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Abstract

Leptospirosis is the most common zoonotic disease and is endemic worldwide. The antibiotic susceptibilities of *Leptospira* isolated from both humans and animals are poorly documented. This issue is particularly important for isolates from food-producing animals which are regularly exposed to antibiotic treatments. In this study, we assess the susceptibility of 35 *Leptospira* strains isolated from food-producing animals of diverse geographical origins between 1936 and 2016 to the antimicrobial agents most commonly used in animals. We used a broth microdilution method to determine the susceptibilities of *Leptospira* strains isolated from livestock to 11 antibiotics. All the isolates were susceptible to penicillin, amoxicillin, clavulanate, cephalexin, ceftriaxone, doxycycline, tetracycline, streptomycin, enrofloxacin, and spectinomycin, but not polymyxin (MIC ≥4mg/L). For tetracycline and doxycycline, the MIC was significantly higher for the recent isolates from Sardinia, Italy than for the other isolates. Antimicrobial susceptibilities were also determined with 10- and 100-fold-higher inocula. High inocula significantly diminished the antibacterial effect by at least ten-fold for enrofloxacin (MIC ≥256mg/L), streptomycin (MIC ≥16mg/L), and tetracycline (MIC ≥32mg/L) suggesting selection of resistant strain for high inoculum. Our findings contribute to the assessment of whether certain antibiotics are potentially useful for the treatment of leptospirosis, and point out the risk of failure for some antibiotics during infection with a high inoculum in both animals and humans. This study strengthens the need to detect and prevent the emergence of antimicrobial resistance of this major emerging zoonotic pathogen.

Keywords : *Leptospira ;* Leptospirosis ; Antibiotic susceptibility ; Livestock ; MIC ; High inoculum

1. Introduction

Leptospirosis is the most common bacterial zoonosis worldwide, although it is most prevalent in tropical and rural environment. There are estimated to be more than one million severe cases of leptospirosis with approximately 60,000 deaths per year [1]. Its largest reservoirs are rodents which are asymptomatic carriers of pathogenic *Leptospira*; and contact with urine from carrier animals, often through contaminated water, is the primary route of transmission to humans. Clinical manifestations of leptospirosis are diverse, ranging from flu-like symptoms to a life-threatening hepato-renal syndrome [2]. Diagnosis of leptospirosis is often difficult because of non-specific clinical manifestations, mimicking other febrile illnesses. In addition, in many endemic areas there is poor access to reference laboratories or rapid diagnostic tests.

Suspected and confirmed cases of leptospirosis are usually treated with antibiotics [3,4], using intravenous penicillin or cephalosporins of third generation for patients with severe leptospirosis and oral agents such as doxycycline or amoxicillin for milder cases. Fluoroquinolone and aminoglycoside antibiotics may also be effective. However, there is controversy over whether antibiotics decrease the severity of the disease. A review of seven randomized trials showed that the duration of the disease appeared to be shorter among patients treated with antibiotics than among those who did not receive antibiotics, but the effects on mortality were not clear [5]. In a retrospective observational study, delayed initiation of antibiotics was associated with more severe disease [6]. Although some studies in hamsters have shown a statistically significant survival advantage in the group of animals treated with antibiotics [7,8], subtherapeutic use of norfloxacin and ciprofloxacin reduced the survival rate in hamsters infected with pathogenic *Leptospira* [10]. In C57BL/6 mice, live imaging of bioluminescent leptospires demonstrates that antibiotic treatment with penicillin, ciprofloxacin, and doxycycline is effective if administered early post-infection but failed to eliminate all the leptospires from the renal tubules during the chronic phase of leptospirosis [9].

Leptospirosis also affects animals other than rodents and in particular food-producing animals, including livestock. The disease is therefore of economic importance. Bovine leptospirosis occurs worldwide and is caused by a wide variety of *Leptospira* serovars. Chronic leptospirosis in cattle is associated with abortion, stillbirth, premature birth and loss of milk production. Leptospirosis is also common in pigs can be a significant cause of reproductive loss [11]. Antibiotics are given to farm animals in order to reduce the number of infected animals, preventing, for example, abortions, and to minimize the urinary shedding and transmission between animals. A combination of penicillin and streptomycin or tetracycline have been the treatment of choice for the treatment of leptospirosis, but ampicillin and third generation cephalosporins have also been used [12].

Antibiotics are also used in agricultures for purposes other than the treatment of infections. Antibiotics are fed to food-producing animals to promote growth and improve feed efficiency. Tetracyclines, for example, have been widely used as a feed supplement. Recent projections indicate that global antimicrobial consumption in food animals will grow by at least 67% by 2030 [12], and significant fraction of this involves antimicrobials use for food production. This overuse of antibiotics in animals can contribute to the emergence of antimicrobial resistance in bacteria that may be subsequently transferred to humans [13].

Data on antimicrobial susceptibilities of *Leptospira* isolates are scarce. This is mainly due to the difficulty of isolating the slow-growing *Leptospira* from biological samples. A standardized microdilution technique has been developed for *Leptospira* for the assessment of antibiotic susceptibility [14]. This technique has been used for the evaluation of MIC for strains isolated from patients, rats, dogs, or swine [15-22]. These previous studies showed that *Leptospira* strains are susceptible to current antibiotics of choice for leptospirosis treatment but resistant to trimethoprim, sulfamethoxazole, and neomycin; these findings allowed the development of a new selective medium for the isolation of *Leptospira*, preventing growth of contaminants [23].

In the current context of antibiotic pressure and regular chronic infections, monitoring of *Leptospira* strains in livestock may help detect the emergence of antimicrobial resistance. The aim of this study was to evaluate the antibiotic susceptibility of *Leptospira* strains isolated from livestock and help in the identification of resistant strains when isolates grow at antibiotic concentrations higher than the range seen for wild-type strains.

2. Materials and Methods

2.1. Strains and culture conditions

Leptospira strains were selected from the collection of the "Biology of Spirochetes" unit which comprises reference strains and strains received at the French National Reference Center for Leptospirosis (Institut Pasteur, Paris) for identification and characterization. Other strains were obtained from Sardinia, Italy (N. Ponti, Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy) and Uruguay (A. Buschiazzo, on behalf of the multicentric consortium Grupo de Trabajo Interinstitucional de Leptospirosis, Institut Pasteur Montevideo/UdelaR/INIA/MGAP, Uruguay).. Strains were cultivated in in-house made liquid Ellinghausen-McCullough-Johnson-Harris (EMJH) medium or on 1% agar plates at 30°C. Isolates were characterized by 16S rRNA sequencing and microscopic agglutination test (MAT) using a standard battery of rabbit antisera against reference serovars representing 24 serogroups. Our final collection was composed of 35 *Leptospira* strains, representing six species and nine serogroups (Table 1). The strains were isolated from cattle (19 strains), swine (10 strains), donkey (one strain), deer (one strain), and human patients (3 strains; associated with contact with cattle and/or prevalent in cattle) between 1936 and 2016 and came from different areas: Europe (13 strains), South America (11 strains), Asia and Oceania (6 strains) and United States (3 strains).

2.2. Antibiotics

Penicillins (penicillin G, amoxicillin, clavulanate), first and third generation cephalosporin (cephalexin, ceftriaxone), aminoglycosides (streptomycin, spectinomycin), cyclines (tetracycline, doxycycline), fluoroquinolone (ernofloxacin) and polypeptide (polymyxin E) were purchased from Sigma-Aldrich. All but ceftriaxone which is used as a therapeutic in humans are used in farms. Antibiotics were stocked in a stock solution at a concentration of 25mg/mL according to the manufacturer's suggestions. The stock solutions of antibiotics were preserved at 4°C and renewed every two weeks.

2.3. MIC Determination

The MIC was determined for all antibtiotics by the broth microdilution method described by Murray and Hospenthal [14]. Briefly, exponential-phase cultures of *Leptospira* were counted in a Petroff-Hausser counting chamber (Fisher Scientific) and *Leptospira* was deposited at a final concentration of $2x10^6$ leptospires/mL in each well of 96 well plates (Techno Plastic Product) with serial two-fold dilutions of antibiotics ranging from 32 to 0.016 mg/L in EMJH media; the final volume in each well was 200µL. The plates were incubated for 3 days at 30°C, then 20µl of Alamar Blue® (Invitrogen, Thermo Fisher Scientific) was added in each well and the samples incubated at 30°C for 2 days: any change in color from blue to pink was considered to indicate cell growth. The minimal inhibitory concentration (MIC) was recorded as the concentration in the well containing the lowest concentration without a blue to pink color change. Each strain-drug combination was tested in duplicate and positive (bacteria and no antibiotic added) and negative (no bacteria added) controls were included in each plate. We also determined MICs for two strains with inocula 10^{-1}-, 10- and 100-times the standard *Leptospira* cell density to evaluate the inoculum effect : thus, inocula of $2x10^5$, $2x10^7$, and $2x10^8$ *Leptospira* /ml were tested as described above. These strains are two representative strains of our collection for which we can obtain high cell density cultures (> 10^8 leptospires/mL).

2.4. Statistical analysis

Strain characteristics in this study were described using frequencies and medians with interquartile (IQR) ranges. Univariable linear regression analysis was conducted to investigate factors associated with an increase of MIC compare. Odds ratios (OR) with 95% confidence intervals [95%CI] were calculated. Statistical significance was defined as a P-value < 0.05 (two-tailed). Statistical analyses were performed with the R version 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1. Antibiotic susceptibility

MICs to 11 diverse antimicrobial agents were determined. They include penicillins (penicillin G, amoxicillin, clavulanate), first and third generation cephalosporins (cephalexin, ceftriaxone), aminoglycosides (streptomycin, spectinomycin), cyclines (tetracycline, doxycycline), fluoroquinolone (ernofloxacin) and polypeptide (polymyxin E). Distribution and median MIC are reported in Figure S1 and Table 2.

All strains were susceptible to amoxicillin, clavulanate and cefalexin with median MICs of 0.02 (IQR [0.02-0.03]), 0.5 (IQR [0.25-0.5]) and 4 mg/L (IQR [3-4]), respectively. Strains were generally very susceptible to penicillin G with a median MIC of 0.06 mg/L (IQR [0.03-0.12]) although one strain recently isolated from swine in Sardinia (N°21) had a MIC of 1 mg/L; this strain and a strain isolated in the United States (N°18) had a high ceftriaxone MIC of 1 mg/L. All the others strains were very susceptible to ceftriaxone. Five of the eight strains recently isolated in Sardinia had a high MIC for cyclines; three strains had a tetracyline MIC of 4 mg/L (N°20, N°21, N°22); one strain had a doxycycline MIC of 2 mg/L (N°26) and one strain had both tetracycline MIC of 4 mg/L and a doxycycline MIC of 2 mg/L (N°7). One strain recently isolated from cattle in Uruguay also had a tetracycline MIC of 4 mg/L (N°27). The medians MIC for enrofloxacin was 1 mg/L (IQR [0.5-1]) and no strains had a MIC above to 1 mg/L. Spectinomycin and streptomycin were effective against all isolates. All the strains seemed to be naturally resistant to polymyxin B with a median MIC of 8 mg/L (IQR [4-8]), which is higher than the MIC breakpoint of this antibiotic (24).

3.2. Factors associated with high MIC in Leptospira isolates

In univariate analysis, susceptibility to antibiotics was generally similar for the three main species of *Leptospira* (Table 3). Although *L. interrogans* had lower MIC to clavulanate than the others strains (OR=0.8, 95% IC [0,6-0,9]), the overall susceptibility to antibiotics of *Leptospira* strains was independent of the species. MICs to streptomycin (OR=4.3, 95% IC

[1.4-13.1]) and polymyxin (OR=610, 95% IC [43.9-8466.6]) were higher in strains isolated from swine than cattle (Table 3). Median MICs did not differ greatly according to the continent of isolation (Table 3). Strains isolated in 2016 presented higher MICs for tetracycline (OR=4.0, 95% IC [1.8-8.7] and spectinomycin (OR=24.1, 95% IC [2-295.6]) than strains isolated earlier (Table 3). The pooled MIC to cyclines of the Sardinia strains is 2.8 times higher than the others strains (OR = 2.8, 95% CI [1.8-4.5]) (Figure 1) and was very close to the MIC breakpoint of cyclines (24). In total, five strains isolated form Sardinia (N°7, N°20, N°21, N°22, N°26) and one from Uruguay showed decreased susceptibility to cyclines (Table S1).

3.3. Impact of high inoculum density on antibiotic susceptibility

To further investigate the effectiveness of antibiotics, we also determined MICs with lower and higher inocula (from $2x10^5$ to $2x10^8$ *Leptospira* per mL) for two representative strains or our collection (Table 4). The observed MIC of most antibiotics increased with inoculum density. Amoxicillin was the only antibiotic for which the size of the inoculum had no impact on MIC values. The MICs of other beta-lactams increased gradually with inoculum density, but remained below the MIC breakpoint of these antibiotics (24) suggesting effectiveness even for large inocula. The MICs of doxycycline and tetracycline were at least 8-fold higher for inoculum densities of $2x10^7$ bacteria per mL or more than for the standard inoculum. The MICs of enrofloxacin, streptomycin and spectinomycin were higher for high inocula: in particular the MICs of enrofloxacin and streptomycin were ≥256 mg/L at an inoculum of $2x10^8$ *Leptospira* per mL. This pattern differs from that for cephalexin, ceftriaxone, polymyxin, penicillin, clavulanate, and spectinomycin for which the MICs increased only slowly with inoculum density (Table 4).

4. Discussion

Veterinary use of antimicrobials is believed to contribute to the emergence of antimicrobial-resistant strains. Pathogenic *Leptospira* is responsible for chronic infections in livestock leading to abortions and other reproduction disorders. Some serovars are particularly associated with chronic infection, for example Hardjobovis in cattle and Pomona in swine. Re-infections of animals in the same farm are frequent [11]. There is currently no comprehensive data about the antibiotic susceptibility of *Leptospira* strains in farm animals. Evaluating the antibiotic susceptibility of *Leptospira* strains in livestock provides a baseline for monitoring trends in antimicrobial resistance in this species. We therefore analyzed susceptibilities of strains isolated worldwide over an 80-year period to the antibiotics most commonly used in veterinary medicine. Our findings are consistent with prior reports of *Leptospira* susceptibility to penicillin, amoxicillin, cephalexin, ceftriaxone, doxycycline, tetracycline, streptomycin, spectinomycin, and polymyxin [18-20,25]. Previous studies showed low enrofloxacin and its active metabolite ciprofloxacin MIC values (≤0.6 mg/L) [15, 17-19], but most of our isolates showed a MIC of 1 mg/L. Similarly, *L. interrogans* serovar Pomona isolates from swine in Brazil were found to be resistant to fluoroquinolones (enrofloxacin MIC > 4mg/L) [26]. We report the good *in vitro* susceptibility of *Leptospira* strains to the beta-lactamase inhibitor clavulanate (IQR [0.25-0.5]) and this is a novel finding. MICs were not significantly different between *Leptospira* species, sources (cattle, swine or others), or place of isolation (countries and continents). However, the MICs were higher for the most recent isolates; mostly recovered from Sardinia in Italy. Strains isolated in Sardinia had high MICs to cyclines. Similar pattern of resistance has been observed on isolates from patients in Egypt [18]; the authors suggested that the substantial exposure of the population to cyclines in this country might explain the high MIC values. We also report one strain (strain 21) with high MICs for ceftriaxone and penicillin G. This resistance profile has already been described in an *L. interrogans* strain isolated from rats in the Philippines [19]. The killing rate of the antibiotics declined with the cell density, but the MICs, for example of beta-lactams, remained below the MIC breakpoint [24], consistent with good efficacy. Amoxicillin presented the lowest MICs in our study and its efficacy was independent of the inoculum size, suggesting that amoxicillin might be a treatment of choice for leptospirosis. In contrast, high inoculum density significantly diminished (at least 10-fold increase of MIC) the antibacterial effects of tetracycline, enrofloxacin, and streptomycin. This inoculum effect may possibly be the consequence of selection and proliferation of spontaneous resistant mutants for these antibiotics in large inocula. However, the inoculum effect is an *in vitro* laboratory phenomenon and should be interpreted with caution. Trailing end points may be seen when bacteriostatic antibiotics such as tetracycline are tested. Nevertheless, our observations suggest that these antibiotics may not be the most reliable agents for therapy in cases of high bacterial load *Leptospira* infections. In animal models *Leptospira* can reach peaks of 10^8 leptospires per gram or per milliliter in blood and all tissues after intraperitoneal inoculation with pathogenic strains [27]. The bacterial load in the blood of patients can exceed 10^6 leptospires/mL, and such high loads are usually associated with poor outcomes [28-30]. *Leptospira* can also form biofilms with high cell densities [31] that can resist antibiotic treatment [32], thereby reducing the effectiveness of antimicrobial drugs for treating human disease. The standard method for assessing the *in vitro* activity of antimicrobial agents against *Leptospira* species might not be efficient for predicting the therapeutic efficacy of antibiotics.

Further genomic exploration is required to elucidate the molecular mechanisms responsible for antimicrobial resistance in *Leptospira*. Only very few plasmids have been isolated so far in pathogenic *Leptospira* and they do not carry genes conferring resistance [33,34]. In addition, *Leptospira* are not naturally competent for transformation. The ability of *Leptospira* to acquire resistance genes such as modifying- or degrading-enzymes by horizontal transfer is thus probably limited. Alteration in the target sites of antibiotics may therefore be the major cause of antibiotic resistance in *Leptospira*. It has been shown experimentally that *in vitro* selection can result in the development of resistance to spectinomycin and streptomycin in *Leptospira* due to spontaneous mutation of the target gene 16S rRNA and *rpsL*, respectively [35,36].

It would be useful to analyze a larger collection of *Leptospira* strains of different origins to determine more precisely the epidemiological cut-off (ECOFF) value which is the MIC value at the upper limit of the wild-type population [37]. This is particularly important because there is actually no clearly established correlation between clinical breakpoints and clinical outcome of antimicrobial agents in leptospirosis.

5. Conclusion

There appears to be a heterogeneity of MICs to some antibiotics among *Leptospira* strains isolated from food-producing animals of diverse geographical origins between 1936 and 2016. Substantial inoculum effects on efficacy were also observed for at least five antibiotics and point out the risk of failure for some antibiotics during infection with high inoculum in both animals and humans. Additional studies are required to better discriminate between susceptible and resistant *Leptospira* strains.

Acknowledgments

We thank the consortium "Grupo de Trabajo Interinstitucional de Leptospirosis" (Institut Pasteur Montevideo/UdelaR/INIA/MGAP, Uruguay) and Nicoletta Ponti for providing *Leptospira* strains. Thanks are also due to the technicians of the French National Reference Center for Leptospirosis for culture and identification of strains.

Declarations

Funding: This work was supported by a grant from French ministry of health (Agence régionale de Santé of Guadeloupe) to G.L. and by Institut Pasteur and Santé Publique France (Centre National de Référence de la Leptospirose).

Competing interests: No conflict

Ethical Approval: Not required

Appendix: Supplementary data

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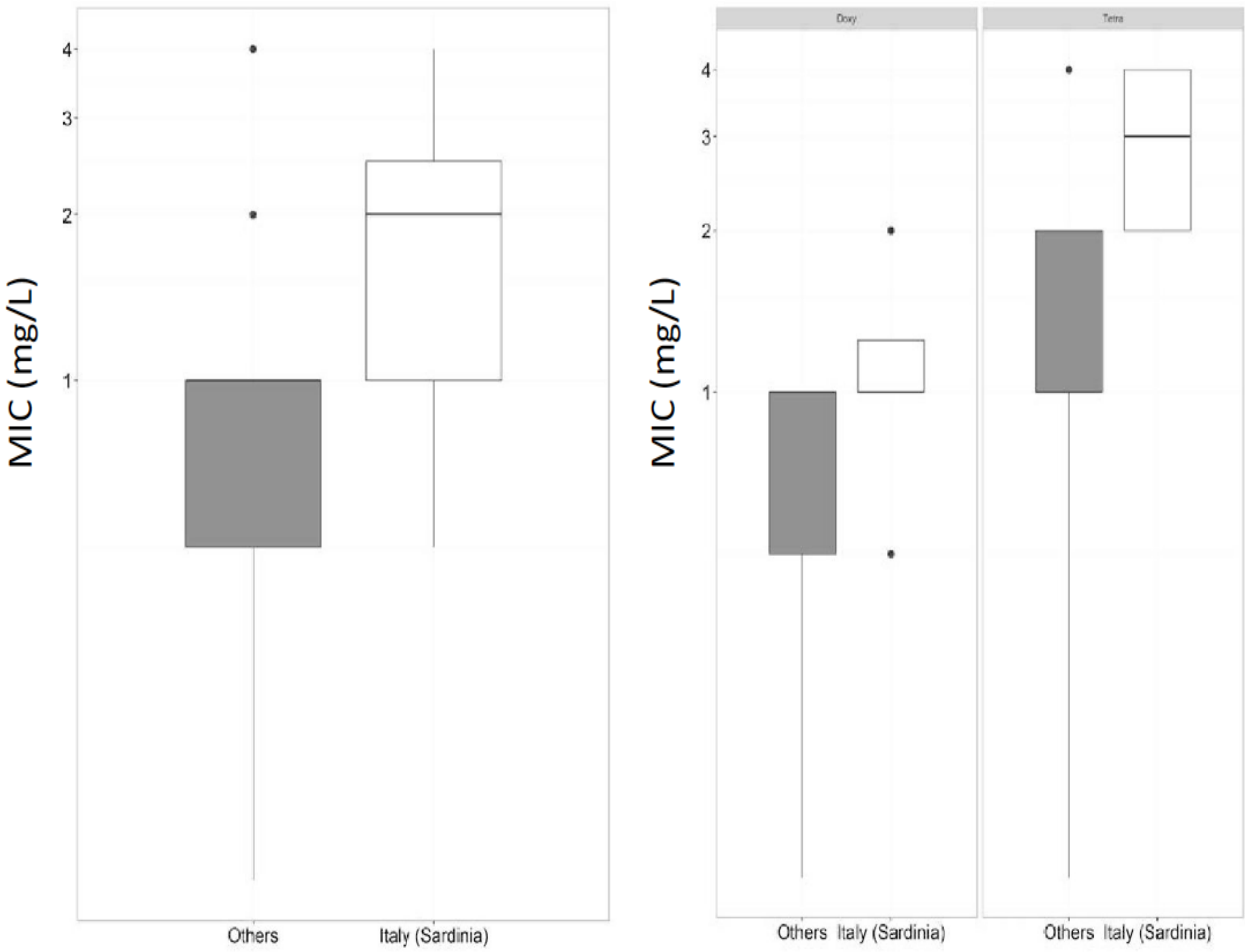
Figure 1. Box plot of MIC for cyclines for strains isolated in Italy (Sardinia)

MICs of strains isolated in Sardinia, Italy was compared with other strains for cyclines, including pooled MICs for doxycycline and tetracycline (A) and individually for each antibiotic doxycycline (Doxy) and Tetracycline (Tetra) (B).

Boxes encompass all data points between the 25th and 75th percentiles (interquartile range, IQR). Thick bars in boxes indicate the median data value. The vertical bar indicates the maximum/minimum values. Data points outside this range ('outliers') are plotted individually as dots.



MIC (mg/L)
4
3
2
1
Others
Italy (Sardinia)
Doxy
Tetra
MIC (mg/L)
4
3
2
1
Others
Italy (Sardinia)
Others
Italy (Sardinia)

Tables

Table 1. *Leptospira* strains used in this study

Strains	Species	Source	Serogroups	Location	Isolation date	Strains
1	*L. borgpetersenii*	Swine	Tarrassovi	Brazil	1948	RP29
2	*L. borgpetersenii*	Cattle	Sejroe	England	1980	Hardjo
3	*L. borgpetersenii*	Human[1]	Sejroe	Australia	1999	L550
4	*L. borgpetersenii*	Swine	Tarrassovi	Brazil	1993	PC 10
5[2]	*L. borgpetersenii*	Cattle	Sejroe	Uruguay	2016	IP1512012
6	*L. borgpetersenii*	Swine	Tarrassovi	China	1986	L100
7	*L. borgpetersenii*	Swine	Tarrassovi	Italy	2015	27562
8	*L. borgpetersenii*	Cattle	Sejroe	Spain	2000	P399
9	*L. broomii*	Breeding deer	Unknown	New Caledonia	1988	10840
10	*L. interrogans*	Swine	Unknown	Brazil	1993	PC 33
11	*L. interrogans*	Cattle	Pomona	USA	1965	164
12	*L. interrogans*	Human[1]	Sejroe	Indonesia	1938	Hardjoprajitno
13	*L. interrogans*	Cattle	Sejroe	Ireland	1980	S.80.1441
14	*L. interrogans*	Swine	Pomona	Croatia	2002	200205138
15	*L. interrogans*	Human[1]	Pomona	Australia	1936	Pomona
16	*L. interrogans*	Swine	Pomona	New Caledonia	1991	LTDV 5
17	*L. interrogans*	Cattle	Australis	Spain	2000	P399
18	*L. interrogans*	Cattle	Pomona	USA	1964	LT1026
19	*L. interrogans*	Cattle	Pomona	New Caledonia	1988	D10613
20	*L. interrogans*	Cattle	Unknown	Italy	2016	75022-5
21	*L. interrogans*	Swine	Unknown	Italy	2016	71516-5
22	*L. interrogans*	Swine	Pomona	Italy	2016	71516-1
23	*L. interrogans*	Cattle	Pomona	Italy	2016	77219-2
24	*L. interrogans*	Cattle	Pomona	Italy	2016	77219-3
25	*L. interrogans*	Cattle	Pomona	Italy	2016	77219-4
26	*L. interrogans*	Donkey	Pomona	Italy	2016	85282
27[2]	*L. interrogans*	Cattle	Pomona	Uruguay	2016	IP1507003
28	*L. kirschneri*	Cattle	Canicola	Argentina	1969	LT1014
29	*L. kirschneri*	Cattle	Grippotyphosa	Turkey	1990	Daclas 1
30	*L. noguchii*	Cattle	Panama	Brazil	2013	U73
31	*L. noguchii*	Cattle	Australis	Peru	1962	V42
32[2]	*L. noguchii*	Cattle	Unknown	Uruguay	2016	IP1512017
33[2]	*L. noguchii*	Cattle	Pyrogenes	Uruguay	2016	IP1605021
34[2]	*L. noguchii*	Cattle	Australis	Uruguay	2016	IP1611024
35	*L. santarosai*	Cattle	Mini	USA	1974	Oregon

[1]Strains isolated from human after close contact with cattle breeding animals, [2]Species identification and serogrouping communicated by the Grupo de Trabajo Interinstitucional de Leptospirosis (Uruguay) (manuscript under preparation).

Table 2. Distribution of MIC for 11 antimicrobial agents among the 35 *Leptospira* strains.

Antimicrobial agent	No. of strains with MIC (mg/L) of:											Median MIC [IQR]
	<0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	
Penicillin	3	7	13	6	4	1	1					0.06 [0.03-0.12]
Amoxicillin	24	8	3									0.02 [0.02-0.03]
Clavulanate				2	9	21	3					0.5 [0.25-0.5]
Cephalexin							1	8	24	2		4 [3-4]
Ceftriaxone		2	4	13	8	6	2					0.12 [0.12-0.25]
Doxycycline				1	3	11	18	2				1 [0.5-1]
Tetracycline				1		5	9	15	5			2 [1-2]
Enrofloxacin				2	3	12	18					1 [0.5-1]
Spectinomycin							2	12	8	11	2	4 [2-8]
Streptomycin						4	7	16	7	1		2 [1-2]
Polymyxin E								1	10	17	7	8 [4-8]

Table 3. Characteristics of *Leptospira* strains by MIC**.**
Associations are reported using Odds Ratio (OR) and their 95% confidence intervals [95%CI]. Values in bold indicate significant associations in univariate analysis

Variable	n (%)	Penicillin	Amoxicillin	Clavulanate	Cefalexin	Ceftriaxone	Doxycycline	Tetracycline	Enrofloxacin	Spectinomycin	Streptomycin	Polymyxin E
Period												
1936-1987 (ref) [a]	11 (31%)	-	-	-	-	-	-	-	-	-	-	-
1988-2015	12 (34%)	1 [0.9-1.1]	1 [1-1]	**0.8 [0.7-1]**	1.9 [0.6-6.2]	0.9 [0.8-1.2]	0.9 [0.7-1.3]	1.3 [0.6-2.8]	0.8 [0.6-1.1]	0.1 [0-1.1]	2.8 [0.8-9.4]	0.2 [0-6.4]
≥2016	12 (34%)	**1.2 [1-1.3]**	1 [1-1.1]	**0.8 [0.7-1]**	1.8 [0.5-5.7]	1 [0.8-1.2]	1.3 [0.9-1.8]	**4 [1.8-8.7]**	0.9 [0.7-1.2]	**24.1 [2-296]**	1.3 [0.4-4.3]	0.1 [0-2.8]
Continent												
America (ref)	14 (40%)	-	-	-	-	-	-	-	-	-	-	-
Oceania / Asia	7 (20%)	1 [0.8-1.1]	1 [1-1]	0.9 [0.8-1.1]	1.8 [0.5-6.5]	0.9 [0.7-1.1]	0.9 [0.6-1.4]	0.8 [0.3-2.1]	1.3 [1-1.7]	0.6 [0-19.4]	0.5 [0.1-1.9]	1.8 [0-96]
Europe	14 (40%)	1 [0.9-1.2]	1 [1-1]	0.9 [0.8-1.1]	2 [0.7-5.9]	0.9 [0.8-1.1]	1.2 [0.9-1.6]	2 [0.9-4.4]	1.2 [1-1.5]	1.2 [0.1-20.7]	0.4 [0.1-1.2]	1 [0-26]
Strains from Sardinia												
Others (ref) [b]	27 (77%)	-	-	-	-	-	-	-	-	-	-	-
Italy / Sardinia	8 (23%)	1.1 [1-1.3]	1 [1-1.1]	1 [0.8-1.1]	1.5 [0.5-4.7]	1 [0.8-1.2]	**1.6 [1.2-2.1]**	**5 [2.5-10]**	1.2 [0.9-1.5]	**22.9 [1.3-392]**	0.7 [0.2-2.2]	4.2 [0.1-124]
Animals												
Cattle (ref)	21 (60%)	-	-	-	-	-	-	-	-	-	-	-
Swine	9 (26%)	1.1 [0.9-1.3]	1 [1-1]	1.1 [0.9-1.3]	2 [0.7-5.9]	1 [0.9-1.3]	1.2 [0.9-1.6]	1.3 [0.5-3.2]	1 [0.8-1.3]	0.2 [0-4.1]	**4.3 [1.4-13.1]**	609.5 [43.9-8467]
Others [c]	5 (14%)	1 [0.8-1.2]	1 [1-1]	1.1 [0.9-1.3]	**4.5 [1.2-17.1]**	1 [0.8-1.3]	1.3 [0.9-1.9]	0.8 [0.3-2.4]	**1.4 [1-1.9]**	2.9 [0.1-118.9]	1.5 [0.4-6.2]	6.8 [0.3-183]
Species												
Others (ref) [d]	4 (11%)	-	-	-	-	-	-	-	-	-	-	-
L. interrogans	18 (51%)	1.1 [0.9-1.3]	1 [1-1.1]	**0.8 [0.6-0.9]**	0.5 [0.1-2.2]	0.9 [0.7-1.2]	1 [0.6-1.5]	2.8 [0.9-9.2]	0.9 [0.7-1.3]	16.5 [0.4-768]	1.9 [0.4-9.3]	5.6 [0.1-501]
L. borgpetersenii	8 (23%)	1 [0.8-1.2]	1 [1-1.1]	1 [0.8-1.2]	0.3 [0.1-1.6]	0.8 [0.6-1.1]	1 [0.6-1.7]	2.3 [0.6-8.4]	1.2 [0.8-1.7]	0.3 [0-20.1]	5.8 [1-34.2]	90 [0.6-13 072]
L. noguchii	5 (14%)	1.1 [0.9-1.4]	1 [1-1.1]	0.8 [0.6-1]	0.4 [0.1-2.7]	1 [0.7-1.3]	0.8 [0.5-1.5]	1.3 [0.3-5.7]	0.7 [0.5-1.1]	3.5 [0-368]	2.1 [0.3-14.5]	0.9 [0-211.3]
Serogroups												
Others (ref) [e]	23 (66%)	-	-	-	-	-	-	-	-	-	-	-
Pomona	12 (34%)	0.9 [0.8-1.1]	1 [1-1]	0.9 [0.8-1]	1.1 [0.4-3]	1 [0.8-1.2]	1.1 [0.9-1.5]	1.4 [0.6-2.9]	1.1 [0.9-1.4]	11.1 [0.9-143]	1.1 [0.4-3.2]	0.1 [0-1.3]

a Two strains with unknown identification date were included in this category
b Including: Argentina (1), Australia (2), Brazil (4), China (1), Croatia (1), England (1), Indonesia (1), Ireland (1), New Caledonia (3), Peru (1), Spain (2), Turkey (1), Uruguay (5), USA (3)
c Including : Breeding deer (1), Donkey (1), Human (3)
d Including: *L. broomii* (1), *L. kirschneri* (2), *L. santarosai* (1)
e Including: serogroup Australis (3), Canicola (1), Grippotyphosa (1), Mini (1), Panama (1), Pyrogenes (1), Sejroe (6), Tarrassovi (4), Unknown (5)

Table 4. Variation of MIC according to the size of the inoculum

Antibiotics	Concentration of *Leptospira* (bacteria per mL)							
	L. borgpetersenii (strain n°6)				*L. noguchi* (strain n°34)			
	$2x10^5$	$2x10^6$	$2x10^7$	$2x10^8$	$2x10^5$	$2x10^6$	$2x10^7$	$2x10^8$
Penicillin	0.016	0.032	0.125	0.25	0.125	0.25	0.5	1
Amoxicillin	<0.016	<0.016	<0.016	<0.016	<0.016	<0.016	<0.016	<0.016
Clavulanate	0.25	0.5	1	4	0.25	0.5	2	4
Cephalexin	2	4	8	16	2	4	8	16
Ceftriaxone	0.064	0.125	0.25	0.5	0.125	0.5	0.5	1
Doxycycline	0.5	1	8	16	0.25	0.5	4	16
Tetracycline	0.5	1	32	64	0.5	2	8	64
Enrofloxacin	0.25	0.5	256	512	0.25	0.5	1	512
Spectinomycin	1	4	8	32	4	8	16	32
Streptomycin	1	2	16	512	1	2	8	256
Polymyxin E	4	8	16	16	2	4	4	4

Figure

Figure 1