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Renal carriage of *Leptospira* species in rodents from Mediterranean Chile: The Norway rat (*Rattus norvegicus*) as a relevant host in agricultural lands

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A B S T R A C T

We evaluated the renal carriage of *Leptospira* species in rodent communities from Mediterranean Chile using a PCR technique. We found that animals inhabiting agricultural areas were almost three times more infected than in wild areas (14.4% *vs.* 4.4%). The Norwegian rat (*Rattus norvegicus*), an invasive murid ubiquitous in the country, was the most infected species (38.1%).

1. Introduction

Leptospirosis is a widespread zoonosis caused by pathogenic species of the genus *Leptospira*. The disease risk of susceptible hosts depends on several factors, including the presence of maintenance hosts and favorable environmental conditions for bacteria (Levett, 2001). Some small mammals are recognized maintenance hosts of *Leptospira*, probably because of natural host adaptation to some serovars (Faine et al., 1999; Levett, 2004) and also because their behavior could increase the chance of contact with susceptible hosts (Perez et al., 2011). Some species of the genus *Rattus* play a relevant role in the epidemiological cycle of *Leptospira* in places where they are present, acting as a chronic reservoir and the main transmission source to humans in urban environments (Athanazio et al., 2008). The environmental persistence of *Leptospira* is enhanced when favorable environmental conditions are present, such as humid soil and warm temperatures. This has been linked to higher incidence of leptospirosis in tropical areas, but recently there has been an increase in human cases associated with several economic and recreational activities (Bharti et al., 2003; Hartskeerl et al., 2011; Levett, 2001).

A low incidence of disease in the human population has been reported in Chile, but high seroprevalence (reaching up 22%) was found in people whose activities are associated with agriculture and animal husbandry (Pappas et al., 2008; Zamora et al., 1990). This could be explained by the exposure of this human subpopulation to some maintenance hosts or contaminated environments (Levett, 2004; Zamora et al., 1994). Infection has been described in several orders of mammals; rodents are one of the more studied groups (Zamora and Riedemann, 1999). Within this group, *Leptospira* infection has been reported in the introduced rodents *Rattus rattus* (black rat), *Rattus norvegicus* (Norway rat), *Mus musculus* (house mouse), and in the native *Abrothrix olivaceus* (olive grass mouse), *Abrothrix longipilis* (long-haired grass mouse), *Oligoryzomys longicaudatus* (long-tailed pygmy rice rat), *Phyllotis darwini* (Darwin's pericote), *Geoxus valdivianus* (long-clawed mole mouse) and *Octodon degus* (degu) (Correa et al., 2017; Muñoz-Zanzi et al., 2014; Zamora and Riedemann, 1999).

Almost all the studies on *Leptospira* hosts in Chile have been performed in the Valdivian temperate rainforest ecoregion (south of 37° S Latitude) (Medina et al., 2009) where the rainy climate could favor environmental persistence of *Leptospira* (Zamora et al., 1994). North of this zone, the Mediterranean ecoregion (33°–37° S) has a temperate climate characterized by a short and cold winter where almost all the precipitations is concentrated, and a hot summer where there is virtual absence of rains (Bailey, 1998; Di Castri and Hajek, 1976). This ecoregion has a natural vegetation cover with predominance of shrublands known locally as *matorral*, with high soil fertility and very high topographic heterogeneity (Cowling et al., 1996). The climatic features in this area translate into environmental conditions that usually would not

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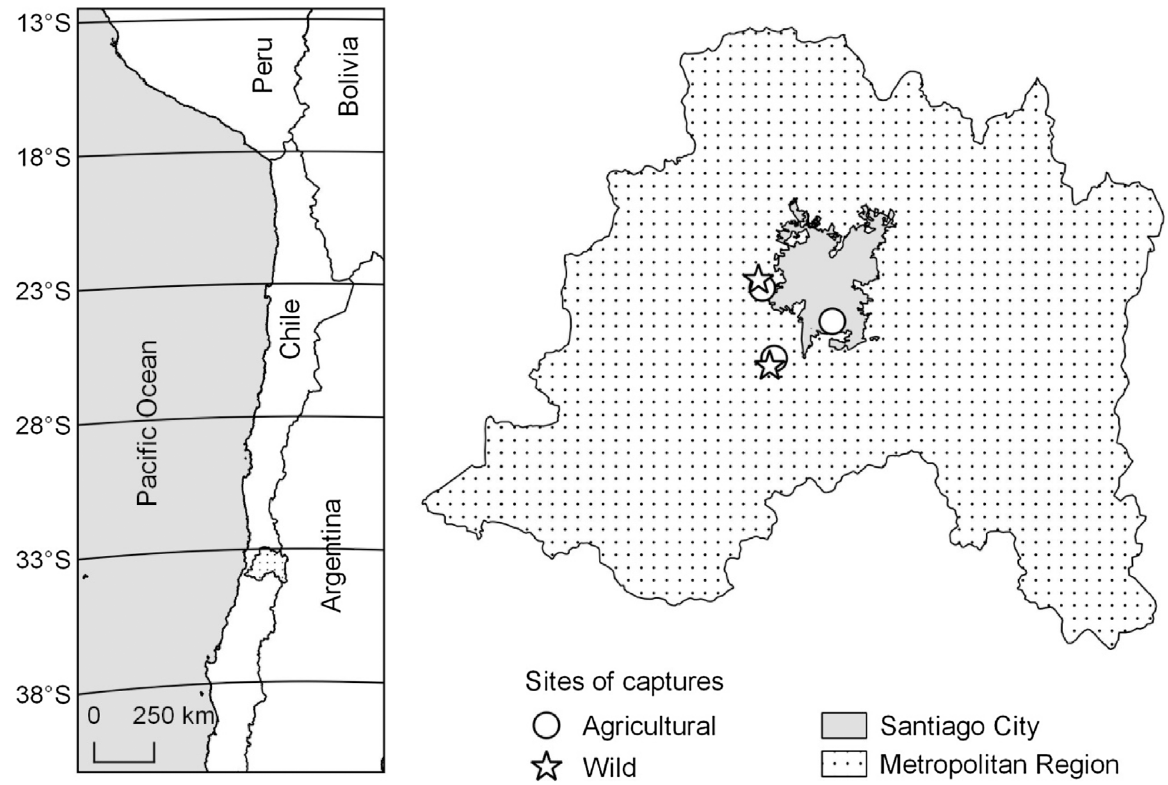

Fig. 1. Map of locations where *Leptospira* infection in rodents was studied. On the left is shown the location of the Metropolitan Region in southern South America. On the right is shown the agricultural (white circles) and wild locations (white stars) where the rodents where captured. The capital of Chile (Santiago, dark gray) is shown as a reference point.

favor the persistence of *Leptospira*. Nevertheless, the widespread agricultural activity in this zone could create micro-climatic and ecological conditions for *Leptospira* persistence, such as humid soils, which could lead to high prevalence among its hosts. Because of this, the aim of this study was to evaluate the effect of the agricultural activities on the frequency of renal carriage of *Leptospira* in rodents in Mediterranean zone of Chile.

2. Materials and methods

2.1. Animal sampling

In 2010 and 2011 trapping sessions were performed in three agricultural and two wild locations near Santiago, Metropolitan Region, Chile (Fig. 1). A minimum sampling number (n = 123) was calculated for each location using the Snedecor and Cochran, 1980 to evaluate differences in proportions (prevalence of 4.8% (Songer et al., 1983) and 14% (Perret et al., 2005), using a confidence level of 90% and a power of 80%). An average of 100 live-traps were used for each location (Rodentrap®, Forma Ltda., Santiago, Chile), baited with rolled oats and cotton bedding. Each captured animal was euthanized by an overdose of isoflurane and cervical dislocation, and the sex and species was determined. Kidneys were removed aseptically and stored in an individual tube with 95% ethanol at −20° C until processing.

2.2. PCR protocol

DNA extraction was performed in 60–80 mg of kidney tissue using a standard phenol–chloroform method. DNA quality and concentration were quantified by spectrophotometry. Renal carriage status was determined by a nested PCR using the primers described by Jouglard et al. (2006), which amplify a 183-bp internal fragment spanning the LipL32 gene (present only in pathogenic species of *Leptospira*). The amplification products were separated by electrophoresis in a 1.5% agarose gel stained with GelRed® (Biotium) and visualized under ultraviolet light. Each sample was assayed in triplicate and was considered positive if at least two reactions presented a strong signal.

2.3. Statistical analysis

Differences in renal carriage frequencies among species, type of location (agricultural or wild) and sex were evaluated with Fisher's exact test with a significance level of $\alpha = 0.05$.

3. Results

A total of 632 rodents were captured, belonging to three exotic species (the murids *R. rattus*, *R. norvegicus* and *M. musculus*) and four native species (the cricetids *A. olivaceus*, *P. darwini* and *O. longicaudatus*; the octodontidae *O. degus*). Three of these species (*A. olivaceus*, *R. rattus* and *O. degus*) represented almost 66% of captures (Table 1). Only native species were captured in wild locations, which had lower species richness than agricultural locations (three species *vs*. five species respectively).

The overall infection frequency was 10.8%, but in agricultural locations the rodents were almost three times more infected than in wild areas (14.4% *vs*. 4.4%; $p < 0.001$). Considering the species with more than 20 captures by site type, *R. norvegicus* had a frequency of infection significantly greater than other species in agricultural lands (38.1%, $p < 0.05$). The most abundant native species (*A. olivaceus*), had the second highest infection frequency after *R. norvegicus* (13.1%) but without significant differences with the other species. *Rattus rattus* – the most abundant exotic species in agricultural locations- had only 6% infection. The most abundant species in wild locations (*O. degus*) was also the most infected (5%). Comparing infection frequencies in species between type of location, only *A. olivaceus* showed significant differences being more infected in agricultural than in wild areas (13.1% *vs*. 0.0%; $p = 0.047$) (see Table 1 for more details).There were no significant differences between sexes.

Table 1
Number of captured rodents and frequency of renal carriage of *Leptospira* by type of location and rodent species. "–" indicates that the species was not captured.

Type of location Species	Agricultural N° captured	Infection Frequency (%)	Wild N° captured	Infection Frequency (%)
Introduced				
Rattus norvegicus	63	38.1	–	–
Rattus rattus	84	6.0	–	–
Mus musculus	47	12.8	–	–
Native				
Abrothrix olivaceus	160	13.1	27	0.0
Oligoryzomys longicaudatus	45	4.4	–	–
Phyllotis darwini	–	0.0	62	4.8
Octodon degus	4	0.0	140	5.0
Total	403	14.4	229	4.4

4. Discussion

4.1. *Leptospira infection in rodent communities of Mediterranean Chile*

This study shows evidence of renal carriage of pathogenic *Leptospira* in rodents in the Mediterranean zone of Central Chile. While the overall *Leptospira* infection frequency was lower than other studies performed in rodents from a different ecoregion of Chile (20.4% Muñoz-Zanzi et al., 2014; 41.4% Zamora and Riedemann, 1999), we found that infection frequency in agricultural areas was significantly greater than in wild areas. In the same region, Perret et al. (2005) linked an outbreak of human leptospirosis at a farm to some rodent species, including four of the seven species captured in this study. Although this association was made using serological methods that do not show direct evidence of renal carriage, the frequency of infected animals (14%) was similar to the present study. While our study was not designed to compare absolute abundances between populations, comparing between agricultural and wild locations we detected differences in the structure of rodent communities in the studied area. We found the three introduced murid species in Chile (*R. rattus*, *R. norvegicus* and *M. musculus*) only in the agricultural areas, where they accounted for almost 48% of the captures and 60% of infected rodents. These species have been mentioned frequently as relevant *Leptospira* hosts in different zones (Cosson et al., 2014; Vanasco et al., 2003) and have been described as species capable of invading wild areas in Mediterranean Chile (Lobos et al., 2005).

4.2. *Rattus norvegicus as a relevant host for Leptospira in agricultural settings*

In this study *R.norvegicus* explained the higher infection frequency in rodent communities of agricultural sites. In spite of not being the most abundant species (15.6% of captures), it made up 38.1% of infected animals and could represent a potential infection risk factor for other cohabiting rodents. In fact the cricetid *A. olivaceus*, the most abundant rodent in agricultural locations (39.7% of captures), was more infected in agricultural sites than in wild ones (13.1% *vs*. 0% infection respectively). This situation cannot be explained only as a consequence of the exposition to an environment with potentially more suitable conditions for *Leptospira* survival (agroecosystems), but also by the phylogenetic relatedness of *A. olivaceus* and *R. norvegicus*. These species are closer to each other (suborder Myomorpha) than to the second most abundant native rodent (*O. degus*, suborder Hystricomorpha). This may lead to *A. olivaceus* being more susceptible to acquiring *Leptospira* species infecting *R. norvegicus*, especially since both species are active at the night when lower temperatures do not favor the evaporation of contaminated urine, thus facilitating the transmission of the pathogen (Feng and Himsworth, 2014; Muñoz-Pedreros and Gil, 2009). It has also been observed that cricetid rodents have higher infection levels than hystrocomorphs in other ecological settings (Aviat et al., 2009; Bunnell et al., 2000).

Rattus norvegicus has been proposed as a relevant maintenance host of *Leptospira*, although its frequency of renal carriage varies among studies (Athanazio et al., 2008; Aviat et al., 2009; Bharti et al., 2003; Krojgaard et al., 2009; Sunbul et al., 2001). This may be explained by differences in the structure of host communities, environmental conditions and infectivity of serovars. The infection levels of the two *Rattus* species found in our study (38.1% for *R. norvegicus* and 6.0% for *R. rattus*), contrasts with what has been reported in southern Chile, where *R. rattus* has been detected more infected than *R. norvegicus* in farms and rural villages (21.3% *vs*. 10.3% respectively; Muñoz-Zanzi et al., 2014). These two *Rattus* species are widespread in continental Chile, only being absent in the most extreme environments of the country (hot deserts in the north, high altitude ecosystems and cold southern zones). It has been proposed that *R. norvegicus* is dominant over *R. rattus* in Mediterranean Chile due to its ability to use a wider range of habitats than *R. rattus*, which can facilitate its presence in more arid environments (Lobos et al., 2005). Nevertheless in this study these two *Rattus* species were captured only in agricultural lands, where crops could enhance the food and refuge availability to micromammals and the use of irrigation may increase the viability of *Leptospira* in soil and water.

Agricultural activities have been considered as a risk factor for human leptospirosis because in these areas animals such as rodents can be attracted by the availability of food and shelter, and workers can become infected by means of exposure of eroded skin to soil or water contaminated with the bacteria (Haake and Levett, 2014). We found a high proportion of invasive rodents infected with *Leptospira* in agroecosystems of Mediterranean Chile. The high infection levels in *R. norvegicus* added to their ability to live in close contact with humans in different environments, could favor the dissemination of *Leptospira* between natural and anthropic locations, putting agricultural workers of this zone of Chile at risk.

4.3. *Final remarks*

Although our methodology was able to determine *Leptospira* infection by detecting bacterial DNA in kidneys of rodents, we were not able to genotype our positive samples. Some studies have shown differences in the distribution of *Leptospira* species infecting rodents in various habitat types. Cosson et al. (2014) found that in Southeast Asia the two most abundant *Leptospira* species have different habitat preferences, with *L. interrogans* being more common in humid zones and *L. borgpetesenii* in humid and dry areas. Dietrich et al. (2014) showed that native small mammals harbored more and different *Leptospira* species than the introduced rodents in Madagascar. Considering this evidence, genotyping could be a valuable tool to understand in more detail the specific interactions between rodent species and *Leptospira* species in the Mediterranean ecoregion of Chile in order to get a better understanding of the real impact of these micromammals as hosts in this ecological setting.

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