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Research article

Utility evaluation of two molecular methods for *Leptospira* spp. typing in human serum samples

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ABSTRACT

Most of the available genotyping methods were applied and evaluated in *Leptospira* isolates and only few of them in a relevant sample size of blood specimens but not of sera. The objective of this study was to evaluate the utility of one partial 16S rRNA gene sequencing assay (16S rRNA) and an optimized. Multilocus sequence typing scheme (MLST) for *Leptospira* typing directly in serum samples. Confirmed leptospirosis patients (n = 228) from Argentina (2005–2016) were randomly included. Septicemic-phase serum samples (n = 228) were studied by two genotyping methods. Available immune-phase serum samples of the included patients (n = 159) were studied by MAT to compare serological and molecular results. In culture-proven cases (n = 8), genotyping results between clinical samples and isolates were compared. Typing success rate (TSR) was 21.9% for 16S rRNA and 11.4% for MLST (full allelic profile) and a positive trend in both TSR during the study period was observed. Two species (*L. interrogans* and *L. borgpertesenii)* were identified by both methods and MLST assigned 8 different STs. The probable serogroups identified by MLST were coincident with the presumptive infecting serogroups identified by MAT, but with different frequencies. The three serogroups (Canicola, Sejroe and Icterohaemorrhagiae) most frequently identified by MAT were also genotyped by MLST. Typing results via 16S rRNA and MLST in clinical samples and isolates of culture-proven cases, were consistent except for one case. Performance of partial 16S rRNA gene sequencing assay and the optimized MLST scheme directly in sera may increase and improve the knowledge about species and serogroups causing human leptospirosis, especially in countries with low rates of culture sample collection or *Leptospira* isolation.

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1. Introduction

Leptospirosis is one of the main zoonotic causes of morbidity and mortality in humans, with 1,030,000 cases and 58,900 deaths per year estimated in the world (Costa et al., 2015). Thus, the burden of leptospirosis is comparable to, or higher than, that of other important neglected tropical diseases, such as severe dengue (Picardeau, 2017). Geography, climate and poverty contribute to the worldwide distribution of this disease, being more frequent in tropical regions (Costa et al., 2015).

The causative agent is the bacteria belonging to genus *Leptospira* of the phylum Spirochaetales (Agampodi and Vinetz, 2021). Infected animals maintained the leptospires in their renal tubules and shed them through the urine into the environment. Humans can be infected directly through contact with the urine of these animals or indirectly through contact with the environment contaminated by pathogens (Soo et al., 2020). The clinical course in humans ranges from mild to lethal with a broad spectrum of symptoms and clinical signs (Bharti et al., 2003).

Leptospira spp. identification and typing play an important role in understanding disease epidemiology and pathogenicity, together with the development of diagnostic tools, effective vaccines, and preventive strategies (Ahmed et al., 2011). Many methods have been proposed for typing isolates. Traditionally, *Leptospira* isolates were classified according to the structural heterogeneity of the carbohydrate component of the lipopolysaccharide. The serological classification includes serovars and serogroups comprising antigenically related serovars (Agampodi and Vinetz, 2021). The classical method based on serological techniques are Cross-Agglutinin Absorption Test (CAAT) and Microscopic Agglutination Test (MAT). However, these methods are laborious, time-consuming and no sufficient to identify at species level (Zhang et al., 2015). Modern technology has greatly improved laboratory procedures, particularly those for the detection, identification and typing of epidemiologic strains (Cerqueira and Picardeau, 2009). The genus *Leptospira* comprised a large number of infectious and noninfectious species (Agampodi and Vinetz, 2021). Molecular techniques allowed the re-classification of this genus and opened an important question for the taxonomy of the genus since the genetic characterization showed a low correlation with the existing serological classification (Caimi and Ruybal, 2020). In addition to the methodological difficulties of traditional typing methods, culturing is slow, laborious, and insensitive (Bharti et al., 2003; Cerqueira and Picardeau, 2009).

The MAT is also used for serodiagnosis and is the reference serological technique to confirmation of clinically suspected leptospirosis cases. This test assesses the capacity of the patient serum to agglutinate live *Leptospira* among a battery of strains (Goarant, 2016; Hartskeerl et al., 2011). The panel of *Leptospira* strains used in the diagnostic process represent circulating serogroups and/or serovars. However, the MAT antigen panels are often not regionally optimized (Agampodi and Vinetz, 2021).

The continuous research into more accessible typing methods has led to the development of culture-independent molecular techniques to provide digital data that can be compared among countries (Hartskeerl and Smythe, 2015). The ribosomal 16S rRNA gene (*rrs*) is most frequently used for typing of *Leptospira* species from clinical samples (Guernier et al., 2018). Multilocus sequence typing (MLST) is the genotyping method of choice for many bacterial pathogens and the most robust method for determining *Leptospira* strain diversity (Ahmed et al., 2006; Boonsilp et al., 2013).

In the last three decades, several strategies for *Leptospira* genotyping in clinical samples including different PCR assays targeting partial 16S rRNA gene and/or MLST schemes were applied (Agampodi et al., 2011, 2013, 2014, 2011; Ahmed et al., 2006; Boonsilp et al., 2011, 2013; Chiani et al., 2016; Guernier et al., 2018; Merien et al., 1995, 2005; Mérien et al., 1992; Perez and Goarant, 2010; Philip et al., 2020; Thaipadungpanit et al., 2007, 2011; Varni et al., 2014; Weiss et al., 2016). However, only a few of them were applied and evaluated a conventional PCR assay in a relevant sample size of blood specimens but not of sera (Boonsilp et al., 2011).

In Argentina, leptospirosis causes individual cases and outbreaks during periods of seasonal abundant rains and floods (Cudós et al., 2014; Vanasco et al., 2008). Although, a system of enhanced surveillance was implemented in the two provinces where most cases occur (Cudós et al., 2014), human culture sample collection is already too low and thus the *Leptospira* isolation rate (<2% of the confirmed cases, unpublished results). Hence, information about species and serogroups causing human infections is limited and is mainly based on the MAT titers. Therefore, the aim of this study was to evaluate the utility of one partial 16S rRNA gene sequencing assay and an optimized MLST scheme for *Leptospira* molecular typing directly in human serum samples.

2. Materials and methods

2.1. Case definition

A confirmed case of human leptospirosis was defined as a patient with acute febrile illness, symptoms and epidemiology consistent with leptospirosis plus: a) one titer of MAT≥ 1/200 and/or bacterial isolate and/or detection of the bacterial genome, or b) MAT seroconversion in two or more samples (Ministerio de Salud Nación, 2014).

2.2. Population and sampling

This retrospective study included 228 patients from Argentina, with confirmed leptospirosis between January 1 2005 and December 31 2016, and with available serum sample obtained during the septicemic-phase of illness (up to 7 days post onset of symptoms – DPO). Significative sample size was obtained by Epidat (2016). Cases were randomly selected from the sample collection database of the *"Laboratorio Nacional de Referencia de Leptospirosis"* – LNRL ("*Instituto Nacional de Enfermedades Respiratorias*" – INER, Santa Fe, Argentina). The LNRL collection includes samples of all suspected leptospirosis patients from the *"Red Nacional de Laboratorios de Leptospirosis"*. Serum samples were stored at −70 °C until processing. Included cases were confirmed by MAT (182, 79.8%), by Real Time PCR targeting the LipL32 gene (LipL32qPCR) (28, 12.3%), by MAT plus LipL32qPCR (15, 6.6%), by LipL32qPCR plus

Leptospira interrogans isolates (2, 0.9%) and by MAT plus LipL32qPCR plus *L. interrogans* isolates (1, 0.4%).

Of the selected patients, 228 septicemic-phase sera were studied by genotyping methods (partial 16S rRNA gene sequencing assay and an optimized MLST scheme) and 159 immune-phase sera were studied by MAT titers analysis. Eight culture-proven cases, with available isolates and septicemic-phase samples, were studied to compare the genotyping results by these two methods.

2.3. DNA extraction

Genomic DNA was extracted from 200μl of the septicemic-phase serum samples using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) commercial kit, following the manufacturer's instructions.

2.4. 16S rRNA amplification

DNA samples were processed using the partial 16S rRNA gene amplification assay (16S rRNA) described by Mérien et al. (1992) modified by Varni et al. (2018). DreamTaq Green DNA Polymerase (1U) (ThermoFisher Scientific, USA), deoxyribonucleotides triphosphate (dNTPs) (200μM), primers (1μM), and DNA (5μl) were added to reach a final volume of 50μl. The SimpliAmp™ Thermal Cycler (Applied Biosystems, USA) was used and the amplification products were analyzed on 2% agarose gels.

2.5. Multilocus sequence typing

DNA samples were processed using a previously published extended MLST scheme based on the amplification of seven housekeeping genes (*caiB*, *glmU*, *pntA*, *sucA*, *tpiA*, *pfkB*, *mreA*) developed by Weiss et al. (2016). These authors reported that *caiB* was the most often amplified locus, followed by *glmU*. Philip et al. (2020) reported that the *glmU* and *pntA* loci were the two most frequently amplified. Due to these reports and previous unpublished data of our laboratory, amplification of *glmU* was selected as a first step, and then, the other six loci were performed in positives samples. This protocol was applied to confident serogroup assignment, considering only complete MLST profiles (sequences from all seven loci). DreamTaq Green DNA Polymerase (1.25U) (ThermoFisher Scientific, USA), dNTPs (200 μM), primers (1 μM), and DNA (5μl) were added to reach a final volume of 25 μl. The nested PCR was performed in 50 μl reaction using 5 μl of the first-round PCR product. SimpliAmp™ Thermal Cycler (Applied Biosystems, USA) was used and the amplified products were analyzed on 2% agarose gels.

Positive controls (5μl of DNA from 10^8 *Leptospira interrogans* serovar Canicola strain Hond Utrech IV/ml), negative controls (ultrapure distilled water, DNAse, RNAse free), and PCR non-template controls were included in each 16S rRNA and MLST amplification.

2.6. Sequencing and sequence analysis

The amplicons obtained with 16S rRNA amplification and MLST were purified using GeneJET PCR Purification Kit (Thermo Scientific, Waltham, MA, USA) and sequenced in Macrogen Inc. (Seoul, Korea). The sequences were edited with CHROMAS LITE 2.1.1 (Technelysium Pty Ltd., Australia), assembled with STADEN Package Software (MRC-LMB, UK), and aligned with MEGA 5.0 (Tamura et al., 2011). The 16S rRNA assembled sequences were compared with "The Ribosomal Database Project (RDP)" to identify the *Leptospira* species (Wang et al., 2007). The MLST assembled sequences of the seven loci were analyzed with *Leptospira* spp. database of the "Public databases for molecular typing and microbial genome diversity (PubMLST)" to assign the sequence types (ST) (Jolley et al., 2018) and to identify the probable serogroups.

2.7. Utility evaluation of typing methods

Partial 16S rRNA gene sequencing and an optimized MLST scheme were applied in the 228 septicemic-phase serum samples of the included confirmed leptospirosis patients. Typing success rate (TSR) of 16S rRNA and MLST for the study period (2005–2016) and per year was calculated. TSR was the percentage of clinical samples with *Leptospira* successfully typed by each method, from all serum samples of the included confirmed cases.

The relative proportions of probable and presumptive infecting serogroup identified by MLST and MAT were compared. To identify presumptive infecting serogroups by MAT, 159 available immune-phase serum samples of the included patients were studied. A presumptive infecting serogroup was defined as the serogroup with the maximum MAT titer (Levett, 2003). The serogroup was considered "undefined" in cases confirmed by MAT seroconversion and if two or more different serogroups showed the maximum titer. MAT was performed with 12 of the 19 reference strains recommended by World Health Organization (2003) and evaluated to be used in Argentina (Jacob et al., 2015), including 8 strains of the *L. interrogans* serogroups Canicola, Icterohaemorrhagiae, Pomona, Pyrogenes, Sejroe and Bataviae; 2 strains of *L. borgpetersenii* serogroup Ballum and Tarassovi; 1 strain of *L. kirschneri* serogroup Grippotyphosa, and 1 strain of *L. biflexa* serogroup Semaranga. Buffered saline solution was used as negative control for each reaction.

In culture-proven cases (n = 8), genotyping results between clinical samples and isolates were compared.

2.8. Ethical statement

This study was revised and approved by the Ethics Committee of the "*Facultad de Bioquímica y Ciencias Biológicas*" of the "*Universidad Nacional del Litoral, Santa Fe, Argentina*". Act 02/16, March 10 2016.

Table 1
Typing success and results of 16S rRNA and MLST per year in septicemic-phase sera of leptospirosis cases, Argentina, 2005–2016.

		16S rRNA			MLST		
Year	Studied samples	Positive	Typing success rate (%)	Specie (No)	Full allelic profiles	Typing success rate (%)	ST (No)
2005	2	0	0.0	N/A	0	0.0	N/A
2006	2	0	0.0	N/A	0	0.0	N/A
2007	44	3	6.8	*L. interrogans* (3)	2	4.5	ST37 (1); ST140 (1)
2008	9	0	0.0	N/A	0	0.0	N/A
2009	7	2	28.6	*L. interrogans* (2)	0	0.0	N/A
2010	41	8	19.5	*L. interrogans* (8)	4	9.8	ST37 (2); ST20 (1); ST37/118[a] (1)
2011	17	3	17.6	*L. interrogans* (3)	2	11.8	ST13 (2)
2012	11	6	54.5	*L. interrogans* (6)	6	54.5	ST13[b] (1); ST37 (4); ST38[b] (1)
2013	15	5	33.3	*L. interrogans* (5)	2	13.3	ST20 (1); ST37 (1)
2014	18	7	38.9	*L. interrogans* (7)	4	22.2	ST20 (2); ST17 (2)
2015	36	7	19.4	*L. interrogans* (6) *L. borgpetersenii* (1)	6	16.7	ST37 (4); ST17 (1); ST149 (1)
2016	26	9	34.6	*L. interrogans* (9)	0	0.0	N/A
Study period	228	50	21.9	*L. interrogans* (49), *L. borgpetersenii* (1)	26	11.4	ST37 (12); ST20 (4); ST17 (3); ST13 (3); ST38 (1); ST140 (1); ST37/118 (1); ST149 (1)

N/A: not applicable.
[a] ST: the obtained MLST full allelic profile shared six alleles with ST37 and six alleles with ST118 and with a sucA and a caiB loci variants, respectively.
[b] ST: resulted by 6 loci match, poor quality sequence was obtained from one locus.

3. Results and discussion

Genotyping methods were assessed in 228 septicemic-phase serum samples of confirmed leptospirosis patients (DPO mean: 4.6, median: 5.0, maximum value: 7 and minimum value: 0).

Partial 16S rRNA sequencing identified *Leptospira* spp. in 50 sera (16S rRNA-TSR = 21.9%, CI [16.3–27.5]). MLST locus *glmU* were amplified from 43 sera, full allelic profiles were obtained from 26 samples (MLST-TSR = 11.4%, CI [7.1–15.7]) and 2 or more loci were amplified from the others specimens. Seven 16S rRNA negative sera were MLST positive (2 full allelic profiles and 5 partial allelic profiles). Table 1 summarizes the 16S rRNA and MLST typing success and results per year of the study period. In order to compare with other authors who only applied MLST in 16S rRNA positive samples, the percentage of positive MLST was calculated. Full and partial allelic profiles were obtained in 24 (48.0%) and 12 (38.0%) 16S rRNA positive samples, respectively.

This report presents the utility evaluation results of one partial 16S rRNA gene sequencing and an optimized MLST for typing *Leptospira* directly from serum samples. The 16S rRNA-TSR and the MLST-TSR allowed to know the performance of each one when the serum sample is the only available clinical specimen. Most of the strategies for typing *Leptospira* use different variants of these methods and samples types to detect only pathogen species (Agampodi et al., 2011, 2013, 2014; Chiani et al., 2016; Guernier et al., 2016; Mendoza and Rivera, 2021; Perez and Goarant, 2010; Varni et al., 2018). This last situation does not allow to know with certainty the usefulness of each method for typing all *Leptospira* species in serum samples. In order to detect pathogenic, intermediate and saprophytic species in the present study, the genotyping methods were performed in sera without any previous molecular assays and this procedure could be the main cause of the low 16S rRNA-TSR. In a comparable study, Boonsilp et al. (2011) evaluated a conventional PCR targeting 16S rRNA gene, without any previous molecular test, in a relevant sample size and in clinical samples. Although these authors did not study serum samples and amplified another region of 16S rRNA gene, the reported positivity rate (28.2%) in EDTA blood samples from 379 culture-negative cases was comparable with the 21.9% found in the present study. Philip et al. (2020) evaluated the same nested PCR in serum and blood samples of suspected cases.

On the other hand, Merien et al. (1995) amplified the same region of the 16S rRNA gene, without any molecular screening, in the same clinical sample type but using nested PCR and Dot Blot hybridization. These authors obtained a positivity rate of 82.1% in 28 serum samples. This high rate could be attributed to nested PCR and Dot Blot hybridization that improve the 16s RNA performance (Mérien et al., 1992). Thaipadungpanit et al. (2011) also reported a high positivity rate (56%) but using Real Time PCR. These reports and the seven 16S rRNA-negative samples but MLST-positive suggest that the 16S rRNA sensitivity should be improved, especially if the typing strategies involve the use of MLST in 16S rRNA-positive samples.

Even without any previous screening test, the MLST-TSR (11,4%) obtained in this study was similar or higher than the 11.7% (Varni et al., 2018), 9.8% (Chiani et al., 2016), and 5.2% (Agampodi et al., 2013) reported in pathogenic *Leptospira* positive samples applying different MLST schemes. Moreover, the 48% of full allelic MLST profile obtained in 16S rRNA positive samples was much higher than all the proportions mentioned above and even higher than the typing rates (17.2% and 1.59%) obtained by Weiss et al. (2016) and Philip et al. (2020) respectively, applying the same extended nested MLST. In the present study, the MLST performance evaluation was improved by analyzing a large serum sample size.

A positive trend in the TSR of both typing methods during 12-year study period was observed. This could be due to DNA degradation during long-time storage and repeated freeze and thaw cycles (Schröder and Steimer, 2018; Shao et al., 2012). However, other factors may influence the success rates such as the low bacterial counts in blood, the absence of DNA because of the short bacteremia period, or an error in the disease-phase identification due to the memory bias of patients in relation to the onset of symptoms.

Forty-nine (98.0%) 16S rRNA sequences presented similarity with *L. interrogans* and one (2.0%) with *L. borgpetersenii*. The species identified in this study are two of the three pathogenic species most frequently identified as worldwide causative agents of leptospirosis

Table 2
Frequency of probable serogroups and presumptive infecting serogroups identified by MLST and MAT in sera of leptospirosis cases, Argentina, 2005–2016.

Method (successfully typed samples/studied samples)	Serogroups	N° of typed samples	Relative proportion (%)
MLST(26/228)	Canicola-ST37 (a)	12	46.2
	Sejroe-ST20 (a)	4	15.4
	Icterohaemorrhagiae-ST17 (a)	3	11.6
	Pyrogenes-ST13 (a)	3	11.6
	Pomona-ST38 (a)	1	3.8
	Ballum-ST149 (b)	1	3.8
	Pomona/Grippotyphosa/Pyrogenes/Hebdomadis-ST140 (a)	1	3.8
	Canicola/Hebdomadis-ST37/118 (a)	1	3.8
MAT(124/159)	Sejroe	39	31.5
	Canicola	35	28.2
	Icterohaemorrhagiae	27	21.8
	Pomona	16	12.9
	Ballum	4	3.2
	Grippotyphosa	2	1.6
	Pyrogenes	1	0.8

Species identified by 16S rRNA and MLST: (a) *L. interrogans* and (b) *L. borgpetersenii*.

(Boonsilp et al., 2013) and are consistent with the species reported in Argentina (Chiani et al., 2016; Colombo et al., 2018; Loffler et al., 2014; Ricardo et al., 2018; Rossetti et al., 2005; Scialfa et al., 2013; Varni et al., 2018).

Among the serum samples, MLST assigned a total of 8 different STs, 7 STs representing *L. interrogans* and 1 ST *L. borgpetersenii.* Typing results of 16S rRNA and MLST were consistent at species level. The 8 STs correspond to 8 different probable serogroups that confirm the circulation of a large variety of genotypes in Argentina. The ST37 was the most frequent genotype.

One hundred and fifty-nine immune-phase serum samples, from all the included patients, were available. MAT indicated the presumptive infecting serogroups in 124 sera (78.0%) and serogroups were undefined in 32 (20.1%). The relative proportion of probable and presumptive infecting serogroups identified by MLST and MAT, respectively, were shown in Table 2. Canicola was the serogroup most frequently identified by MLST followed by Sejroe, Icterohaemorrhagiae, and Pyrogenes. The most frequently presumptive infectious serogroups determined by MAT were Sejroe, Canicola, Icterohaemorrhagiae, and Pomona. The probable serogroups identified by MLST were coincident with the presumptive infecting serogroups identified by MAT, but with different frequencies. The three serogroups (Canicola, Sejroe and Icterohaemorrhagiae) most frequently identified by MAT and also genotyped by MLST differed from previous reports in Argentina (Vanasco et al., 2008).

Table 3 shows the comparison of the genotyping results by 16S rRNA and MLST between septicemic-phase clinical samples and isolates of culture-proven patients. In 5 clinical samples of the 8 available culture-proven cases, genotyping of the causative agent was possible. The lower number of clinical samples with successfully identified *Leptospira* compared to typed isolates was expected due to the low amount of bacterial DNA present in a clinical sample compared to isolates. In these 5 cases, the results of 16S rRNA typing in isolates and clinical samples were consistent. MLST results were also consistent except in one case, in which Canicola serogroup (ST37) and Australis serogroup (ST120) were identified in the isolate and the septicemic phase sample, respectively. ST37 and ST120 differ only in the locus pfkB. The similarity of the two alleles obtained for that locus and the low quality of the obtained sequence might explain the difficulty in identifying the correct serogroup from this blood specimen.

On the other hand, MLST apparently failed to define the serogroup in a Pomona isolate by assigning the sequence type ST140 (probable serogroup: Pomona-Grippotyphosa-Pyrogenes-Hebdomadis). However, previous studies also reported the assignment of ST140 to isolates and reference strains of Pomona serogroup (Bertasio et al., 2020; Ferreira et al., 2020; Guernier et al., 2016; Weiss et al., 2016), so it is necessary to enrich the available databases with a greater number of serotyped and genotyped *Leptospira* isolates, in order to improve the STs assignment and the identification of the causative serogroups.

Understanding the epidemiology, pathogenesis, and new approaches to treating and preventing leptospirosis require detailed knowledge of regionally circulating *Leptospira* in highly endemic settings (Agampodi and Vinetz, 2021). These authors express that the culture-independent typing based on Next-generation sequencing overcomes the limitation of *Leptospira* isolation from clinical samples, providing public health information applicable to leptospirosis-endemic low/middle-income settings. The genotyping methods evaluated in this study are part of these culture-independent methods based on next-generation sequencing and constitute are unambiguous tools that could increase the number of leptospirosis cases with identified causative *Leptospira*, at species and subspecies level, and improve the knowledge of their relative frequency. Additionally, the typing strategy evaluated in this study could by very useful to improving the MAT diagnosis panel. The antigen panel used in the diagnostic process is often not regionally optimized, especially in countries with unknown circulating leptospires, moreover in resource limited low/middle income regions. The development of efficient methods to precisely identify the *Leptospira* species and serogroup/serovar is important to replace imprecise serology (Agampodi and Vinetz, 2021; Philip et al., 2020).

4. Conclusions

Performance of partial 16S rRNA gene sequencing assay and the optimized MLST scheme directly in sera may increase and improve the knowledge about species and serogroups causing human leptospirosis, especially in countries with low rates of culture sample collection or *Leptospira* isolation. Access to high-performance genotyping methods is even more relevant if serum sample is the only available clinical specimen.

The use of the evaluated typing strategy could be a useful tool to optimize the MAT diagnosis panel in regions with unknown

Table 3
Species and serogroups identified by 16S rRNA and MLST, in clinical samples of eight culture-proven patients, Argentina, 2005–2016.

Isolates typing			Clinical samples typing	
Species by 16S rRNA	ST- Probable serogroup by MLST	Serogroup by serotyping	Species by 16S rRNA	ST - Probable serogroup by MLST
L. interrogans	37-Canicola	Canicola	*L. interrogans*	37-Canicola
L. interrogans	37-Canicola	Canicola	*L. interrogans*	120-Australis
L. interrogans	17-Icterohaemorrhagiae	Icterohaemorrhagiae	*L. interrogans*	17-Icterohaemorrhagiae
L. interrogans	37-Canicola	Canicola	*L. interrogans*	37-Canicola
L. interrogans	17-Icterohaemorrhagiae	Icterohaemorrhagiae	*L. interrogans*	Partial allelic profile
L. interrogans	17-Icterohaemorrhagiae	Icterohaemorrhagiae	N	N
L. interrogans	37-Canicola	Canicola	N	N
L. interrogans	140-Pomona/Grippotyphosa/Pyrogenes/ Hebdomadis	Pomona	N	N

N: negative.

circulating leptospires.

However, the percentage of samples with unsuccessfully typing results highlights the need for further optimization of these methods (especially the 16S rRNA) to increase the typing rate of *Leptospira* in serum samples.

Identification of infecting *Leptospira* specie and serogroup by these methods has clinical, public health, epidemiological, and agricultural implications for diagnosis, treatment, control, and prevention.

Declarations

Author contribution statement

LANDOLT, Noelia Yolanda: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

CHIANI, Yosena Teresita; PUJATO Nazarena; JACOB, Paulina; SCHMELING, María Fernanda: Performed the experiments; Wrote the paper.

GARCÍA EFFRON, Guillermo: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

VANASCO, Norma Bibiana: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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