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# Natural Infection of *Leptospira* Species in the Native Rodents Degu (*Octodon degus*) and Darwin's Pericote (*Phyllotis darwini*) in Mediterranean Ecosystem of Chile

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ABSTRACT: We report natural infections by pathogenic *Leptospira* of two rodent species endemic to Chile: the degu (*Octodon degus*) and Darwin's pericote (*Phyllotis darwini*). We detected *Leptospira* DNA in kidney and urine samples taken in different years and sites, reaching 33% infection. The effects of infection in these species requires further evaluation.

*Leptospira* is a zoonotic genus of bacteria with infections reported worldwide in several orders of mammals (Andersen-Ranberg et al. 2016). Because of their ubiquity and close contact with susceptible hosts, small mammals are among the most relevant maintenance hosts—those chronically infected animals that release the bacteria into the environment (Levett 2001). Although the humid environment can favor higher disease incidence of leptospirosis in tropical ecosystems, infection has also been described in temperate and arid areas (Levett 2001; Bharti et al. 2003). *Leptospira* has been reported infecting several mammals in Chile, including the rodents *Rattus rattus*, *Rattus norvegicus*, *Mus musculus*, *Abrothrix olivaceus*, *Abrothrix longipilis*, *Oligoryzomys longicaudatus*, and *Geoxus valdivianus* (Zamora and Riedemann 1999; Muñoz-Zanzi et al. 2014). We report natural *Leptospira* infections in two endemic rodents of the Mediterranean ecosystem of Chile: degu (*Octodon degus*) and Darwin's pericote (*Phyllotis darwini*).

Between 2010 and 2011, trapping sessions of three to five nights were performed in one agricultural (Amancay, 33°09′S, 70°53′W) and two wild (Lonquen, 33°39′S, 70°48′W; Rinconada, 33°28′S, 70°49′W) locations near Santiago (Fig. 1). We used 80 live animal traps (Rodentrap®, Forma Ltd., Santiago, Chile) baited with rolled oats and with cotton bedding, distributed in transects. Traps were activated at sunset and checked the following morning. Animals were euthanized with an overdose of isoflurane and cervical dislocation. Kidneys were removed aseptically and stored in 95% ethanol at –20 C. Blood samples were withdrawn by cardiac puncture and sera were stored at –20 C. In the summer and spring of 2014 another trapping session using 100 traps for four nights was performed in Amancay. Animals were weighed and anesthetized with an intramuscular injection of 40–85 mg/kg ketamine and 5–21 mg/kg xylazine (Kohn et al. 1997). Urine samples were collected from the urethra by pressing the bladder externally, mixed with phosphate buffered saline 1% (1:3 v:v) and stored at –20 C. Blood samples were obtained by puncture of the saphenous (degu) or masseteric veins (Darwin's pericote) and sera were stored at –20 C. Animals were ear-tagged and released at the point of capture. We purified DNA of kidney samples using phenol-chloroform, whereas DNA of urine samples was extracted with a commercial kit (Qiagen® DNeasy Blood & Tissue Kit, Hilden, Germany). Concentration and purity of DNA was evaluated by spectrophotometry. A nested PCR was performed using primers targeted to the *LipL32* gene, present only in pathogenic *Leptospira*, following the protocol described by Jouglard et al. (2006), with modifications in reagent concentrations (1X

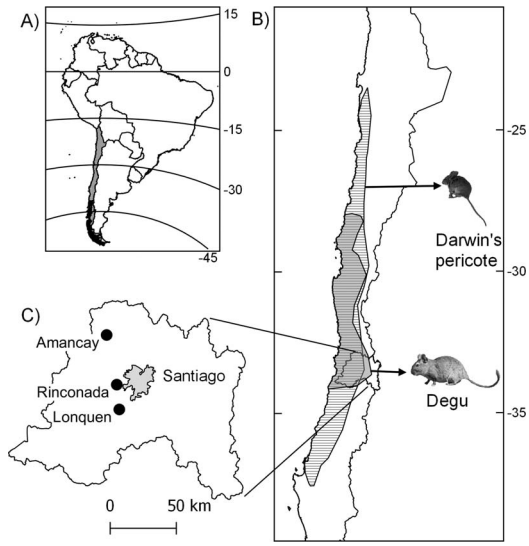


FIGURE 1. (A) Map of South America highlighting the location of Chile (dark gray). (B) Map showing the geographical distribution of the rodents Darwin's pericote (*Phyllotis darwini;* horizontal lines) and degu (*Octodon degus;* dark gray). (C) Map of the Metropolitan Region showing the locations (black circles) where Darwin's pericote and degu were captured and tested for *Leptospira* infection. Santiago City (light gray) is shown as reference.

PCR buffer, 0.5 µmol/L of each oligonucleotide, 0.2 mmol/L dNTPs, 1.25 units *Taq* polymerase, and 100 ng of kidney or 10 ng of urine DNA). Amplicons were separated by electrophoresis in agarose gels and visualized in an ultraviolet transilluminator.

Anti-*Leptospira* antibodies were detected in an initial sera dilution of 1:25 by a microscopic agglutination test (MAT), with a panel of 15 serovars available at the National Institute of Public Health (Santiago, Chile), representing 11 serogroups and five species (*Leptospira biflexa*, *Leptospira borgpetersenii*, *Leptospira interrogans*, *Leptospira kirschneri*, and *Leptospira. santarosai*). Fisher's exact test was used to compare differences in frequency of infection, with a significance level of $\alpha$=0.05.

The PCR results indicated that 10.0% of degus and 5.9% of Darwin's pericotes were infected. In 2010–11, 4.5% of all rodents were infected ($n$=247) and 23.5% in 2014 ($n$=81). Infection frequencies did not differ between species within a sampling period or habitat (2010–11 wild sites, 2010–11 agricultural sites, 2014 agricultural site; $P$=1) or between type of habitat within rodent species in 2010–11 ($P$>0.5). In 2014 we detected a higher positive percentage in spring (32.1%) than in summer (4.0%) in degus ($P$=0.008; Table 1).

Only two degus showed agglutination in MAT against bratislava (*L. interrogans*) and ballum (*L. borgpetersenii*) serovars at 1:50 dilution.

Darwin's pericote and the degu are common rodents in Mediterranean Chile; they inhabit mainly wild areas and are syntopic in some locations (Fig. 1). *Leptospira* infection had not been described in those species, even though degu is maintained in captivity as a pet and an animal model for some human diseases (Suckow et al. 2012). Although they have fundamental behavioral differences (degus are diurnal and social whereas Darwin's pericotes are nocturnal and solitary), *Leptospira* is not the only pathogen shared by these rodent species: both are hosts of *Trypanosoma cruzi*, the etiological agent of Chagas' disease (Botto-Mahan et al. 2012). The social behavior of degu could favor the transmission of *Leptospira* both directly and indirectly, because they form groups with cooperative breeding (Bauer et al. 2015). Thus, in 2014 we found higher infection levels in spring,

TABLE 1. Distribution in the Mediterranean ecosystem of Chile of the rodents, degu (*Octodon degus*) and Darwin's pericote (*Phyllotis darwini*) captured in 2010–11 and 2014 according to species, study location, land status, type of sample, number of analyzed animals by PCR, and frequency of infection with *Leptospira* spp.

| Species | Study location | Land status | Sample type | No. of animals | Prevalence (%) |
|---|---|---|---|---|---|
| Degu | Amancay | Agricultural | Kidney[a] | 42 | 2 |
| | Amancay | Agricultural | Urine[b] | 78 | 23 |
| | Rinconada | Wild | Kidney[a] | 64 | 9 |
| | Lonquen | Wild | Kidney[a] | 76 | 1 |
| Darwin's pericote | Amancay | Agricultural | Kidney[a] | 3 | 0 |
| | Amancay | Agricultural | Urine[b] | 3 | 33 |
| | Rinconada | Wild | Kidney[a] | 39 | 0 |
| | Lonquen | Wild | Kidney[a] | 23 | 13 |

[a] Animals captured in 2010–11.

[b] Animals captured in 2014.

coinciding with a peak in the abundance of degus, sustained by a higher proportion of juveniles (82% of captures in spring versus 0% in summer). In addition, in spring the soil is wetter than in summer; the virtual absence of rain in summer might reduce the environmental persistence of *Leptospira*. Because of the solitary behavior of Darwin's pericote, their infection might be acquired mainly by environmental exposure.

The MAT had low efficiency detecting *Leptospira* exposure, probably because the animals had low antibody titers or were exposed to serovars not represented in the panel. This has been mentioned as a limitation of this test when dealing with chronically infected animals (Office International des Epizooties 2008) and could be ameliorated by efforts to isolate native serovars.

The two species we studied are abundant in Central Chile. As the encroachment of humans in wild habitat increases the chance of human contact with them, it is necessary to elucidate their role in the transmission cycle of *Leptospira* and the effects of infection and land use change in rodents.

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