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1 **Antibiotic susceptibilities of livestock isolates of *Leptospira***

2

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25

26 **Running head:** Antibiotic Susceptibilities of *Leptospira* strains

27

28 **Abstract**

29 Leptospirosis is the most common zoonotic disease and is endemic worldwide. The antibiotic
30 susceptibilities of *Leptospira* isolated from both humans and animals are poorly documented.
31 This issue is particularly important for isolates from food-producing animals which are
32 regularly exposed to antibiotic treatments. In this study, we assess the susceptibility of 35
33 *Leptospira* strains isolated from food-producing animals of diverse geographical origins
34 between 1936 and 2016 to the antimicrobial agents most commonly used in animals. We
35 used a broth microdilution method to determine the susceptibilities of *Leptospira* strains
36 isolated from livestock to 11 antibiotics. All the isolates were susceptible to penicillin,
37 amoxicillin, clavulanate, cephalexin, ceftriaxone, doxycycline, tetracycline, streptomycin,
38 enrofloxacin, and spectinomycin, but not polymyxin (MIC ≥ 4 mg/L). For tetracycline and
39 doxycycline, the MIC was significantly higher for the recent isolates from Sardinia, Italy than
40 for the other isolates. Antimicrobial susceptibilities were also determined with 10- and 100-
41 fold-higher inocula. High inocula significantly diminished the antibacterial effect by at least
42 ten-fold for enrofloxacin (MIC ≥ 256 mg/L), streptomycin (MIC ≥ 16 mg/L), and tetracycline (MIC
43 ≥ 32 mg/L) suggesting selection of resistant strain for high inoculum. Our findings contribute to
44 the assessment of whether certain antibiotics are potentially useful for the treatment of
45 leptospirosis, and point out the risk of failure for some antibiotics during infection with a high
46 inoculum in both animals and humans. This study strengthens the need to detect and
47 prevent the emergence of antimicrobial resistance of this major emerging zoonotic pathogen.

48
49 **Keywords :** *Leptospira* ; Leptospirosis ; Antibiotic susceptibility ; Livestock ; MIC ; High inoculum

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55 **1. Introduction**

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

66

67 Suspected and confirmed cases of leptospirosis are usually treated with antibiotics [3,4],
68 using intravenous penicillin or cephalosporins of third generation for patients with severe
69 leptospirosis and oral agents such as doxycycline or amoxicillin for milder cases.
70 Fluoroquinolone and aminoglycoside antibiotics may also be effective. However, there is
71 controversy over whether antibiotics decrease the severity of the disease. A review of seven
72 randomized trials showed that the duration of the disease appeared to be shorter among
73 patients treated with antibiotics than among those who did not receive antibiotics, but the
74 effects on mortality were not clear [5]. In a retrospective observational study, delayed
75 initiation of antibiotics was associated with more severe disease [6]. Although some studies
76 in hamsters have shown a statistically significant survival advantage in the group of animals
77 treated with antibiotics [7,8], subtherapeutic use of norfloxacin and ciprofloxacin reduced the
78 survival rate in hamsters infected with pathogenic *Leptospira* [10]. In C57BL/6 mice, live
79 imaging of bioluminescent leptospires demonstrates that antibiotic treatment with penicillin,
80 ciprofloxacin, and doxycycline is effective if administered early post-infection but failed to
81 eliminate all the leptospires from the renal tubules during the chronic phase of leptospirosis
82 [9].

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

93

94 Antibiotics are also used in agricultures for purposes other than the treatment of infections.
95 Antibiotics are fed to food-producing animals to promote growth and improve feed efficiency.
96 Tetracyclines, for example, have been widely used as a feed supplement. Recent projections
97 indicate that global antimicrobial consumption in food animals will grow by at least 67% by
98 2030 [12], and significant fraction of this involves antimicrobials use for food production. This
99 overuse of antibiotics in animals can contribute to the emergence of antimicrobial resistance
100 in bacteria that may be subsequently transferred to humans [13].

101

102 Data on antimicrobial susceptibilities of *Leptospira* isolates are scarce. This is mainly due to
103 the difficulty of isolating the slow-growing *Leptospira* from biological samples. A standardized
104 microdilution technique has been developed for *Leptospira* for the assessment of antibiotic
105 susceptibility [14]. This technique has been used for the evaluation of MIC for strains isolated
106 from patients, rats, dogs, or swine [15-22]. These previous studies showed that *Leptospira*
107 strains are susceptible to current antibiotics of choice for leptospirosis treatment but resistant
108 to trimethoprim, sulfamethoxazole, and neomycin; these findings allowed the development of
109 a new selective medium for the isolation of *Leptospira*, preventing growth of contaminants
110 [23].

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

116

117

118 **2. Materials and Methods**

119 *2.1. Strains and culture conditions*

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

136

137 *2.2. Antibiotics*

138 Penicillins (penicillin G, amoxicillin, clavulanate), first and third generation cephalosporin
139 (cephalexin, ceftriaxone), aminoglycosides (streptomycin, spectinomycin), cyclines
140 (tetracycline, doxycycline), fluoroquinolone (ernofloxacin) and polypeptide (polymyxin E)
141 were purchased from Sigma-Aldrich. All but ceftriaxone which is used as a therapeutic in
142 humans are used in farms. Antibiotics were stocked in a stock solution at a concentration of
143 25mg/mL according to the manufacturer’s suggestions. The stock solutions of antibiotics
144 were preserved at 4°C and renewed every two weeks.

145

146 2.3. *MIC Determination*

147 The MIC was determined for all antibiotics by the broth microdilution method described by
148 Murray and Hospenthal [14]. Briefly, exponential-phase cultures of *Leptospira* were counted
149 in a Petroff-Hausser counting chamber (Fisher Scientific) and *Leptospira* was deposited at a
150 final concentration of 2×10^6 leptospores/mL in each well of 96 well plates (Techno Plastic
151 Product) with serial two-fold dilutions of antibiotics ranging from 32 to 0.016 mg/L in EMJH
152 media; the final volume in each well was 200 μ L. The plates were incubated for 3 days at
153 30°C, then 20 μ l of Alamar Blue® (Invitrogen, Thermo Fisher Scientific) was added in each
154 well and the samples incubated at 30°C for 2 days: any change in color from blue to pink
155 was considered to indicate cell growth. The minimal inhibitory concentration (MIC) was
156 recorded as the concentration in the well containing the lowest concentration without a blue
157 to pink color change. Each strain-drug combination was tested in duplicate and positive
158 (bacteria and no antibiotic added) and negative (no bacteria added) controls were included in
159 each plate. We also determined MICs for two strains with inocula 10^{-1} -, 10^{-} and 100-times
160 the standard *Leptospira* cell density to evaluate the inoculum effect : thus, inocula of 2×10^5 ,
161 2×10^7 , and 2×10^8 *Leptospira* /ml were tested as described above. These strains are two
162 representative strains of our collection for which we can obtain high cell density cultures (>
163 10^8 leptospores/mL).

164

165 2.4. *Statistical analysis*

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

172

173

174 3. Results

175 3.1. Antibiotic susceptibility

176

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

182 All strains were susceptible to amoxicillin, clavulanate and cefalexin with median MICs of
183 0.02 (IQR [0.02-0.03]), 0.5 (IQR [0.25-0.5]) and 4 mg/L (IQR [3-4]), respectively. Strains were
184 generally very susceptible to penicillin G with a median MIC of 0.06 mg/L (IQR [0.03-0.12])
185 although one strain recently isolated from swine in Sardinia (N°21) had a MIC of 1 mg/L; this
186 strain and a strain isolated in the United States (N°18) had a high ceftriaxone MIC of 1 mg/L.
187 All the others strains were very susceptible to ceftriaxone. Five of the eight strains recently
188 isolated in Sardinia had a high MIC for cyclines; three strains had a tetracycline MIC of 4 mg/L
189 (N°20, N°21, N°22); one strain had a doxycycline MIC of 2 mg/L (N°26) and one strain had
190 both tetracycline MIC of 4 mg/L and a doxycycline MIC of 2 mg/L (N°7). One strain recently
191 isolated from cattle in Uruguay also had a tetracycline MIC of 4 mg/L (N°27). The medians
192 MIC for enrofloxacin was 1 mg/L (IQR [0.5-1]) and no strains had a MIC above to 1 mg/L.
193 Spectinomycin and streptomycin were effective against all isolates. All the strains seemed to
194 be naturally resistant to polymyxin B with a median MIC of 8 mg/L (IQR [4-8]), which is higher
195 than the MIC breakpoint of this antibiotic (24).

196

197 3.2. Factors associated with high MIC in *Leptospira* isolates

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

202 [1.4-13.1]) and polymyxin (OR=610, 95% IC [43.9-8466.6]) were higher in strains isolated
203 from swine than cattle (Table 3). Median MICs did not differ greatly according to the
204 continent of isolation (Table 3). Strains isolated in 2016 presented higher MICs for
205 tetracycline (OR=4.0, 95% IC [1.8-8.7] and spectinomycin (OR=24.1, 95% IC [2-295.6]) than
206 strains isolated earlier (Table 3). The pooled MIC to cyclines of the Sardinia strains is 2.8
207 times higher than the others strains (OR = 2.8, 95% CI [1.8-4.5]) (Figure 1) and was very
208 close to the MIC breakpoint of cyclines (24). In total, five strains isolated from Sardinia (N°7,
209 N°20, N°21, N°22, N°26) and one from Uruguay showed decreased susceptibility to cyclines
210 (Table S1).

211

212 3.3. *Impact of high inoculum density on antibiotic susceptibility*

213 To further investigate the effectiveness of antibiotics, we also determined MICs with lower
214 and higher inocula (from 2×10^5 to 2×10^8 *Leptospira* per mL) for two representative strains or
215 our collection (Table 4). The observed MIC of most antibiotics increased with inoculum
216 density. Amoxicillin was the only antibiotic for which the size of the inoculum had no impact
217 on MIC values. The MICs of other beta-lactams increased gradually with inoculum density,
218 but remained below the MIC breakpoint of these antibiotics (24) suggesting effectiveness
219 even for large inocula. The MICs of doxycycline and tetracycline were at least 8-fold higher
220 for inoculum densities of 2×10^7 bacteria per mL or more than for the standard inoculum. The
221 MICs of enrofloxacin, streptomycin and spectinomycin were higher for high inocula: in
222 particular the MICs of enrofloxacin and streptomycin were ≥ 256 mg/L at an inoculum of
223 2×10^8 *Leptospira* per mL. This pattern differs from that for cephalexin, ceftriaxone, polymyxin,
224 penicillin, clavulanate, and spectinomycin for which the MICs increased only slowly with
225 inoculum density (Table 4).

226

227 4. Discussion

228 Veterinary use of antimicrobials is believed to contribute to the emergence of antimicrobial-
229 resistant strains. Pathogenic *Leptospira* is responsible for chronic infections in livestock

230 leading to abortions and other reproduction disorders. Some serovars are particularly
231 associated with chronic infection, for example Hardjobovis in cattle and Pomona in swine.
232 Re-infections of animals in the same farm are frequent [11]. There is currently no
233 comprehensive data about the antibiotic susceptibility of *Leptospira* strains in farm animals.
234 Evaluating the antibiotic susceptibility of *Leptospira* strains in livestock provides a baseline
235 for monitoring trends in antimicrobial resistance in this species. We therefore analyzed
236 susceptibilities of strains isolated worldwide over an 80-year period to the antibiotics most
237 commonly used in veterinary medicine. Our findings are consistent with prior reports of
238 *Leptospira* susceptibility to penicillin, amoxicillin, cephalexin, ceftriaxone, doxycycline,
239 tetracycline, streptomycin, spectinomycin, and polymyxin [18-20,25]. Previous studies
240 showed low enrofloxacin and its active metabolite ciprofloxacin MIC values (≤ 0.6 mg/L) [15,
241 17-19], but most of our isolates showed a MIC of 1 mg/L. Similarly, *L. interrogans* serovar
242 Pomona isolates from swine in Brazil were found to be resistant to fluoroquinolones
243 (enrofloxacin MIC > 4mg/L) [26]. We report the good *in vitro* susceptibility of *Leptospira*
244 strains to the beta-lactamase inhibitor clavulanate (IQR [0.25-0.5]) and this is a novel finding.
245 MICs were not significantly different between *Leptospira* species, sources (cattle, swine or
246 others), or place of isolation (countries and continents). However, the MICs were higher for
247 the most recent isolates; mostly recovered from Sardinia in Italy. Strains isolated in Sardinia
248 had high MICs to cyclines. Similar pattern of resistance has been observed on isolates from
249 patients in Egypt [18]; the authors suggested that the substantial exposure of the population
250 to cyclines in this country might explain the high MIC values. We also report one strain (strain
251 21) with high MICs for ceftriaxone and penicillin G. This resistance profile has already been
252 described in an *L. interrogans* strain isolated from rats in the Philippines [19]. The killing rate
253 of the antibiotics declined with the cell density, but the MICs, for example of beta-lactams,
254 remained below the MIC breakpoint [24], consistent with good efficacy. Amoxicillin presented
255 the lowest MICs in our study and its efficacy was independent of the inoculum size,
256 suggesting that amoxicillin might be a treatment of choice for leptospirosis. In contrast, high
257 inoculum density significantly diminished (at least 10-fold increase of MIC) the antibacterial

258 effects of tetracycline, enrofloxacin, and streptomycin. This inoculum effect may possibly be
259 the consequence of selection and proliferation of spontaneous resistant mutants for these
260 antibiotics in large inocula. However, the inoculum effect is an *in vitro* laboratory
261 phenomenon and should be interpreted with caution. Trailing end points may be seen when
262 bacteriostatic antibiotics such as tetracycline are tested. Nevertheless, our observations
263 suggest that these antibiotics may not be the most reliable agents for therapy in cases of
264 high bacterial load *Leptospira* infections. In animal models *Leptospira* can reach peaks of 10^8
265 leptospores per gram or per milliliter in blood and all tissues after intraperitoneal inoculation
266 with pathogenic strains [27]. The bacterial load in the blood of patients can exceed 10^6
267 leptospores/mL, and such high loads are usually associated with poor outcomes [28-30].
268 *Leptospira* can also form biofilms with high cell densities [31] that can resist antibiotic
269 treatment [32], thereby reducing the effectiveness of antimicrobial drugs for treating human
270 disease. The standard method for assessing the *in vitro* activity of antimicrobial agents
271 against *Leptospira* species might not be efficient for predicting the therapeutic efficacy of
272 antibiotics.

273

274 Further genomic exploration is required to elucidate the molecular mechanisms responsible
275 for antimicrobial resistance in *Leptospira*. Only very few plasmids have been isolated so far
276 in pathogenic *Leptospira* and they do not carry genes conferring resistance [33,34]. In
277 addition, *Leptospira* are not naturally competent for transformation. The ability of *Leptospira*
278 to acquire resistance genes such as modifying- or degrading-enzymes by horizontal transfer
279 is thus probably limited. Alteration in the target sites of antibiotics may therefore be the major
280 cause of antibiotic resistance in *Leptospira*. It has been shown experimentally that *in vitro*
281 selection can result in the development of resistance to spectinomycin and streptomycin in
282 *Leptospira* due to spontaneous mutation of the target gene 16S rRNA and *rpsL*, respectively
283 [35,36].

284

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

290

291

292

293 **5. Conclusion**

294 There appears to be a heterogeneity of MICs to some antibiotics among *Leptospira* strains
295 isolated from food-producing animals of diverse geographical origins between 1936 and
296 2016. Substantial inoculum effects on efficacy were also observed for at least five antibiotics
297 and point out the risk of failure for some antibiotics during infection with high inoculum in both
298 animals and humans. Additional studies are required to better discriminate between
299 susceptible and resistant *Leptospira* strains.

300

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306

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311 **Competing interests:** No conflict

312 **Ethical Approval:** Not required

313

314 **Appendix: Supplementary data**

315

316

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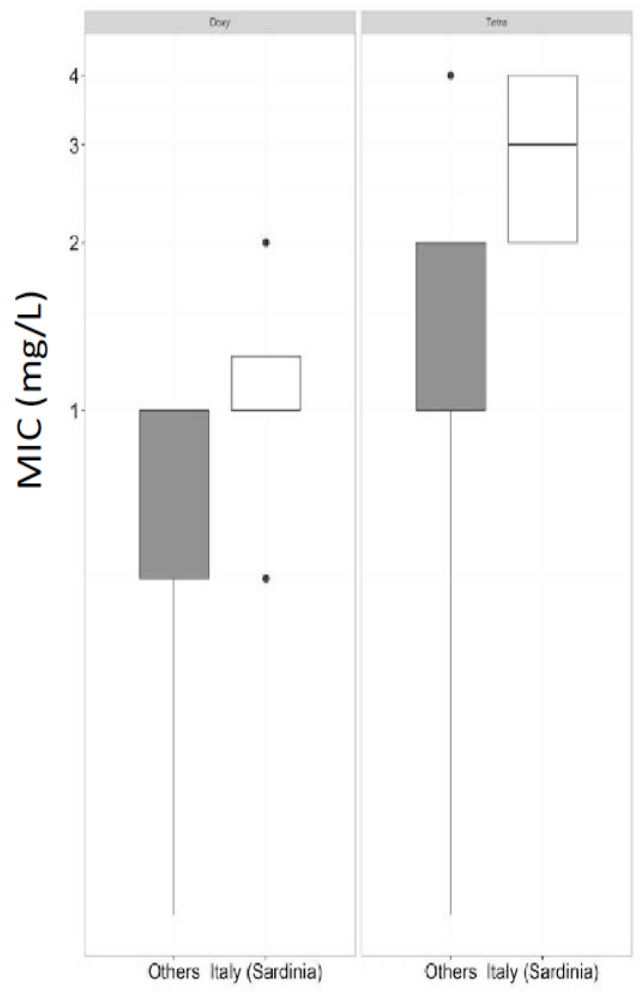
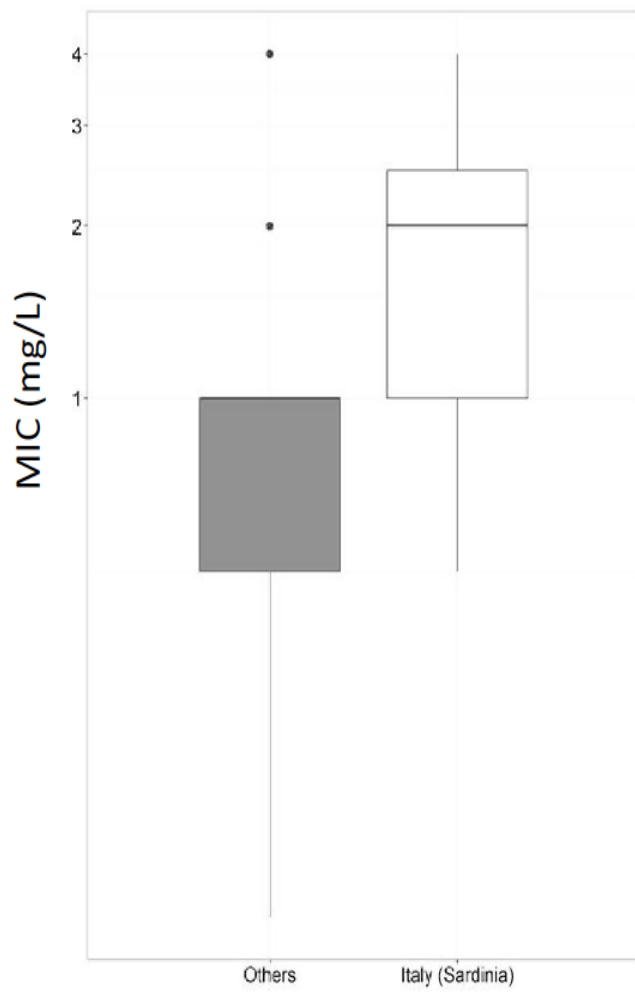
430 **Figure 1. Box plot of MIC for cyclines for strains isolated in Italy (Sardinia)**

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

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

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Tables

Table 1. *Leptospira* strains used in this study

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

¹Strains isolated from human after close contact with cattle breeding animals, ²Species 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	Aminocillin	Clavulanate	Cefalexin	Ceftriaxone	Doxycycline	Tetracycline	Enrofloxacin	Spectinomycin	Streptomycin	Polymyxin E
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 [0-9.6]
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 [0-2.6]
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 [1.2-17.4]	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%)	-	-	-	-	-	-	-	-	-	-	-
<i>L. interrogans</i>	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]
<i>L. borgpetersenii</i>	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]
<i>L. noguchii</i>	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 [0.3-68]	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]

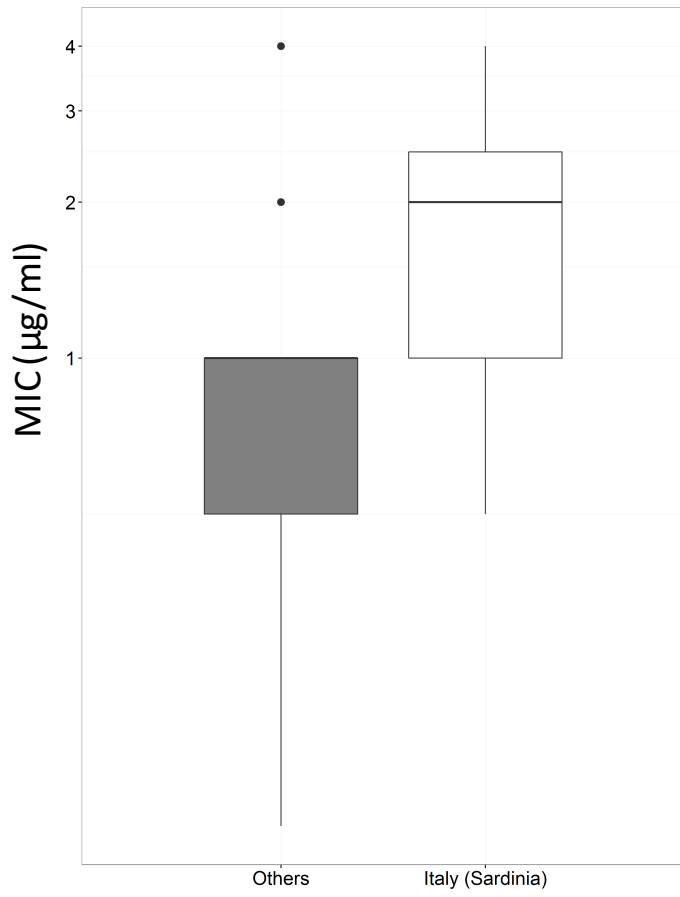
- a Two strains with unknown identification date were included in this category
- b Including: Argentina (1), Australia (2), Brazil (4), China (1), Croatia (1), England (1), Indonesia (1), Ireland (1), New Caledonia (3), Peru (1), Spain (2), Turkey (1), Uruguay (5), USA (3)
- c Including : Breeding deer (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), Tarrasovi (4), Unknown (5)

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

Antibiotics	Concentration of <i>Leptospira</i> (bacteria per mL)							
	<i>L. borgpetersenii</i> (strain n°6)				<i>L. noguchi</i> (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 1

A



B

