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Short communication

New host species for *Leptospira borgpetersenii* and *Leptospira interrogans* serovar Copenhageni

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ABSTRACT

We investigated the presence of infection by *Leptospira* spp. in an assembly of Sigmodontinae rodents from the Paraná Delta, Argentina. Rodents were captured in places with natural grassland, implanted forest, with and without raising cattle and in sites prone and not prone to flooding. The DNA was amplified from cultured isolates by PCR and *Leptospira* spp. strains were genotyped using Multiple – Locus Variable Number Tandem Repeat Analysis (MLVA). We isolated *Leptospira interrogans* serovar Copenhageni from *Oligoryzomys nigripes*, *Leptospira borgpetersenii* from *Scapteromys aquaticus* and *Leptospira interrogans* serovar Icterohaemorrhagiae from *Akodon azarae*. The zoonotic *Leptospira* isolated and genotyped from *O. nigripes* and *S. aquaticus* are the first reports from these species. The geographic range of these rodent species include, in addition to Argentina, the countries of Paraguay, Uruguay and Brazil, suggesting that these rodents might be involved in the transmission of spirochetes in other regions. Human and animal health care professionals should be alert to the potential occurrence of leptospirosis in areas where these rodent species are present.

1. Introduction

Leptospirosis is a worldwide re-emerging disease that affects human and animal health principally in humid and warm environments. Rodents are one of the most important reservoir hosts. Infected rodents can shed leptospires throughout their life, acting as spreaders of the disease and as source of infection for humans and domestic animals (Bharti et al., 2003; Cosson et al., 2014). The ecology of *Leptospira* spp. in Muridae rodent species has been studied intensively (e.g. Bharti et al., 2003; Cosson et al., 2014). However, only a few studies have investigated the occurrence of *Leptospira* spp. infection in Cricetidae, especially in Sigmodontinae rodents (Vanasco et al., 2003; Dos Santos et al., 2017). This subfamily of rodent, consisting of more than 400 species, is endemic to and distributed throughout the American continent, being the living Neotropical mammal group most diverse (Pardiñas et al., 2017). Considering that zoonotic pathogens originating from wildlife represent the most significant source of emerging infectious diseases (Jones et al., 2008), understanding the dynamics of *Leptospira* spp. infection in wild reservoirs can warn human and animal health care professionals about the potential risk of leptospirosis infection in other than already recognized hosts. In the present study, we investigated the presence of infection by *Leptospira* spp. in an assembly of wild Sigmodontinae rodents from the Paraná Delta, Argentina.

2. Materials and methods

2.1. Study area

The study was conducted in the Estación Experimental Agropecuaria Delta, Instituto Nacional de Tecnología Agropecuaria (INTA), Campana (34°11S, 58°50W), Buenos Aires, Argentina. The site is located in the lower Parana River Delta region, which is the southern extension of the Paranense Province of the Amazonic Phytogeo-graphic Dominion (Cabrera, 1994). The site is characterized by levees that surround dry areas as well as temporarily or permanently flooded marshes. Besides the native vegetation, the site has areas with commercial forestations of *Populus* spp. and *Salix* spp. In addition, in the study area there is a herd of beef cattle consisting of twenty-one

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Table 1
Host species (number of rodents in parentheses), prevalences of infection, *Leptospira* species, serovar and strain typed in the present study. The flanking regions of the variable number tandem repeat (VNTR) loci 4, 7, 10, Lb4, Lb5 are mentioned in parentheses.

Host species	Prevalence (%)	Leptospira species	Serovar	Strain
A. azarae (2)	1	*L. interrogans*	Icterohaemorrhagiae	RGA (2,1,7,-,-)
O. nigripes (1)	5	*L. interrogans*	Copenhageni	Fiocruz L1 130 (2,1,7,-,6)
S. aquaticus (1)	7	*L. borgpetersenii*	–	–

Aberdeen Angus cows maintained at a density of approximately one cow per hectare. The climate is temperate with a mean annual temperature of 16.7 °C and a mean annual rainfall of 1000 mm with an undefined rainy season (Kandus and Malvárez, 2004).

Rodents were captured from November 2010 through October 2012 in 3-night trapping sessions carried out every 5 weeks as described in Colombo et al. (2015). Grids were located in places with natural grassland, implanted forest and with and without farmed cattle. Some of the sites (5/16) were located in areas prone to flooding. Trapped rodents were transported to a field lab, anesthetized by inhalation of Isoflurane and sacrificed by cervical dislocation. Later they were identified to the species level by assessing cranium morphology. From each rodent, whole kidneys were taken at necropsy, and placed in individual tubes in liquid nitrogen and then maintained at − 80 °c at the Leptospirosis Laboratory at the Pathobiology Institute of the CICyA-INTA, Argentina. Kidneys were first cultured in liquid Ellinghausen-McCullough-Johnson-Harris (EMJH) medium and the isolates grown in Fletcher semi-solid medium (Difco Laboratories). The DNA was extracted and amplified by polymerase chain reaction (PCR) as described in Loffler et al. (2017). *Leptospira* spp. strains were genotyped using Multiple Locus Variable-number tandem repeat Analysis (MLVA). This method consists of amplifying variable-number tandem repeat loci (VNTR), showing polymorphism to differentiate the repeated copy number through the size of the resultant amplicon (Pavan et al., 2011). MLVA is an economic and simple technique for the identification of *Leptospira* strains.

All procedures were carried out under the approval of the Dirección de Flora y Fauna de la Provincia de Buenos Aires and the Ethic and Biosafety Committee of the Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral, Argentina.

3. Results

We trapped 782 Sigmodontinae rodents (Rodentia: Cricetidae) in all trapping sessions, but only 317 kidney samples were attained from 189 *Akodon azarae*, 77 *Oxymycterus rufus*, 20 *Oligoryzomys nigripes*, 17 *Oligoryzomys flavescens*, 13 *Scapteromys aquaticus* and 1 *Holochilus brasiliensis*. *Leptospira* spp. isolations were successful in 4 rodents as described in Table 1. For the isolate from *S. aquaticus*, it was not possible to determine the serogroup of *L. borgpetersenii* by MLVA. The environmental characteristics of the sites where the positive rodents were trapped are described in Table 2.

Table 2
Environmental conditions of the sites where we trapped the *Leptospira* positive rodents. *Leptospira* species and serovar, type of vegetation, presence or absence of cattle and the proneness to flooding of the site are mentioned.

L. species/Serovar	Type of vegetation	Presence of cattle	Prone to flooding
L. interrogans/ Icterohaemorrhagiae	Natural grassland	Yes	No
L. interrogans/ Icterohaemorrhagiae	Natural grassland	No	Yes
L. interrogans/Copenhageni	Natural grassland	Yes	No
L. borgpetersenii	Implanted forest	Yes	No

4. Discussion

Both *Leptospira* species isolated in the present study are pathogenic. They have been previously found infecting humans (Thornley et al., 2002; Bharti et al., 2003), wild and domestic animals (Suepaul et al., 2010; Houwers et al., 2011; Jorge et al., 2012). To our knowledge, the present study is the first isolation of *L. interrogans* serovar Copenhageni and *L. borgpetersenii* from *O. nigripes* and *S. aquaticus*, respectively. Both rodent species geographical ranges includes the countries of Argentina, Paraguay, Uruguay and Brazil (Délia and Pardiñas, 2015; Weksler and Bonvicino, 2015), adding two potential *Leptospira* spp. hosts in these countries. The finding of *L. interrogans* serovar Icterohaemorrhagiae in *A. azarae* was previously reported in Buenos Aires province (Cacchione, 1973), while seropositive results were found in rodents of that species captured in Santa Fe province (Vanasco et al., 2003). Besides these reports, the only other isolation of *Leptospira* spp. in the Sigmodontinae species here investigated was done in *O. nigripes* from Brazil, but the serovar involved was Pomona (Cordeiro et al., 1981). The few isolates we obtained in this study precludes drawing conclusions concerning the association between *Leptospira* infection and environmental and host variables. However, it is worth noting that *L. Icterohaemorrhagiae* serogroup isolates were obtained from rodents found in both floodable and non-floodable sites, indicating that *Leptospira* spp. infections are not exclusive to floodable lands as previously suggested (Cosson et al., 2014). Regarding the prevalence of *Leptospira* spp infection, the estimates reported here are similar to those reported in other Sigmodontinae (Zamora and Riedemann, 1999; Vanasco et al., 2003), but lower than the prevalence observed in urban rats (Vanasco et al., 2003; Agudelo-Flórez et al., 2009; Costa et al., 2014). Further studies are needed to establish potential associations between *Leptospira* spp. infection and host and environmental factors, as well as the role of *O. nigripes* and *S. aquaticus* as reservoirs.

5. Conclusion

The findings of the present study contribute to the knowledge of the ecoepidemiology of leptospirosis, adding two rodent species as hosts of *L. interrogans* serovar Copenhageni and *L. borgpetersenii*. This enables human and animal health care professionals to be alert to the potential occurrence of *Leptospira* in areas where these rodent species are present, and highlights the importance of studying the role of Sigmodontinae rodents in the transmission dynamics of Leptospirosis.

Conflict of interest

None.

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