers: OP273650 – OP273655. The five sequences exhibited a 99% (429/430 bp) similarity with sequences of *L. interrogans* deposited in GenBank. Additionally, the phylogenetic reconstruction grouped our sequences with those of the *L. interrogans* group in a monophyletic subgroup with a support value of 98% (FIG. 1).

TABLE III
Frequency and distribution of positive sera by antibody titles by *Leptospira* serovars.

Serovar	Antibody titration							F% (CI95%)
	1:50	1:100	1:200	1:400	1:800	1:1600	1:3200	
L. borgpetersenii								
*Mini	34	13	2	2	3	--	--	25.0 (15.9-35.9)
Sejroe	13	6	2	1	1	--	1	13.7 (7.0-23.2)
Ballum	4	--	--	--	--	--	--	0 (0-4.5)
L. interrogans								
**Hardjo	41	6	3	4	1	1	--	18.7 (10.9-29.0)
Icterohaemorrhagiae	18	8	1	--	1	--	--	12.5 (6.1-21.7)
Bratislava	37	1	3	1	--	--	--	6.2 (2.0-13.9)
Pomona	6	2	--	1	1	--	--	5.0 (1.3-12.3)
Hardjo	10	1	--	1	--	2	--	5.0 (1.3-12.3)
L. santarosai								
*Tarassovi	16	11	9	4	1	4	1	37.5 (26.9-49.0)
Mini	24	10	9	1	3	1	--	30.0 (20.2-41.2)

Positive seroreactor with titles ≥ 1:100, *serovars with a higher frequency of seroreactors by *Leptospira* species, **Hardjo serovar isolated in cattle from the State of Veracruz, Mexico, F: frequency, Confidence Interval at 95%: ($CI_{95\%}$)

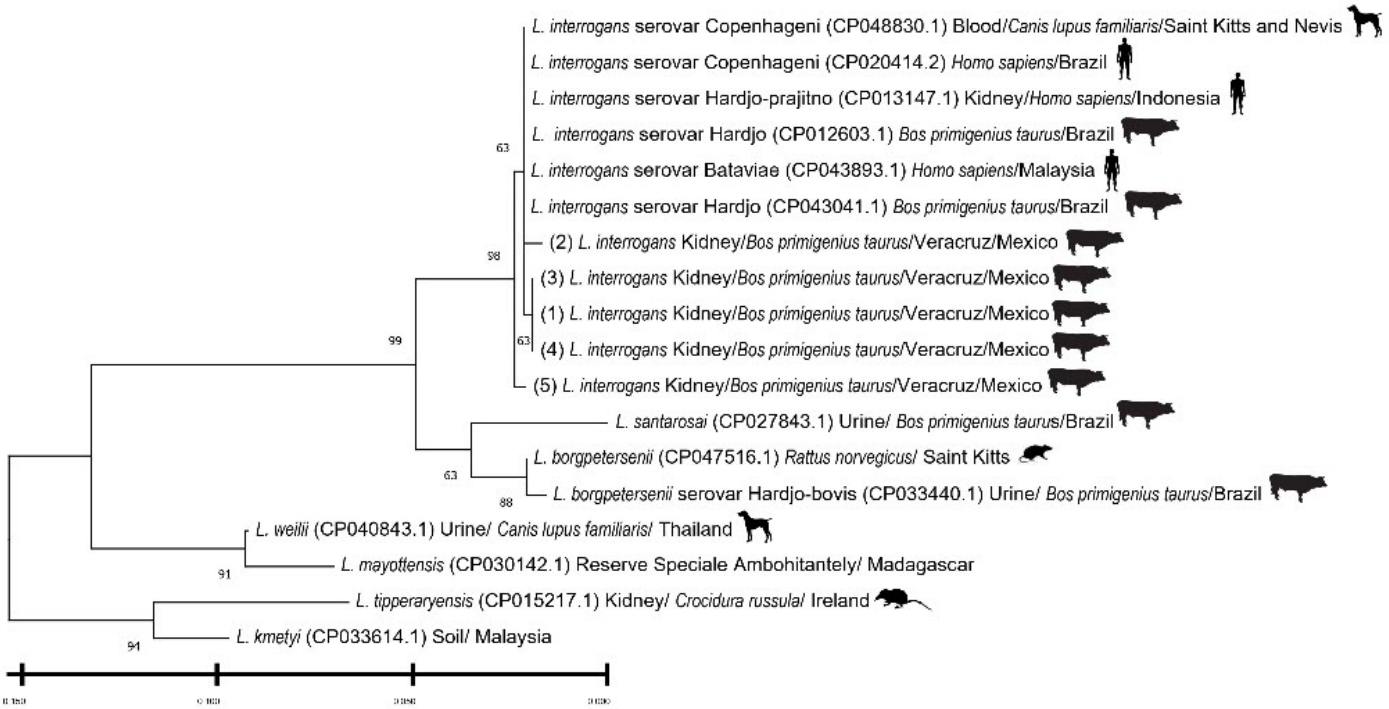

FIGURE 1. Phylogenetic tree by the maximum likelihood (ML) method based on the 2-parameter Kimura model. A discrete Gamma distribution (+ G, parameter = 0.2798) was used. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The numbers in parentheses from one to five correspond to the sequences recovered from the *Leptospira* hosts

Due to the fact that the positive animals presented the same *Leptospira* species, and the recovered genetic variants have low polymorphism, it is possible to assume that there is an extensive dissemination of the pathogen due to contamination of water sources or due to the movement of animals that are infected in the wild across these 17 Municipalities. It is important to emphasize that the positive animals came from MS 1 and 2, with 15 positive animals from the first one and five from the second. Globally, there are few studies focused on the detection of *Leptospira* spp. in cattle for human consumption, particularly in animals from MS, within which, various kind of samples have been screened (urine, kidney, vaginal fluid, whole blood, blood serum, uterine tissue, and *foetal* tissue). Also, many diagnostic techniques have been implemented, such as serology (MAT, ELISA, AFD and IF), molecular (cPCR, nested PCR, real-time PCR, sequencing, among other ones) and bacterial isolation (TABLE I).

The prevalence of *Leptospira* spp. fluctuates depending on the technique used. It is recognized that serological methods tend to over register cases due to the fact that animals that arrive at MS for human consumption may present with antibodies as a result of an immunological memory process generated by previous infections or by vaccines. However, by means of bacteriological isolation and molecular methods, it is possible to confirm the circulation of *Leptospira* spp. that ranges from 5 to 10%. Since molecular techniques recognize the presence of bacteria, however, it depends on the amount of tissue, as well as the extraction method and the number of bacteria in which they may have a lower sensitivity. In the same way, isolation conceals a confirmatory technique, but with low yield since the transportation of the sample, as well as the selection processes of the variants present, can generate variable results [58, 59].This is important because MS represent centres of concentrations of animals from different farms in the same geographic region (facility), which is why multiple pathogenic species of *Leptospira* can be present in these in a short period of time enough for a detectable human chronic infection occur.

In relation to the serovars historically identified by serology of animals from MS, there are several that have been reported in the different studies, namely *L. interrogans* serovar Hardjo (the most frequently reported)(TABLE I). In the present study, it was identified the presence of three serovars: *L. interrogans* serovar Hardjo, being similar to that reported in bovines slaughtered in the United Kingdom [12], USA [35, 52], Canada [22, 27], Czech Republic [53], Tanzania [49], Chile [21], Jamaica [5] and Nigeria [1, 36]; *L. borgpetersenii* serovar Mini that, as far it was known, does not coincide with cattle slaughtered in other Countries (however, there is evidence of other serovars belonging to this *Leptospira* species such as Hardjo bovis [8, 10, 16, 32, 35, 54], Tarassovi [10, 54], Ballum [25] and Sejroe [40]; and *L. santarosai* serovar Tarassovi.

Compared to the rest of the studies carried out worldwide in cattle from MS, in the present study, it was possible to identify the species *L. interrogans* in kidney samples using molecular essays (PCR), since, as mentioned above, the recovered sequences presented a 98% similarity with sequences of *L. interrogans* deposited in GenBank—three of them from studies carried out in Brazil (two of them from *Bos taurus* and one from *Homo sapiens*), one from *Canis lupus familiaris* reported in Saint Kitts, and finally two from *H. sapiens* from Malaysia and Indonesia. This same species of *Leptospira* has been reported, by the same diagnostic method, in similar studies carried out in Countries such as New Zealand [15] and Brazil [6, 40].

It is recognized that *L. interrogans* is the species most frequently associated with human leptospirosis, especially with the presence of Weil's syndrome, which is the most serious complication of this disease [57]. For this reason, it is important to consider the risk that MS workers were exposed to when they come into contact with organs and fluids that may be infected with this bacterium. Likewise, this study was the first carried out in the State of Veracruz and the second in Mexico, although it should be noted that the first similar study carried out in Mexico was 25 years ago and exclusively serological. A previous study showed high antibody titres (24.7%) in one MS workers in the State of Jalisco, Mexico, for which it is reaffirmed that it is a risk group to which a greater number of control studies should be carried out and provide more protection elements to avoid human leptospirosis cases [8]. Other studies [2, 23, 27, 32] performed in the USA, Brazil, and Uganda identified *L. borgpetersenii, L. kirschneri, L. santarosai, L. noguchii, and L. alstonii,* in different cattle organs and fluid from animals destinated for consumption (TABLE I).

CONCLUSIONS

With the results obtained and comparing them with what was reported in other similar studies, it was conclude that *L. interrogans* is one of the pathogenic species of this bacterium that is circulating in cattle slaughtered for consumption in the Coastal State of Veracruz, Mexico. This study is a pioneer for the detection of pathogenic species (*L. interrogans*) that can be found in cattle slaughtered in MS and in Federal Inspection Type (TIF) traces, which are regulated by the Mexican government. This study confirmed the presence of *L. interrogans* in at least two MS by molecular methods and demonstrated antibody titters in animals from the three sites, thus it can be identified that the central region of the state is endemic for the presence of the microorganism and probably of the disease. Therefore, it is important to intensify efforts for epidemiological surveillance against this pathogen in traces so they continue to fulfil their function as sentinel units to safeguard Public Health, since it represents a potential risk, not only for the workers of these MS but also the health risk represented by the management of waste from these MS, which can affect Public (final consumers) and Animal Health.

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Conflict of interest

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest, nonfinancial interest in the subject matter or materials discussed in this manuscript.

Code or data availability

Sequences generated in the present study are deposited in GenBank under the following Accession numbers: OM060690, OM108474, OM108475 and OM108476.

Ethical statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. This project was approved by the Bioethics and Animal Welfare Committee of the University of Veracruz, School of Veterinary Medicine and Animal Husbandry in Veracruz, Veracruz, Mexico.

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