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Isolation and clinical sample typing of human leptospirosis cases in Argentina

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ABSTRACT

Leptospira typing is carried out using isolated strains. Because of difficulties in obtaining them, direct identification of infective *Leptospira* in clinical samples is a high priority. Multilocus sequence typing (MLST) proved highly discriminatory for seven pathogenic species of *Leptospira*, allowing isolate characterization and robust assignment to species, in addition to phylogenetic evidence for the relatedness between species. In this study we characterized *Leptospira* strains circulating in Argentina, using typing methods applied to human clinical samples and isolates. Phylogenetic studies based on 16S ribosomal RNA gene sequences enabled typing of 8 isolates (6 *Leptospira interrogans*, one *Leptospira wolffii* and one *Leptospira broomii*) and 58 out of 85 (68.2%) clinical samples (55 *L. interrogans*, 2 *Leptospira meyeri*, and one *Leptospira kirschneri*). MLST results for the *L. interrogans* isolates indicated that five were probably Canicola serogroup (ST37) and one was probably Icterohaemorrhagiae serogroup (ST17). Eleven clinical samples (21.6%), provided MLST interpretable data: five were probably Pyrogenes serogroup (ST13), four Sejroe (ST20), one Autumnalis (ST22) and one Canicola (ST37). To the best of our knowledge this study is the first report of the use of an MLST typing scheme with seven loci to identify *Leptospira* directly from clinical samples in Argentina. The use of clinical samples presents the advantage of the possibility of knowing the infecting strain without resorting to isolates. This study also allowed, for the first time, the characterization of isolates of intermediate pathogenicity species (*L. wolffii* and *L. broomii*) from symptomatic patients.

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1. Introduction

Leptospirosis is a zoonotic disease of global distribution caused by spirochetes of the genus *Leptospira* spp. (Levett, 2007). It is maintained by chronic carrier hosts that excrete the organism into the environment, and human infection results from direct contact with infected animals or with a contaminated environment (Levett, 2007; Ko et al., 2009). Leptospirosis in humans can vary in severity according to the infecting *Leptospira* serovar, inoculum dosage and the patient's age, health and immunological competence. Clinical manifestations, when present, vary from a mild flu-like febrile illness to a severe disease with symptoms that may include jaundice, renal failure and pulmonary hemorrhage (Adler and de la Peña Moctezuma, 2010).

In recent decades typing and detection of *Leptospira* spp. based on molecular techniques has been introduced and widely applied to the field of leptospirosis study. Moreover, molecular methods have been exploited as an alternative or supplementary approach to the currently existing serological methods. The cross-agglutination absorption test (CAAT) analysis that led to the definition of serovar, is today considered to be the basic systematic unit for *Leptospira* spp. typing (Adler and de la Peña Moctezuma, 2010). However, CAAT is cumbersome and time-consuming for routine typing, mostly due to the time required for the preparation of rabbit immune sera. Therefore, few laboratories are able to perform CAAT (Terpstra et al., 1985). For this reason, most isolates are identified at serogroup level, using the Microscopic agglutination test (MAT) (Cerqueira and Picardeau, 2009).

Since the 1990s speciation of leptospires based on genomic DNA homology has been available. Molecular approaches such as heterologous DNA hybridization (Brenner et al., 1999; Levett, 2006; Ahmed et al., 2012) and more recently, average nucleotide identity and genome-to-genome distances (Bourhy et al., 2014), have led to the identification of 22 *Leptospira* species, ten pathogenic: *Leptospira interrogans*, *Leptospira borgpetersenii*, *Leptospira santarosai*, *Leptospira noguchii*, *Leptospira weilii*, *Leptospira kirschneri*, *Leptospira alexanderi*, *Leptospira alstonii*, *Leptospira kmetyi* and *Leptospira mayottensis*; five intermediate: *Leptospira inadai*, *Leptospira broomii*, *Leptospira fainei*, *Leptospira wolffii*, *Leptospira licerasiae*; and seven non-pathogenic: *Leptospira biflexa*,

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Leptospira wolbachii, *Leptospira vanthielii*, *Leptospira terpstrae*, *Leptospira meyeri*, *Leptospira idonii*, and *Leptospira yanagawae*. The intermediate group produces mild clinical symptoms or no symptoms at all. The pathogenic implication of this group is yet unknown.

DNA hybridization is a complicated method that requires the use of considerable amounts of isotope-labeled DNA of high quality. Other techniques based on PCR amplification, such as multilocus sequence analysis, are gaining importance as molecular tools for the speciation of *Leptospira* (Ahmed et al., 2012). The 16S ribosomal RNA (rRNA) gene allows identification due to inter-species differences and low intra-species variability. The comparison of 16S rRNA sequences is a powerful tool for deducing phylogenetic and evolutionary relationships among bacteria (Backstedt et al., 2015). Due to multiple benefits, Multilocus Sequence Typing (MLST), based on the generation of sequences from different genes and allowing a high-throughput scale, has become the method of choice. MLST is unambiguous and suitable for epidemiological research, population studies and the investigation of maintenance hosts during outbreaks (Ahmed et al., 2006, 2011; Maiden, 2006; Thaipadungpanit et al., 2007; Leon et al., 2010; Boonsilp et al., 2013). MLST has proved highly discriminatory for seven pathogenic species of *Leptospira*, providing both isolate characterization and robust species assignment in addition to phylogenetic evidence for relatedness between species. Crucially, this scheme is also supported by a public website (http://Leptospira.mlst.net/) (Boonsilp et al., 2013).

Leptospira typing is carried out using isolated strains. Although culture is rarely performed in routine clinical practice and is only positive in a minor number of cases, it continues to have an important role in defining the global epidemiology of infection (Thaipadungpanit et al., 2007; Slack et al., 2007; Adler and de la Peña Moctezuma, 2010; Thaipadunpanit et al., 2011). Identification of the infecting serovar can provide clues as to the chronic carrier host, since certain serovars may be associated with either a single or small number of mammalian or other species (Levett, 2001; Bharti et al., 2003). Such information makes an important contribution to the development of prevention strategies (Levett, 2001; Bharti et al., 2003; Ko et al., 2009). Nonetheless, because of difficulties in obtaining isolates, direct identification of infective *Leptospira* in clinical samples is a high priority for clinical, epidemiological, and basic scientific purposes (Agampodi et al., 2013).

In Argentina, there have been a few studies concerning species prevalence and many of these studies reported a number of cases with agglutination titers against multiple serogroups. In a study by Vanasco et al. (2008), Icterohaemorrhagiae and Pomona were identified as the major serogroups involved in human leptospirosis. Recently, Caimi et al. (2012) demonstrated the presence of serogroup Canicola and Icterohaemorrhagiae, using variable number tandem repeat and MLST characterization in bovine and porcine isolates.

In this context, the aim of this study was to characterize *Leptospira* strains circulating within Argentina. This was performed by the use of molecular typing methods applied to human clinical samples and isolates.

2. Materials and methods

2.1. Bacterial isolates and clinical samples

Human isolates (culture) and samples obtained between January 2004 and March 2014 were selected from a collection at the Leptospirosis laboratory of the National Respiratory Disease Institute (Instituto Nacional de Enfermedades Respiratorias, INER), Santa Fe, Argentina. Characteristics of participants are shown in Table 1. Eight isolates, and eighty five samples (78 serum samples and 7 whole blood) diagnosed as positive by real-time quantitative polymerase chain reaction (qPCR), were selected from the INER collection. The qPCR assay was performed according to Stoddard et al. (2009) using a TaqMan probe targeting *LipL32*.

Eight isolates were obtained from 0.2 ml of human whole blood cultured into Ellinghausen–McCullough–Johnson–Harris semisolid medium (EMJH). The cultures were incubated at 28 °C and examined weekly for 4 months (World Health Organization and International Leptospirosis Society, 2003).

Leptospires in serum samples were quantified using the threshold cycle (CT) of the qPCR reaction. Leptospiral culture dilutions from 10^8 to 1 *Leptospira*/ml were prepared and the number of cells in culture was determined as described by Smythe et al. (2002), except for the use of a Neubauer bacterial counting chamber. The CT of each culture was obtained by qPCR as described above, and the calibration curves were constructed.

2.2. Serological characterization of isolates

Serological characterization of isolates into serogroups was carried out by MAT using rabbit antisera against reference serovars (Faine, 1999), representing a standard battery of 23 serogroups: Grippotyphosa, Pomona, Ballum, Pyrogenes, Icterohaemorrhagiae, Sejroe, Tarassovi, Canicola, Bataviae, Semaranga, Autumnalis, Australis, Cynopteri, Javanica, Panama, Hebdomadis, Sarmin, Ranarum, Louisiana, Mini, Celledoni, Djasiman, Shermani. Each isolate was assigned to the serogroup of the group serum with the highest titer.

2.3. DNA extraction

Genomic DNA was extracted from serum, whole blood and EMJH cultures using QIAamp DNA Blood Mini Kits (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions.

2.4. 16S rRNA

Determination of species was performed as a first step, using 16S rRNA as the amplification target (Mérien et al., 1992). For each reaction, 1 U of GoTaq DNA polymerase (Promega, Madison, WI, USA), 200 μM deoxynucleoside triphosphates (dNTPs), and 1 μM of primers, were added to a total volume of 50 μl. Amplification was carried out using a Veriti Thermal Cycler (Applied Biosystems, Foster City, CA, USA) and the PCR products were analyzed on 2% agarose gels.

2.5. MLST

A previously published MLST scheme based on the amplification of seven housekeeping genes (*mreA*, *pfkB*, *pntA*, *sucA*, *tpiA*, *glmU* and *caiB*) was used (Boonsilp et al., 2013). Reaction mixtures were prepared using 1.25 U GoTaq DNA polymerase (Promega, Madison, WI, USA) with 5 pmol of each primer and 5 μl of DNA in a total volume of 50 μl. Amplification was carried out using a Veriti Thermal Cycler. The PCR products were analyzed on 2% agarose gels.

2.6. Sequencing and sequence analysis

PCR amplification products of 16S rRNA and MLST genes were purified using GeneJET PCR Purification Kit (Thermo Scientific, Waltham, MA, USA) prior to DNA sequencing. PCR products were then sequenced by Macrogen Inc. (Seoul, Korea). The sequences were edited using Chromas Lite 2.1.1 (Technelysium Pty Ltd., Australia). The contigs were assembled using the Staden Package software (MRC-LMB, UK) and the alignment and construction of phylogenetic trees was performed using MEGA 5 (Tamura et al., 2011). To obtain the *Leptospira* species, the assembled sequences of 16S rRNA were analyzed using the Ribosomal Database Project (RDP) (http://rdp.cme.msu.edu/seqmatch/seqmatch_intro.jsp).

Phylogenetic relationship among concatenated sequences of 16S rRNA was inferred using the maximum-likelihood (ML) method and the Tamura–Nei parameter, using MEGA 5.0 (Tamura et al., 2011).

Table 1
Characteristics of participants. Argentina (2004–2014).

N°	Year	Type of sample	DPO	Fatal Case	Culture	Age	Sex	Province
1	2010	S	3	Y	UR	57	M	Santa Fe
2	2010	S	5	Y	UR	52	F	Santa Fe
5	2010	S	5	Y	UR	25	M	Salta
6	2010	S	5	Y	UR	43	M	Entre Ríos
7	2010	S	2	Y	UR	55	M	Santa Fe
8	2010	S	1	N	N	27	M	Santa Fe
12	2010	S	4	N	UR	42	M	Santa Fe
13	2010	S	5	Y	UR	50	M	Santa Fe
14	2010	S	4	N	UR	47	M	Santa Fe
21	2010	S	5	N	UR	59	M	Santa Fe
22	2010	S	4	N	UR	18	M	Santa Fe
28	2010	S	3	N	UR	26	M	Santa Fe
29	2010	S	5	N	UR	43	M	Santa Fe
37	2010	S	3	N	UR	38	M	Santa Fe
38	2010	S	5	Y	UR	23	M	Santa Fe
39	2010	S	1	N	UR	31	M	Santa Fe
40	2010	S	3	Y	N	51	M	Santa Fe
56	2010	S	6	N	UR	31	M	Santa Fe
59	2010	S	5	N	UR	39	M	Santa Fe
64	2010	S	6	Y	UR	34	M	Entre Ríos
73	2010	S	6	N	UR	13	M	Santa Fe
92	2011	S	6	Y	UR	54	M	Santa Fe
99	2011	S	3	N	UR	55	M	Entre Ríos
105	2011	S	2	Y	UR	19	M	Entre Ríos
116	2011	S	11	Y	UR	17	F	Chaco
118	2011	S	20	Y	UR	68	M	Entre Ríos
120	2011	S	5	Y	UR	39	M	Santa Fe
130	2007	S	9	Y	UR	69	M	Entre Ríos
132	2007	S	2	Y	UR	12	M	Entre Ríos
133	2007	S	7	Y	UR	71	M	Entre Ríos
135	2011	S	16	Y	UR	40	F	Entre Ríos
139	2007	WB	4	Y	UR	23	M	Santa Fe
143	2010	S	2	Y	UR	39	M	Entre Ríos
157	2012	S	4	Y	UR	44	M	Entre Ríos
172	2007	S	6	N	UR	34	m	Santa Fe
175	2007	S	5	Y	UR	66	M	Entre Ríos
178	2010	S	4	N	UR	8	M	Santa Fe
179	2012	WB	5	Y	N	56	F	Santa Fe
180	2010	S	4	U	UR	56	M	Santa Fe
182	2010	S	2	N	UR	30	M	Santa Fe
196	2012	WB	5	U	N	15	M	Entre Ríos
197	2012	S	3	Y	UR	42	M	Santa Fe
228	2012	S	5	N	UR	26	M	Santa Fe
234	2013	S	5	Y	UR	46	M	Buenos Aires
246	2013	S	5	Y	UR	34	M	Chaco
258	2013	S	2	U	UR	39	M	Buenos Aires
271	2013	S	4	N	N	21	M	Santa Fe
272	2013	S	5	N	UR	29	M	Buenos Aires
273	2013	S	14	N	UR	1	F	Santa Fe
277	2013	S	6	N	UR	U	M	Santa Fe
282	2012	S	6	U	UR	U	M	Santa Fe
285	2012	S	7	U	UR	U	M	Santa Fe
288	2004	S	6	U	UR	U	M	Entre Ríos
337	2011	S	2	U	UR	46	M	Santa Fe
339	2011	S	4	U	UR	41	M	Entre Ríos
352	2010	S	2	U	UR	U	M	Santa Fe
356	2010	S	6	U	UR	41	F	Entre Ríos
357	2010	S	2	U	UR	23	M	Santa Fe
359	2010	S	4	U	UR	52	M	Entre Ríos
386	2012	S	8	U	UR	50	M	Entre Ríos
388	2012	S	4	U	UR	25	M	Santa Fe
389	2012	S	8	Y	N	33	M	Santa Fe
400	2013	WB	8	N	N	66	M	Santa Fe
401	2013	S	5	Y	UR	60	M	Entre Ríos
402	2013	S	7	Y	UR	36	M	Entre Ríos
409	2012	S	4	U	N	22	M	Santa Fe
411	2012	S	2	U	N	33	M	Santa Fe
412	2012	S	3	U	N	42	M	Santa Fe
413	2013	S	2	U	N	17	M	Santa Fe
414	2013	S	2	U	N	U	F	Santa Fe
415	2013	S	5	U	N	42	M	Santa Fe
416	2013	S	4	U	N	38	F	Santa Fe
425	2012	S	4	U	UR	47	M	Catamarca
426	2013	S	7	U	UR	47	M	Río Negro

(continued on next page)

Table 1 (*continued*)

N°	Year	Type of sample	DPO	Fatal Case	Culture	Age	Sex	Province
427	2012	S	7	U	UR	54	M	Entre Ríos
437	2013	WB	2	N	N	21	M	Santa Fe
439	2013	S	10	U	UR	17	F	Santa Fe
452	2014	S	7	Y	UR	24	M	Santa Fe
464	2014	S	6	Y	UR	21	M	Buenos Aires
466	2014	S	3	U	UR	14	M	Santa Fe
471	2014	S	4	Y	UR	56	M	Santa Fe
477	2014	S	5	Y	UR	U	M	La Pampa
15/A1	2010	S/C	4	Y	P	63	M	Santa Fe
236/A9060	2013	WB/C	3	U	P	61	M	Buenos Aires
A188	2013	C	2	U	P	46	M	Santa Fe
A2	2011	C	2	N	P	16	M	Buenos Aires
A7493	2012	C	12	N	P	22	F	Buenos Aires
A75	2012	C	3	U	P	60	F	Santa Fe
A8223	2013	C	14	N	P	39	M	Buenos Aires
A9197	2013	WB/C	2	Y	P	35	M	Santa Fe

DPO: Days post onset, S: Serum, WB: Whole blood, C: Culture, Y: Yes, N: No, U: Unknown, P: Positive, N: Negative, UR: Unrealized, F: Female, M: Male.

The sequence type (ST) was determined in each case according to the 7 MLST *loci* sequences published in the world database (www.Leptospira.mlst.net).

2.7. Ethical statement

This study was revised and approved by the ethics committee of the Biochemistry and Biological Science College, National University of the Littoral, Santa Fe, Argentina (Facultad de Bioquímica y Ciencias Biológicas-Universidad Nacional del Litoral, FBCB-UNL).

3. Results and discussion

3.1. 16S rRNA

Phylogenetic studies based on 16S rRNA gene sequences (Fig. 1) showed that six isolates were grouped within pathogenic species (*L. interrogans*), while isolates A1 and A2 were within the intermediate species *L. wolffii* and *L. broomii* respectively. To the best of our knowledge this was the first time these latter species had been isolated in Argentina. These intermediate species produce a mild illness that disappears without complications, but their pathogenicity is still unknown. This may be because the virulence determinants of leptospires and the mechanisms by which the bacteria cause illness have been poorly studied (Ko et al., 2009; Ricaldi et al., 2012; Balamurugan et al., 2013). However, the ability of intermediate species to cause illness in humans has been reported previously (Levett, 2006; Slack et al., 2008; Balamurugan et al., 2013). In this study, *L. broomii* was isolated from a patient who required hospitalization without signs of severe disease. On the contrary, *L. woolffii* was isolated from a fatal case of respiratory syndrome. However, the serum sample of this patient was positive to qPCR, and clustered with *L. interrogans* by 16S rRNA (Fig. 1), supporting the idea of double infection. The severe disease was probably caused by *L. interrogans*.

From a total of 85 clinical samples, 53 sera and 5 whole blood samples (68.2%) were positive to 16S rRNA. The difference between *LipL32* qPCR positivity and that of 16S rRNA PCR could be related to the limitations of the conventional PCR used in the 16S rRNA (Merien et al., 2005; Picardeau et al., 2014). Fifty five samples were grouped within *L. interrogans* and one sample (serum 99) within *L. kirschneri*. Two samples from fatal cases (whole blood 139 and serum 175), previously identified as pathogenic by qPCR due to detection of the *LipL32* gene in their genomes, were unexpectedly grouped within the saprophytic species *L. meyeri* by 16S rRNA (Fig. 1). This finding could be explained through two possible situations. The patients could have been double infected with a pathogenic and saprophytic species and 16S rRNA only detected the last because of a larger amount of *L. meyeri* DNA in the sample. In addition, no evidence of environmental contamination was found in any of the reaction controls. Secondly, previous studies have reported that *L. meyeri* is composed of strains with different pathogenic potential, consequently its classification has been controversial, and it could cause illness (Postic et al., 2000; Kositanont et al., 2007; Victoria et al., 2008; Slack et al., 2009). These results should be discussed and analyzed in future research.

3.2. Serotyping of isolates

Results for isolate serotyping showed a predominance of serogroup Canicola (63%) (Table 2). Isolates *L. wolffii* and *L. broomii*, as identified by 16S rRNA, were negative to serotyping, probably due to the fact that only pathogenic species such as *L. interrogans*, *L. kirschneri* and *L. borgpetersenii*, were used for this technique. Patients whose isolated serogroup was Canicola required hospitalization, presenting with different degrees of severity. Only one fatal case associated to serogroup Icterohaemorragiae was reported.

3.3. MLST

The results of MLST from isolates belonging to *L. interrogans* according to 16S rRNA, showed that most of them were ST37, probably Canicola serogroup (Table 2). These findings were similar to those from the serotyping and where in agreement with results obtained by Romero et al. (2011). In Argentina, only one previous study reported using MLST (Varni et al., 2014) on 3 human isolates, probably belonging to Pomona, Icterohaemorragiae and Sejroe serogroups.

Of the 56 clinical samples identified as pathogenic species according to 16S rRNA, MLST was performed on 51 of them, where enough volume was available to run the test. MLST provided interpretable data from 11 samples (21.6%), only 5 of which were positive to the entire locus, and all of these were ST13, probably the Pyrogenes serogroup (Table 3). These results were similar to Agampodi et al. (2013), who identified the ST in 12 out of 58 samples (20.7%), and only 3 were positive to the entire locus. In contrast, Perez and Goarant (2010) did not identify STs from clinical samples.

The difference between the probable serogroup distribution identified by MLST in isolates and clinical samples (Canicola vs. Pyrogenes and Sejroe) could be related to the culture bias of different serogroups. It is probable that Canicola have a greater ability to grow in culture medium than Pyrogenes. In fact, there are very few studies

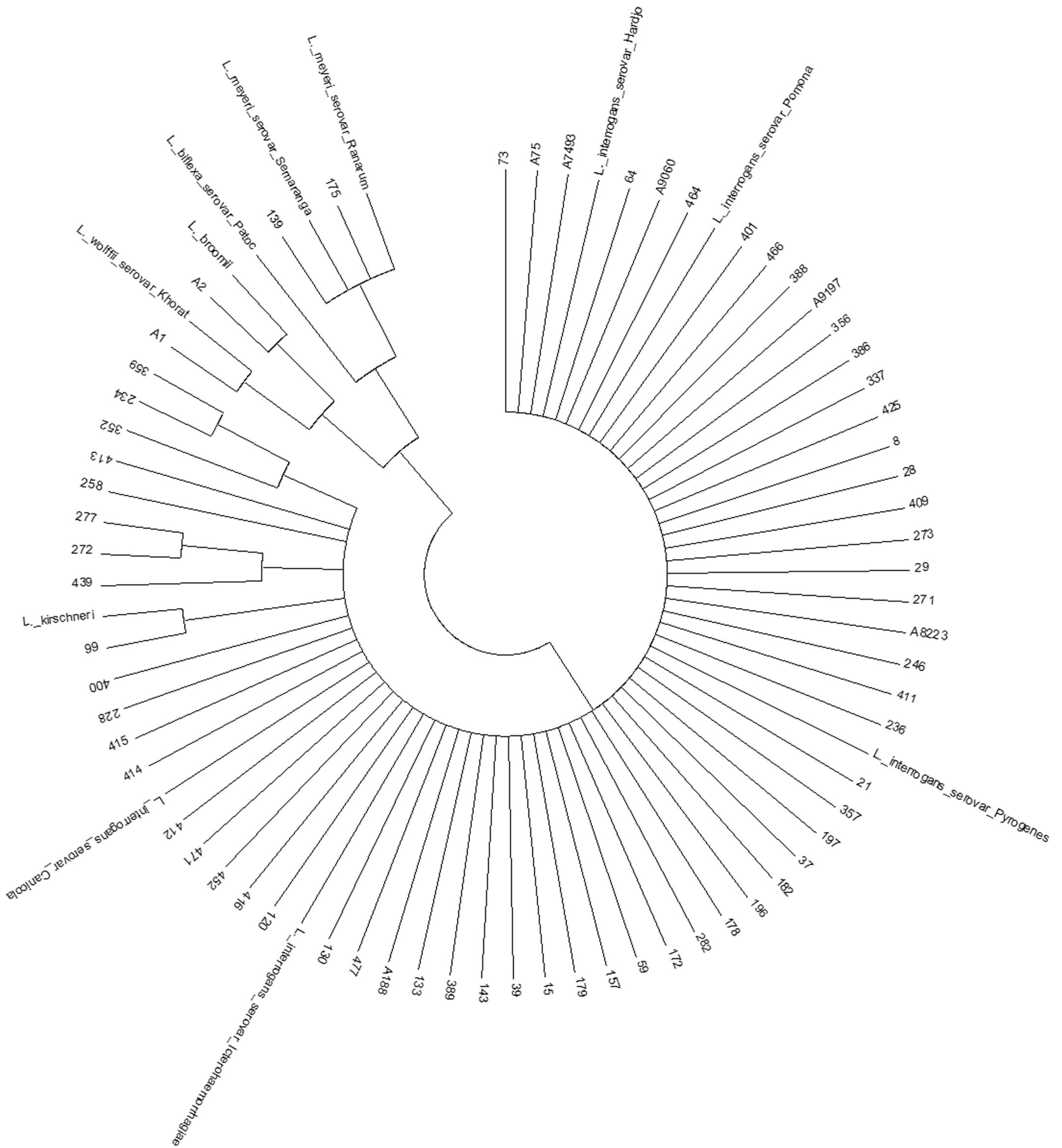

Fig. 1. Phylogenetic studies based on 16S rRNA gene sequences.

showing isolation of Pyrogenes. No relationships were found between the origin of patients, sampling year and serogroup distribution.

Of the seven loci, *tpiA* and *sucA* were the most detected genes, while *pfkB* and *caiB* data were the least reliable. This might be related to the quantity of leptospires/ml, as all samples with the full profile sequenced had more than 5×10^4 leptospires/ml. This finding was also observed by Perez and Goarant (2010). Some authors agree that serum PCR is less sensitive than PCR performed on other types of sample, e.g. whole blood for detection of *Leptospira* DNA (Stoddard et al., 2009; Bourhy et al., 2011). Besides low DNA concentration, the low percentage of typing samples might be due to the use of storage at −70 °C for several years, and the associated freezing and thawing, which can cause DNA damage (Visvikis et al., 1998; Ross et al., 1990). In addition, MLST makes use of PCR amplified DNA segments and thus depends on the success of amplification, which in turn depends on the annealing efficiency of the PCR primers (Nalam et al., 2010).

Table 2
16S rRNA, MLST and serotyping results of Isolates.

Isolate N°	Year	Species (16S rRNA)	ST by MLST	Allelic profile (MLST)							Probably serogroup (MLST)	Serogroup by serotyping (MAT)
				glmU	pntA	sucA	tpiA	pfkB	mreA	caiB		
A1	2010	*L. wolffii*	UD	–	–	–	–	–	–	–	UD	N
A2	2011	*L. broomii*	UD	–	–	–	–	–	–	–	UD	N
A75	2012	*L. interrogans*	37	3	3	3	3	4	5	5	Canicola	Canicola
A7493	2012	*L. interrogans*	37	3	3	3	3	4	5	5	Canicola	Canicola
A188	2013	*L. interrogans*	37	3	3	3	3	4	5	5	Canicola	Canicola
A8223	2013	*L. interrogans*	37	3	3	3	3	4	5	5	Canicola	Canicola
A9060	2013	*L. interrogans*	37	3	3	3	3	4	5	5	Canicola	Canicola
A9197	2013	*L. interrogans*	17	1	1	2	2	10	4	8	Icterohaemorragiae	Icterohaemorragiae

(−): No amplification. UD: undetermimated. N: negative result.

4. Conclusions

This study allowed characterization of human isolates and clinical samples in Argentina through serotyping, 16S rRNA and MLST. Moreover, for the first time, species of intermediate pathogenicity (*L. wolffii* and *L. broomii*) were isolated in symptomatic patients. This finding suggests the need for future investigations concerning the pathogenic capacity of intermediate strains. The correlation observed between the STs of isolates and serotyping supports the implementation of MLST as a complementary approach towards the classification of *Leptospira* strains on a global scale.

MLST was successful on 21.6% of serum samples. According to our results, MLST should be applied in serum samples with leptospiremia higher than 5×10^4 leptospires/ml.

This study is the first report on using an MLST typing scheme with seven loci to identify *Leptospira* from clinical samples in Argentina. The advantage of using clinical samples is the possibility of identifying the infective strain without the need to culture the bacteria. Identification of the strain is important for knowing the epidemiology and probable reservoirs, and for prevention of outbreaks of the disease.

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Table 3
16S rRNA and MLST results of clinical samples. No amplification is indicated (−).

Clinical Sample N°	Year	Species by16S rRNA	ST by MLST	Allelic profile							Probably serogroup (MLST)	*Leptospira*/ml
				glmU	pntA	sucA	tpiA	pfkB	mreA	caiB		
271	2013	*L. interrogans*	13	1	1	1	5	12	2	9	Pyrogenes	$3.5\ 10^5$
273	2013	*L. interrogans*	13	1	1	1	5	12	2	9	Pyrogenes	$5.8\ 10^6$
282	2012	*L. interrogans*	13	1	1	1	5	12	2	9	Pyrogenes	$1.6\ 10^5$
388	2012	*L. interrogans*	13	1	1	1	5	12	2	9	Pyrogenes	$8.3\ 10^4$
389	2012	*L. interrogans*	13	1	1	1	5	12	2	9	Pyrogenes	$5.7\ 10^4$
157	2012	*L. interrogans*	20*	1	1	2	1	-	-	3	Sejroe	$1.8\ 10^4$
178	2010	*L. interrogans*	20*	1	1	2	1	-	4	-	Sejroe	$1.9\ 10^3$
337	2011	*L. interrogans*	20*	1	1	2	1	-	4	3	Sejroe	$4.2\ 10^3$
386	2012	*L. interrogans*	20*	1	1	2	1	-	4	3	Sejroe	$2.9\ 10^2$
414	2013	*L. interrogans*	22*	-	1	3	1	-	6	-	Autumnalis	$2.8\ 10^2$
401	2013	*L. interrogans*	37*	-	3	3	3	-	-	5	Canicola	$1.9\ 10^3$

* Most probably ST due to incomplete allelic profile.

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