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Household Characteristics Associated with Rodent Presence and *Leptospira* Infection in Rural and Urban Communities from Southern Chile

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Abstract. Rodents are well-recognized reservoirs of *Leptospira*, contributing to its maintenance in endemic areas and playing a role in the public health risk associated with the infection. This study sought to provide some insights into rodent populations from Chile and their *Leptospira* carriage. In total, 393 rodents were trapped in 177 households. Higher rodent counts were associated with year 2 of the study, rainfall, and number of rodent signs. There was an inverse correlation with the number of cats. The number of rodents was higher in villages compared with slums (rate ratio = 3.23) but modified by average household age. Eighty rodents (20.4%) tested positive for *Leptospira*: 19.7% on the farms, 25.9% in villages, and 12.3% in the slums. Prevalence was 22.5% in *Mus musculus*, 20.7% in *Rattus rattus*, 21.1% in wild rodents, and 10.3% in *R. norvegicus*. Seasonal and temporal effects were the major determinants of *Leptospira* infection in rodent populations.

INTRODUCTION

Leptospirosis, caused by the pathogenic species of the bacteria *Leptospira,* has been characterized as an emerging zoonosis of global importance by the World Health Organization.[1,2] The clinical course of human leptospirosis ranges in severity from asymptomatic or mild infection to severe illness, including jaundice, renal failure, and hemorrhaging.[3] *Leptospira* bacteria are maintained in the environment through a complex transmission cycle, in which humans and other mammals (domestic and wild) become infected after contact with urine from an infected host or *Leptospira*-contaminated water or damp soil.[3,4] Leptospires can enter a host through skin abrasions or cuts and the mucous membranes.[5]

Although some serovars of *Leptospira* have an affinity for specific livestock and domestic animals, small rodents are recognized as maintenance hosts, because they are generally asymptomatic carriers, and *Leptospira* can be present in the urine for a considerably long period of time.[5] The intermittent but possibly lifelong shedding of the bacteria by rodents into the environment can provide plenty of opportunities for new animal and human infections, which can be magnified by the rodents' ability to adapt, survive in nearly any environment, and coexist in close proximity to humans.[6] Within human settlements, rodents migrate from household to household based on whichever residence is easiest to enter and provides favorable living conditions.[7] Among the human factors associated with increased rodent presence are poor housing quality, inadequate food storage, and poor rubbish disposal within the immediate peridomestic area.[8,9] An extensive review by Meerburg and others[10] highlights the significance of the link between rodents and risks for public health in terms of rodent-associated crop losses, spoilage of food, structural damage, and carriers of zoonotic infections, including leptospirosis.

The impact of leptospirosis in tropical areas is substantial and often associated with severe weather events and poor living conditions.[1] Leptospirosis can also be endemic in temperate areas, although at lower incidence rates, where it manifests as sporadic cases and occasional small outbreaks.[5] In Chile, the first clinical cases of human leptospirosis were recognized and documented as early as 1933.[11] Sporadic outbreaks and reports of seroprevalence in specific populations reveal that human leptospirosis is more common in Chile than the reported annual incidence rates suggest and that geographical variations exist.[12,13] The Los Rios Region in Chile, where this study took place, is a prominently agricultural and farming area with scattered urban settlements. A local serosurvey in people with occupational risk revealed significant evidence of human exposure (22%),[14] and surveys in animals report that leptospirosis in domestic animals, including sheep[15] and dogs,[16] is common. An earlier report also suggested that leptospirosis is widespread in rodents from the region, with prevalence as high as 38%.[17]

The ecology of *Leptospira* infection in temperate climates is as complex as in tropical areas, considering the many factors that influence rodent population dynamics, its relation with maintenance of infection within the rodent population, and subsequently, the opportunities for transmission to other species, including humans. Because of the public health importance of leptospirosis in the peridomestic environment, this study sought to provide some insights into the dynamics of rodent populations in households from rural and slum communities and the socioecological factors associated with leptospirosis in those rodents.

MATERIALS AND METHODS

Study population and data collected. This study corresponds to analysis of data of a larger study on the ecoepidemiology of leptospirosis that is being carried out in the Los Rios Region in the southcentral part of Chile (latitude: 39°15′ S, to 40°33′S, longitude: 73°43′ W to 71°35′ W). The region's climate is characterized as temperate rainforest. Annual cumulative rainfall is 2,588 mm but can range from 1,200 mm in the central valley to 5,500 mm in the Andes Mountains. Average temperate in summer is 17°C, and average temperature in winter is 8°C. Communities (two of each type) were selected based on the following definitions. (1) Slums: informal settlements in the outskirts of a major city

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characterized by substandard housing. (2) Villages: rural community settlements away from major cities where households are clustered together. (3) Farms: dispersed households, typically small family farms, located in a specific rural locality. Communities were selected from areas where most settlements in the region are located—the central valley and near the region's capital. Almost all communities were located at an altitude of 0–100 m, except for one village (C-2) and two farm communities (D-1 and D-3), which had an altitude of 100–200 m (Figure 1).

Households in each community were invited to participate and enrolled (up to 40 households per community) from August of 2010 to March of 2012 based on representativeness and willingness to participate in the study. The heads of each household were approached to obtain informed consent and complete a staff-administered questionnaire. The questionnaire gathered information pertaining to sociodemographic characteristics, living conditions, presence of domestic animals and livestock, and evidence and control of rodents. Variables were created to consider the impact of temporal and climatic effects. To account for the 2-year enrollment period, a variable called sampling year was defined as year 1 (August of 2010 to March of 2011) and year 2 (August of 2011 to March of 2012). A variable called sampling season was defined as spring (August to December) and summer (January to March).

Monthly averages for temperature and rainfall from 2001 to 2012 were obtained from weather stations in Isla Teja (Austral Universidad) and the local airport. Variables were created that provided the historical average temperature and rainfall for each sampling month, and they were assigned to each community depending on closeness to a weather station and time of sampling. The study protocol was approved by the University of Minnesota's Institutional Review Board (No. 0903M62042) and Institutional Animal Care and Use Committee (No. 0904A63201) and the Austral University's Human and Animal Ethics Committee (No. 01/09).

Rodent trapping and sample collection. Rodents were trapped over 3 nights by placing 15 large and small traps (Forma Ltda. Santiago, Chile) within each house (5 traps) and in the peridomestic area (10 traps). Bait consisted of peanut butter, oatmeal, and butter. Traps were checked daily, and live rodents were transported to Universidad Austral for euthanasia, which was performed using a CO_2 chamber (AVMA 2007). Kidneys were harvested and processed for DNA extraction. Blood samples were processed to obtain sera, which were stored at –40°C until testing. Rodent species, total length and head to body length, weight, sex, age, and other physical characteristics were also recorded. Age was determined based on tooth wear following standard methods.[18]

Laboratory detection of *Leptospira*. DNA extraction from 30 mg kidney tissue was carried out using a commercial kit (E.Z.N.A Tissue DNA Kit; Omega Bio-Tek, Norcross, GA) according to the manufacturer's instructions. DNA elution was performed with 200 μL elution buffer, allowing at least 5 minutes of incubation. Two polymerase chain reaction (PCR) protocols were run in all samples. One protocol was a PCR targeting the *lipL32* gene using the previously published

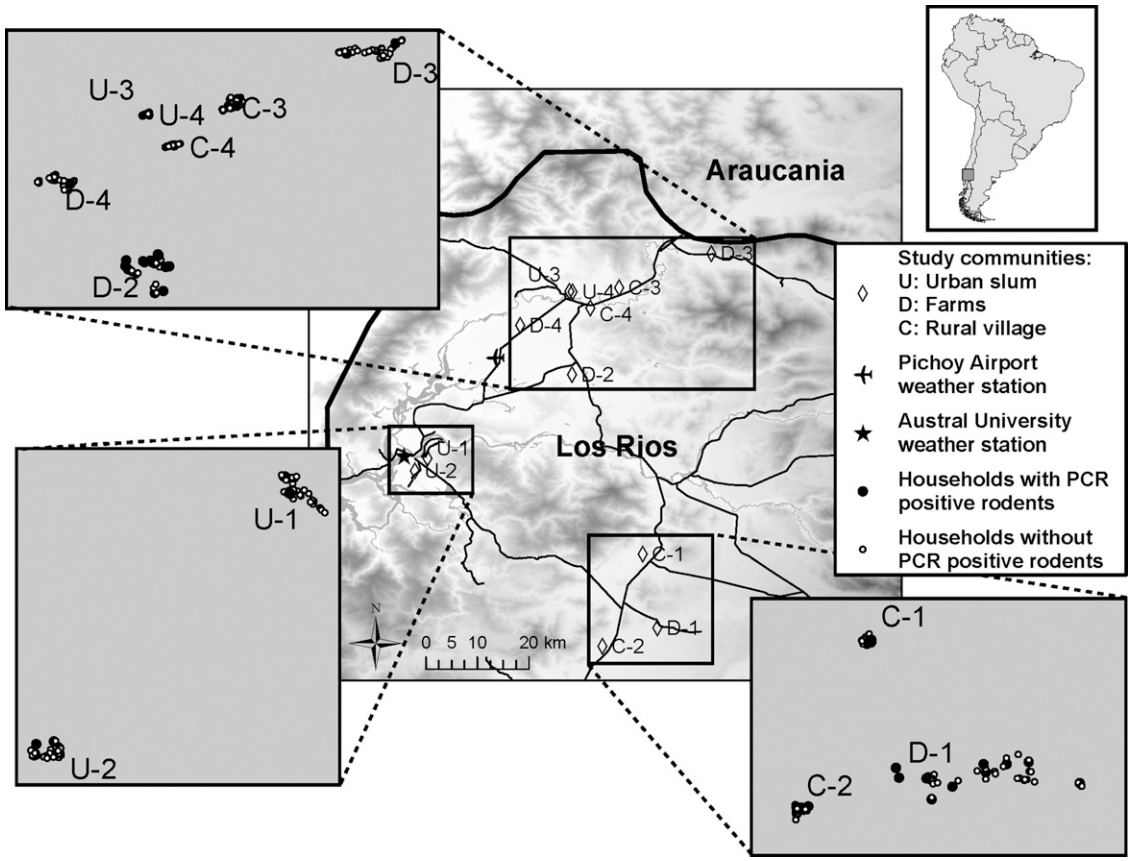

FIGURE 1. Map of the study communities in the Los Rios region of Chile, the location of households, and the households with and without rodents positive for *Leptospira*.

primers LipL32-45F (5′-AAG CAT TAC CGC TTG TGG TG-3′) and LipL32-286R (5′-GAA CTC CCA TTT CAG CGA TT-3′).[19] The PCR reactions were performed in 25 μL mixture containing 5 μL diluted template, 0.25 μM each primer, 0.625 U GoTaq Flexi DNA polymerase in 1× Green Buffer GoTaq (Promega, Madison, WI), 2.5 mM $MgCl_2$, 0.8 mM 2′-deoxynucleoside 5′-triphosphate (dNTP; Promega, Madison, WI), and 400 ng mL^{-1} bovine serum albumin (BSA; BioLabs, Ipswich, England). All samples were tested in duplicate, and template DNA was diluted 1:100 and 1:500. Cycle conditions included an initial denaturation step at 95°C for 5 minutes followed by 40 cycles at 94°C for 1 minute, 57°C for 1 minute, and 72°C for 1 minute and a final elongation step at 72°C for 10 minutes. In addition, a nested PCR was performed to amplify the 16S rRNA gene of pathogenic *Leptospira*. A 510-bp product was amplified in the first PCR round using 16S13 (5′-CGGCGCGTCTTAAACATG-3′) and 16S522 (5′-TCCGCCTACACACCCTTTAC-3′) primers. The mixture included 5 μL diluted template (1:100), 0.5 μM each primer, 0.625 U GoTaq Flexi DNA polymerase in 1× Green Buffer GoTaq (Promega, Madison, WI), 2.5 mM $MgCl_2$, 0.8 mM dNTPs (Promega, Madison, WI), and 400 ng mL^{-1} BSA (BioLabs, Ipswich, England) in 25 μL mix. Reaction conditions were 95°C for 5 minutes followed by 35 cycles at 95°C for 1 minute, 63°C for 1.5 minutes, and 72°C for 1 minute and a final elongation step at 72°C for 10 minutes. For the second amplification round, a 330-bp product was obtained using Lepat1 (5′-GAGTCTGGGATAACTTT-3′) and Lepat2 (5′-TCACATCGYTGCTTATTTT-3′) primers.[20] PCR was performed in 25 μL by adding 1 μL first amplification round diluted 1:100 to the mixture containing 0.5 μM each primer, 0.625 U GoTaq Flexi DNA polymerase in 1× Green Buffer GoTaq (Promega), 2.5 mM $MgCl_2$, 0.8 mM dNTPs (Promega), and 400 ng mL^{-1} BSA (BioLabs). Reaction conditions were 95°C for 5 minutes followed by 35 cycles at 95°C for 1 minute, 51°C for 1 minute, and 72°C for 1 minute and a final elongation step at 72°C for 10 minute. In both PCR protocols, each amplification run contained a negative control, consisting of water, and a plasmid-positive control, consisting of target DNA sequences ligated into a pGEMT Easy Vector (Promega, Madison, WI). The PCR products obtained were separated on 1.5% (wt/vol) agarose gel, stained with Gel Red (GelRed; Biotium Inc., Hayward, CA), and purified using a commercial kit (E.Z.N.A. Gel Extraction Kit; Omega Bio-Tek, Norcross, GA). The sequences were obtained (Macrogen Inc., Seoul, Korea) and used in a basic local alignment search tool (BLAST) search of GenBank to confirm similarity to *Leptospira* spp. sequences.

Statistical analysis. Descriptive statistics for questionnaire responses and rodent characteristics were computed and compared across community types using analysis of variance (ANOVA), χ^2 test, or Fisher exact test as appropriate. Factors associated with the number of rodents trapped per household were examined using a zero-inflated Poisson model, which assumed a logistic regression model for the 0 or ≥1 rodent per household portion of the model and a Poisson regression for the count portion of the model (number of rodents per household).[21,22] Variables examined included socioeconomic and living condition characteristics obtained from the survey as well as climate factors and temporal effects. The same set of initial covariates was considered for the binomial and count portions as part of the model selection process. Model selection was based on statistical significance, comparison of Akaike information criterion (AIC) values, and examination of model assumptions, while considering the need to adjust the model for potential confounders and evaluating a wide range of plausible interactions. Results from the final binomial model were reported as odds ratios (ORs), and results from the count model were reported as rate ratios (RRs) or count percentage differences. The model was run using the pscl R package.[23]

For evaluation of the factors associated with *Leptospira* carriage, data were analyzed at the rodent level, where a rodent was considered positive if tested positive in at least one PCR protocol and negative otherwise. Both mixed effects, with random effects for household and community, and conventional logistic regression were considered. Variables examined included rodent characteristics, relevant household characteristics, and variables for climate and temporal effects. Adjustment for potential confounders and testing for interactions were carried out as appropriate. Final model selection was carried out based on AIC and likelihood ratio tests between nested models. The Cessie–van Houwelingen–Copas–Hosmer unweighted sum of squares test from the rms R package was used for assessment of global goodness of fit of the final model.[24] Mixed effects logistic regression was run using the lme4 package.[25] Statistical significance was set at $P < 0.05$. All analyses were performed using the R 2.15.1 statistical program.[26]

RESULTS

Rodent characteristics. Trapping was carried out in 417 households from the three community types. Detailed characteristics of the households are described in Table 1. In total, 393 rodents were trapped in 177 households, including 228 (58.0%) rodents in farm communities, 108 (27.5%) rodents from villages, and 57 (14.5%) rodents from slums (Figure 2A and Table 2). No marked differences or patterns between community types were found in the number of sprung but empty traps. At the household level, ≥ 1 rodent was trapped in 85 of 144 (59.0%) farms, 55 of 131 (42.0%) households from rural villages, and 37 of 142 (26.1%) households from slums. Number of rodents trapped per household ranged from zero to nine. More rodents were trapped per household in farms (mean = 1.6, SD = 2.0) than villages (mean = 0.8, SD = 1.3) or slums (mean = 0.4, SD = 0.8; $P < 0.01$). The distribution of species captured differed significantly across the three community types ($P < 0.01$). *Rattus rattus* was the most frequently trapped species overall (62.6%; 246/393) and in the farms (66.7%; 152/228) and villages (72.2%; 78/108). Conversely, the largest proportion in the slums corresponded to *Mus musculus* (64.9%; 37/57) (Table 2). Wild rodents (38 in total) were trapped in the peridomestic environment of all community types, corresponding to 10.1% (23/228) of the rodents from farms, 10.2% (11/108) of the rodents from villages, and 7.0% (4 /57) of the rodents from slums (Table 2). Wild rodent species trapped included 20 *Oligoryzomys longicaudatus* (long-tailed pygmy rice rat; family Cricetidae), 4 *Akodon longipilis* (long-haired grass mouse; family Muridae), and 14 *Abrothix* spp. (family Cricetidae). *Rattus* spp. and wild species were consistently trapped in the peridomestic areas (100% of *R. norvegicus* and wild rodents and 86.9% of *R. rattus*). On the contrary,

TABLE 1
Demographic characteristics and living conditions of enrolled households by community type in Los Rios Region, Chile, 2010–2012

HH characteristics	Community type			*P* value
	Farms	Rural villages	Urban slums	
Number of HH	144	131	142	
Monthly income ≥ $417*	72 (50.0%)	55 (42.0%)	31 (21.8%)	< 0.01
Number of HH members†	4.4 (1.9)	4.1 (1.7)	4.1 (1.7)	0.24
Average age of HH members (years)†	39.1 (14.5)	38.8 (16.1)	26.3 (12.0)	< 0.01
Head of HH has high school degree	40 (27.8%)	37 (28.5%)	31 (22.1%)	0.42
Age of head of HH (years)†	51.5 (14.0)	48.5 (17.2)	36.7 (13.4)	< 0.01
Housing type: house‡	142 (98.6%)	128 (97.7%)	24 (16.9%)	< 0.01
With electricity	139 (96.5%)	130 (99.2%)	142 (100%)	0.03
Closed food storage	132 (91.7%)	121 (92.4%)	122 (85.9%)	0.15
Stores water in HH	35 (24.3%)	25 (19.1%)	47 (33.1%)	0.03
Covered trash can	18 (12.5%)	23 (17.6%)	26 (18.3%)	0.35
HH trash removal service	107 (74.3%)	117 (89.3%)	135 (95.1%)	< 0.01
Water source				< 0.01
Surface water	31(21.5%)	9 (6.9%)	1 (0.7%)	
Well	65 (45.1%)	3 (2.3%)	0 (0%)	
Public potable water	48 (33.3%)	119 (90.8%)	141 (99.3%)	
Human waste disposal				< 0.01
None	11 (7.6%)	5 (3.8%)	64 (45.1%)	
Sewer system or septic tank	83 (57.6%)	107 (81.7%)	39 (27.5%)	
Latrine	50 (34.7%)	19 (14.5%)	39 (27.5%)	
Floor type				0.03
Wood	123 (85.4%)	122 (93.1%)	131 (92.3%)	
Tiles, cement, or linoleum	18 (12.5%)	4 (3.1%)	9 (6.3%)	
Dirt or concrete slab	3 (2.1%)	5 (3.8%)	2 (1.4%)	
Good floor condition§	105 (72.9%)	90 (68.7%)	74 (52.1%)	< 0.01
Wall type				< 0.01
Wood	137 (95.1%)	127 (96.9%)	104 (73.2%)	
Concrete	1 (0.7%)	2 (1.5%)	11 (7.7%)	
Scraps or adobe	6 (4.2%)	2 (1.5%)	27 (19.0%)	
Good wall condition§	87 (60.4%)	71 (54.2%)	55 (38.7%)	< 0.01
Roof type: corrugated iron sheet¶	142 (98.6%)	126 (96.2%)	140 (98.6%)	0.41
Good roof condition§	96 (66.4%)	78 (59.5%)	51 (35.9%)	< 0.01

*$1 US = 480 Chilean pesos.
†Mean (SD).
‡Versus shack.
§Versus deteriorated or bad.
¶Versus other materials (scraps or tiles).
HH = household.

the majority of *M. musculus* (64.1%) was trapped inside the households. Overall, the majority of rodents was adults (255/393; 64.9%) and males (286/393; 72.8%), with significant differences by community type for age ($P < 0.01$) but not sex ($P = 0.34$) (Table 2). There was no difference by sex ($P = 0.89$) between wild (28/38 male; 73.7%) and non-wild rodents (258/355 male; 72.7%). Among non-wild rodents species, 62% (222/355) were adults, whereas almost all wild rodents were adults (35/38; 92.1%; $P < 0.01$).

Survey results showed that a larger proportion of farms reported perceived presence of rodents and specific signs of rodent presence, including droppings, urine, and gnawed food, compared with other community types (Table 3), which was consistent with increased rodent control efforts. Traps or poison was used in 81.9% in the farms compared with 61.8% in villages and 56.3% in slums (Table 3).

Factors associated with the number of rodents captured. Model selection yielded a parsimonious final model shown in Table 4 that included the binomial and the count portions of the zero-inflated regression model. In a binomial model adjusted for sampling season, sampling year, and community type, the only variables found to be significantly associated with zero-trapped rodents in a household were average temperature ($P = 0.04$) and an interaction between sampling season and monthly average rainfall ($P = 0.03$). A 1° increase in average temperature was associated with a 76% increase in the odds of having zero rodents trapped (OR = 1.76, 95% confidence interval [95% CI] = 1.03–3.02). Summer sampling season decreased the likelihood of households with zero-trapped rodents compared with spring sampling season, and the magnitude of this effect increased as average rainfall increased. For example, the OR was 0.17 (95% CI = 0.03–0.75) when average rain was 45 mm and 0.11 (95% CI = 0.02–0.64) when average rain was 50 mm (Table 4). The count portion of the model included sampling year ($P < 0.01$), average rain ($P = 0.03$), number of rodent signs ($P < 0.01$), and number of cats ($P < 0.01$) as independent predictors of number of rodents trapped in a household, while adjusting for sampling season, community type, and average temperature. Trapping efforts in sampling year 2 were associated with a 49% decrease in the number of rodents compared with year 1 (RR = 0.51, 95% CI = 0.38–0.70). An increase in average rainfall was associated with an increase in number of rodents, where for example, a 10-mm increase in rainfall corresponded to a 9% (95% CI = 1–18%) increase in the number of rodents captured. Number of rodent signs reported by the heads of households was positively correlated with the number of rodents trapped, where an increase in one of the listed signs corresponded to an increase of 17% in the number of rodents trapped (RR = 1.17, 95% CI = 1.05–1.30). Conversely, an increase of one cat in the household was associated with a decrease by 13% in the number of rodents trapped

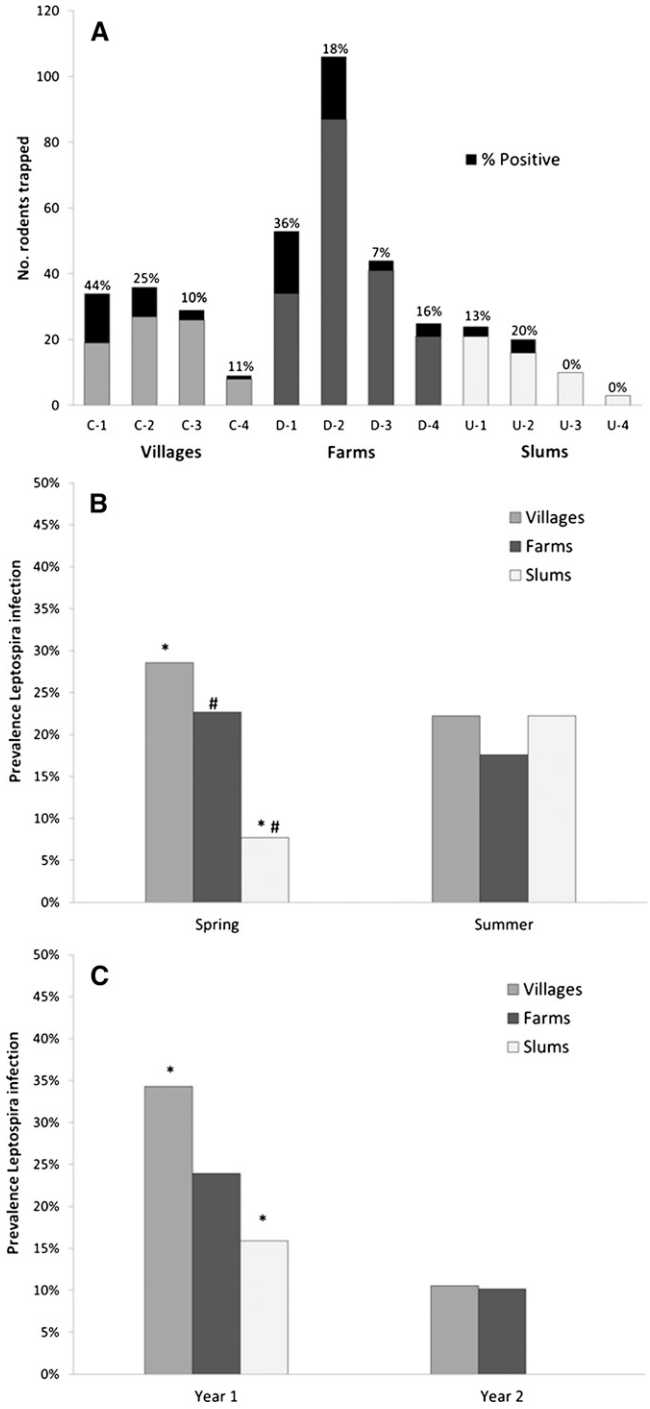

FIGURE 2. Distribution of *Leptospira*-positive rodents by (**A**) community, (**B**) sampling season, and (**C**) year. Bars with the same symbol indicate statistically significant differences. C = villages; D = farms; U = slums.

(RR = 0.87, 95% CI = 0.80–0.95). Although not statistically significant at $\alpha = 5\%$, summer was associated with a 38% increase in the number of rodents trapped compared with spring (RR = 1.38, 95% CI = 0.98–1.93). A statistically significant interaction was found between community type and the average household age, where although more rodents were trapped in both villages ($P = 0.03$) and farms ($P = 0.08$) compared with slums, the magnitude of this effect increased markedly as the average household age increased (Table 4). For example, the number of rodents was three times higher in villages compared with slums (RR = 3.23, 95% CI = 1.54–6.75) when average household age was 15 years and almost five times higher when average age was 25 years (RR = 4.77, 95% CI = 2.46–9.23).

Factors associated with *Leptospira* infection in rodents. In total, 80 of 393 rodents captured (20.4%) tested positive for *Leptospira* with either PCR test. There was an 85.5% agreement between PCR tests, mainly driven by the agreement among negative samples. Seventy-six rodents tested positive by the LipL32 test, resulting in a prevalence of 19.3%. Four rodents were positive by the 16S/Lepat PCR test but negative by the LipL32 test. All additional analyses used any positive PCR test result as a *Leptospira*-positive rodent. Within-community prevalence varied from 0% in slums to 44% in villages (Figure 2A). There was a marked decrease in the proportion of positive rodents from sampling year 1 (25.3%; 69/273) to year 2 (9.2%; 11/120; $P < 0.01$), with a significantly higher prevalence in spring (33%; 37/113) than summer (20%; 32/160) in year 1 ($P = 0.02$) but not year 2 ($P = 0.29$). Across all communities, there were no statistically significant differences by species ($P = 0.59$), sex ($P = 0.78$), or age ($P = 1.0$) (Table 3). On the farms, 19.7% (45/228) of the rodents tested positive, 25.9% (28/108) of the rodents tested positive in rural villages, and 12.3% (7/57) of the rodents tested positive in the slums ($P = 0.11$). Prevalence was significantly higher in farms and villages than slums but depended on sampling year and season (for example, in spring but not summer and in year 1 but not year 2) (Figure 2B and C). Because of the small number of positive rodents in slums, multivariable analysis was done for rodents from farms and villages only. This regression model yielded sampling season but modified by sampling year, was the main predictor of prevalence of infection in rodents (interaction term $P = 0.02$). The odds of *Leptospira* infection were nearly threefold higher in rodents trapped in spring compared with summer (OR = 2.86; 95% CI = 1.52–5.38) in year 1; this effect was opposite but not significant in year 2 (OR = 0.50, 95% CI = 0.14–1.80). The same regression model suggested differences by species, although they were not significant at $\alpha = 5\%$. Adjusted odds of infection in *M. musculus* (OR = 3.49, $P = 0.08$) and wild rodents (OR = 4.15, $P = 0.07$) were higher than in *R. norvegicus*.

DISCUSSION

We described social and ecological factors associated with rodent population dynamics and *Leptospira* infection in three distinct types of communities located in southcentral Chile. Nearly all heads of households in farms, villages, and urban slums reported having rodents and signs of rodent presence (e.g., droppings, noises, or sightings), with a greater percentage in rural areas than slums (Table 3). Overall, as well as in farm and village households, the species most frequently trapped was *R. rattus* followed by *M. musculus*. Wild rodents were consistently trapped in the peridomestic area of households from all three community types, suggesting a close interface between wild and domestic environments (Table 2). Evidence of *Leptospira* infection was found in 20.4% of rodents, all species, community types, and rodents trapped inside and outside the households (Table 2). Results showed

TABLE 2

Characteristics and *Leptospira* infection in rodents trapped in 417 households from rural and urban communities in Los Rios Region, Chile, 2010–2012

	Farms		Rural villages		Urban slums		Total	
	No. trapped	No. PCR+	No. trapped	No. PCR+	No. trapped	No. PCR+	No. trapped	No. PCR+
Total no. of rodents	228/393 (58.0%)*	45/228 (19.7%)	108/393 (27.5%)*	28/108 (25.9%)	57/393 (14.5%)*	7/57 (12.3%)	393 (100%)	80/393 (20.4%)
Season trapped								
Spring	97/228 (42.5%)	22/97 (22.7%)	63/108 (58.3%)	18/63 (28.6%)	39/57 (68.4%)	3/39 (7.7%)	199/393 (50.6%)	43/199 (21.6%)
Summer	131/228 (57.5%)	23/131 (17.6%)	45/108 (41.7%)	10/45 (22.2%)	18/57 (31.6%)	4/18 (22.2%)	194/393 (49.4%)	37/194 (19.1%)
Trap location								
Inside†	9/124 (7.3%)	3/9 (33.3%)	23/78 (29.5%)	8/23 (34.8%)	30/53 (56.6%)	4/30 (13.1%)	62/255 (24.3%)	15/62 (24.2%)
Outside	115/124 (92.7%)	28/115 (24.3%)	55/78 (70.5%)	16/55 (29.1%)	23/53 (43.4%)	3/23 (13.0%)	193/255 (75.7%)	47/193 (24.4%)
R. rattus	152/228 (66.7%)	33/152 (21.7%)	78/108 (72.2%)	16/78 (20.5%)	16/57 (28.1%)	2/16 (12.5%)	246/393 (62.6%)	51/246 (20.7%)
Sex								
Male	107/152 (70.4%)	24/107 (22.4%)	57/78 (73.1%)	12/57 (21.1%)	14/16 (87.5%)	2/14 (14.3%)	178/246 (72.4%)	38/178 (21.3%)
Female	45/152 (29.6%)	9/45 (20.0%)	21/78 (26.9%)	4/21 (19.0%)	2/16 (12.5%)	0/2 (0%)	68/246 (27.6%)	13/68 (19.1%)
Age								
Adult	82/152 (53.9%)	20/82 (24.4%)	47/78 (60.3%)	7/47 (14.9%)	12/16 (75.0%)	1/12 (8.3%)	141/246 (57.3%)	28/141 (19.9%)
Juvenile	70/152 (46.1%)	13/70 (18.6%)	31/78 (39.7%)	9/31 (29.0%)	4/16 (25.0%)	1/4 (25.0%)	105/246 (42.7%)	23/105 (21.9%)
R. norvegicus	23/228 (10.1%)	1/23 (4.3%)	6/108 (5.6%)	2/6 (33.3%)	–	–	29/393 (7.4%)	3/29 (10.3%)
Sex								
Male	15/23 (65.2%)	0/15 (0%)	4/6 (66.7%)	0/4 (0%)	–	–	19/29 (65.5%)	0/19 (0%)
Female	8/23 (34.8%)	1/8 (12.5%)	2/6 (33.3%)	2/2 (100%)	–	–	10/29 (34.5%)	3/10 (30.0%)
Age								
Adult	14/23 (60.9%)	1/14 (7.1%)	5/6 (83.3%)	1/5 (25.0%)	–	–	19/29 (65.5%)	2/19 (10.5%)
Juvenile	9/23 (39.1%)	0/9 (0%)	1/6 (16.7%)	1/1 (100%)	–	–	10/29 (34.5%)	1/10 (10.0%)
M. musculus	30/228 (13.2%)	7/30 (23.3%)	13/108 (12.0%)	7/13 (53.8%)	37/57 (64.9%)	4/37 (10.8%)	80/393 (20.4%)	18/80 (22.5%)
Sex								
Male	22/30 (73.3%)	4/22 (18.2%)	9/13 (69.2%)	5/9 (55.6%)	30/37 (81.1%)	3/30 (10.0%)	61/80 (76.2%)	12/61 (19.7%)
Female	8/30 (26.7%)	3/8 (37.5%)	4/13 (30.8%)	2/4 (50.0%)	7/37 (18.9%)	1/7 (14.3%)	19/80 (23.8%)	6/19 (31.6%)
Age								
Adult	16/30 (53.35)	4/16 (25.0%)	11/13 (84.6%)	7/11 (63.6%)	33/37 (89.2%)	4/33 (12.1%)	60/80 (75.0%)	15/60 (25.0%)
Juvenile	14/30 (46.7%)	3/14 (21.4%)	2/13 (15.4%)	0/2 (0%)	4/37 (10.8%)	0/4 (0%)	20/80 (25.0%)	3/20 (15.0%)
Wild rodents	23/228 (10.1%)	4/23 (17.4%)	11/108 (10.2%)	3/11 (27.3%)	4/57 (7.0%)	1/4 (25.0%)	38/393 (9.7%)	8/38 (21.1%)

*Percentage of the total number of rodents (393).

†Denominator was adjusted to exclude rodents from households that did not allow trap placement inside the house.

TABLE 3

Perception of rodent presence and reported control measures in the study households by community type in Los Rios Region, Chile, 2010–2012

	Community type			
	Farms	Rural villages	Urban slums	*P* value
Perceived rodent presence	139/144 (96.5%)	121/131 (92.4%)	121/142 (85.2%)	< 0.01
Any sign of rodent presence	133/144 (92.4%)	108/131 (82.4%)	115/142 (81.0%)	0.01
Rodent signs				
Droppings	88/144 (61.1%)	60/131 (45.8%)	58/142 (40.8%)	< 0.01
Urine	22/144 (15.3%)	11/131 (8.4%)	8/142 (5.6%)	0.02
Gnawed wood	13/144 (9.0%)	5/131 (3.8%)	6/142 (4.2%)	0.11
Gnawed boxes	8/144 (5.6%)	6/131 (4.6%)	6/142 (4.2%)	0.86
Gnawed food	29/144 (20.1%)	4/131 (3.1%)	6/142 (4.2%)	< 0.01
Holes in walls	8/144 (5.6%)	6/131 (4.6%)	5/142 (3.5%)	0.71
Noises	82/144 (56.9%)	69/131 (52.7%)	67/142 (47.2%)	0.25
Seen rodents	33/144 (22.9%)	46/131 (35.1%)	56/142 (39.4%)	< 0.01
Number of rodent signs*	2.0 (0–5)	1.6 (0–4)	1.5 (0–5)	< 0.01
Does rodent control	138/144 (95.8%)	113/131 (86.3%)	112/142 (78.9%)	< 0.01
Rodent control last 6 months	91/109 (83.5%)	62/83 (74.7%)	75/90 (83.3%)	0.24
Cats in the household	86/144 (59.7%)	75/131 (57.3%)	71/142 (50.0%)	0.23
Number of cats†	1.44 (1.9)	1.03 (1.2)	0.73 (1.0)	< 0.01
Dogs in the household	132/144 (91.7%)	111/131 (84.7%)	98/142 (69.0%)	< 0.01
Number of dogs†	2.4 (2.0)	1.7 (1.6)	1.02 (0.9)	< 0.01
Control measures				
Traps	26/144 (18.1%)	26/131 (19.8%)	8/142 (5.6%)	< 0.01
Poison	108/144 (75.0%)	65/131 (49.6%)	72/142 (50.7%)	< 0.01
Cats	74/144 (51.4%)	66/131 (50.4%)	46/142 (32.4%)	< 0.01
Traps or poison	118/144 (81.9%)	81/131 (61.8%)	80/142 (56.3%)	< 0.01
Number of rodent control measures*	1.4 (0–3)	1.2 (0–3)	0.9 (0–2)	< 0.01

*Mean (range) number of signs of rodent presence, including droppings, urine, gnawing, holes in walls, noises, and HH has seen rodents.
†Mean and SD.

seasonal and temporal effects as the major determinants of rodent captures and *Leptospira* infection.

Trapping efforts were consistently more successful in villages and farms, which represent ideal breeding and nesting grounds[27] compared with slums. Furthermore, there was a strong annual effect not fully explained by any of the examined variables, which yielded fewer rodents during the second sampling period. This finding could reflect cyclical changes in rodent population dynamics as well as external factors, such as changes in environmental conditions, habitat quality, and food access, that affect reproductive success and population densities.[28] Most of what is known about the ecology of the local rodent populations is limited to a few wild species, which show distinct seasonal and 2- to 5-year density cycles, including dramatic increases in the population or ratadas after particularly wet years.[29] Trapping efforts during months of higher average temperature (specifically December and January) led to an increased likelihood of not trapping rodents around the household environment. The opposite (a high number of rodent captures when temperature decreases) has been previously documented, because rodents leave the fields and move to or near the peridomestic environment for protection.[30] Although seemingly contradictory with the previous finding, because higher temperatures are expected in summer, higher captures predicted for what was defined as the summer sampling season (January to April) are concurrent with documented increases in breeding and population size during this time.[31] Generally, breeding rates and population size begin to decline with decreasing temperature (March and April in the current study) after the summer spike and decreasing food availability; however, more active foraging for food and nutrients to maintain metabolic rates may increase capture rates because of higher movement rates. Most rodents avoid direct contact with heavy rainfall, but the resulting vegetation growth and higher abundance of leaves and seeds may subsequently increase their foraging activities.[32,33] The positive correlation between the variable summer sampling season and higher captures was stronger

TABLE 4

Results of a zero-inflated regression model of the environmental and household characteristics associated with the presence of rodents in rural and slum communities in Los Rios Region, Chile, 2010–2012

Variables	Coefficient	SE	*P* value
Poisson count model			
Intercept	–0.278	0.976	0.78
Season: summer*	0.320	0.173	0.07
Sampling year: year 2†	–0.670	0.156	< 0.01
Community type‡			
Farms	1.333	0.513	0.01
Villages	0.587	0.565	0.30
Average temperature	–0.032	0.034	0.35
Average rain	0.009	0.004	0.03
Mean household age (years)	–0.039	0.008	0.02
Number rodent signs	0.158	0.054	< 0.01
Number cats	–0.138	0.046	< 0.01
Community type: farms × mean household age	0.031	0.018	0.08
Community type: villages × mean household age	0.039	0.019	0.03
Binomial model			
Intercept	–12.407	6.629	0.06
Season: summer*	2.234	1.511	0.14
Sampling year: year 2†	0.499	0.508	0.33
Community type‡			
Farms	0.196	1.282	0.88
Villages	1.153	1.410	0.41
Average temperature	0.567	0.275	0.04
Average rain	0.036	0.036	0.09
Season: summer × average rain	–0.089	0.042	0.03

*Spring as reference.
†Sampling year 1 as reference.
‡Slum as reference.

when trapping was done in months of higher rainfall, which could be explained by this rain-induced increase in food availability and movement.[30,34] Similar positive correlations between rainfall and capture rates have been observed in other studies.[35,36]

Slums showed notable differences in rodent capture and species diversity. The number of rodents captured was lower in slums compared with the other community types, particularly in the second year of the field work. Beyond normal cyclical changes not captured by this study, a similar study in China examined the effect of proximity to other households on rodent abundance and also found fewer rodents in households with surrounding houses.[8] In slums, most of the rodents trapped were *M. musculus* (64.9%) from the inside of the households, whereas most rodents in the other community types were *Rattus* spp. and trapped outside of the household (Table 2). This finding is consistent with a study of rodents in Argentina, where *M. musculus* was the most frequently captured species in urban environments.[37] These slum communities in southern Chile are small, and ecologically, they are different from other marginalized communities, such as the large slums in Brazil, where rodents, mostly *R. norvegicus*, are found in high densities and have exceptionally high prevalence of *Leptospira* infection.[38,39] Nevertheless, the public health implications of 23% of mice carrying *Leptospira* in close proximity to people's living environment merits additional exploration.

Rodent presence based on the number of observed signs of infestation reported by the head of household correlated with the number of rodents trapped ($P < 0.01$) (Table 4), suggesting that people were well aware of the problem. Active rodent control (using poison or traps) was reportedly practiced by a large majority of households and to a greater extent, farms, which were the households where more rodents were also trapped (Table 3); however, rodent control did not have an effect on rodent captures in the analysis. Cats commonly move and breed freely and/or are purposely kept for rodent control rather than as pets. Greater numbers of cats in the household did correlate in the analysis with fewer trapped rodents ($P < 0.01$) (Table 4), which been found in other reports.[8,40] Because use of traps or poison for control was based on information reported by the head of household, we cannot rule out information bias and the possibility that people tended to overstate their active rodent control efforts. Also, data show awareness about rodent presence but either ineffective or less than optimal control measures. Interestingly, multivariable analysis revealed that the positive association between rural living and number of rodents trapped was also positively correlated with the age of the household members (Table 4), suggesting that households with older members, particularly elderly living in rural areas, may have difficulties carrying out effective rodent control.

Even in temperate climate, overall prevalence of *Leptospira* infection (20.4%) was similar to or slightly lower than PCR or culture-based estimates from other studies, including 11% in Tanzania,[41] 14.8% on the Canary Islands,[42] 26.7% in New Caledonia,[43] and 29.8% on Mayottee Island in India.[44] However, it was much lower than rodent prevalence from reports associated with increase of human cases or outbreak situations, such as 96% in Tandil, Argentina and 43% in Manila, Philippines.[45] Because of the limited value of serology in rodents,[46] the microagglutination test was performed in a subset of 95 rodents only (data not shown), and 2 rodents were seropositive. One rodent, a mouse from a village, reacted with a titer of 1:6,400 to the serovar Bratislava, and the other rodent, a rat from a farm, reacted with a titer of 1:100 to the serovar Icterohaemorrhagiae. The difference in the total number of positive rodents between the two tests could be explained by the genetic makeup of the circulating strains and random laboratory variability. Prior validation of the two PCR protocols in our laboratory using 30 reference strains showed that the 16S/Lepat PCR detected additional strains (the intermediate strains *L. fainei* and *L. licerasiae*) that were not detected by the LipL32 PCR assay. Because of the unavailability of isolates for full genetic classification, we can only speculate that the four rodents that tested positive by 16S/Lepat PCR but negative by LipL32 PCR may have been carriers of intermediate *Leptospira* species.

Inferences about differences in prevalence of infection by species are challenging because of the multiple factors influencing dynamics of infection within the specific rodent populations as well as differences by locality and study methods. The highest prevalence of infection was for *M. musculus* (22.5%), which has been found in other species comparison studies.[43,47] However, mice have also been found to have the lowest prevalence compared with *Rattus* spp.[48] The relative prevalence in *Rattus* spp. varies by study, where for example, in this study, prevalence in *R. rattus* was high and similar to the prevalence in mice; however, the opposite has also been reported.[43] This study and other studies[49] have found the prevalence in *R. novergicus* to be lower than in the other species. No statistically significant differences by sex or age were detected, which limits the ability to make inferences about within-species dynamics of infection. Other studies, in higher prevalence settings, found higher prevalence with increasing age,[43,50] which implies a role of horizontal transmission in the maintenance of infection.[28]

Overall prevalence in this study (20.4%) (Table 2) was lower than the prevalence reported from a previous study carried out in the mid-1990s in the same region with similar trapping methods (37.8%).[17] These studies are not directly comparable, because the study used a combination of serology, culture, and immunohistochemistry for detection of infected rodents; also, no other data are available to determine if the difference corresponds to actual changes over time. Generally speaking, drivers of decreased prevalence include decrease in rodent population size below the necessary threshold to sustain the same level of infection[51,52] and changes in climatic and other environmental conditions that can influence transmission dynamics. Notably, even under lower prevalence, patterns of infection were still comparable with the previously reported findings. In this study and the study by Zamora and Riedemann,[17] a higher prevalence was found in rodents trapped in the spring compared with the summer, rodents from rural areas compared with urban areas, and *M. musculus*, *R. rattus*, and wild rodents compared with *R. norvegicus*. In a theoretical model in which rodent reproduction and *Leptospira* survival were allowed to exhibit seasonal variation, which was expected in the temperate climate of the study area, both numbers of infected rodents and environmental *Leptospira* numbers increased sharply after the onset of the rainy season.[52] High odds of infection in rodents captured in spring season, a warm and rainy period in the study area, have also been found in other studies.[43]

More extensive research has been done on the within-population dynamics of other pathogens, like in the case of hantavirus, which has shown an increased risk of infection in rodents during winter, suggesting a transmission associated with communal nesting and mutual grooming during cold weather.[28] A higher observed prevalence in spring could, in turn, be the combined result of winter nesting and new indirect (environmental) transmission facilitated by alternating periods of heightened foraging activity and spring rainfall.[52] These seasonal factors are expected to play a bigger role in prevalence of infection in *Rattus* spp. and wild species than *M. musculus*, which are mainly found indoors. The small sample limited formal examination of seasonal factors by species; however, wild rodents showed the largest difference in prevalence of infection between spring (31%) and summer (14%).

A knowledge gap exists in the ecology of leptospirosis in rodents. Finding wild and commensal rodent species sharing the same environment underscores the need to improve the understanding of transmission between these species. Some reports support strong species specificity.[53] Preliminary phylogenetic analysis, based on the *secY* gene, of the sequences from positive rodents has shown high similarities within rodent species with some exceptions (for example, high similarity between a rat and a mouse and a mouse and a wild rodent; data not shown). Cross-sectionally, the findings reported in this study contribute to the knowledge of rodent dynamics and leptospirosis in distinct rural and slum communities from temperate climate areas. Some aspects of leptospirosis dynamics in these rodent populations are surprisingly similar to the infection in rodents from tropical areas, but occurrence of human infection is sporadic.[14] Additional analyses are being carried out to examine the ecoepidemiology of leptospirosis in the study area, including the molecular and spatial epidemiology, while taking into consideration the interrelationships between environmental, animal, and anthropogenic factors to gain insight into infection thresholds and drivers of transmission in people and animals.

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REFERENCES

1. Abela-Ridder B, Sikkema R, Hartskeerl RA, 2010. Estimating the burden of human leptospirosis. *Int J Antimicrob Agents 36 (Suppl 1):* S5–S7.
2. Hartskeerl AR, Collares-Pereira M, Ellis WA, 2011. Emergence, control and re-emerging leptospirosis: dynamics of infection in the changing world. *Clin Microbiol Infect 17:* 494–501.
3. Plank R, Dean D, 2000. Overview of the epidemiology, microbiology, and pathogenesis of *Leptospira* spp. in humans. *Microbes Infect 2:* 1265–1276.
4. Watkins S, 1986. Leptospirosis as an occupational disease. *Br J Ind Med 43:* 721–725.
5. Levett PN, 2001. Leptospirosis. *Clin Microbiol Rev 14*: 296–326.
6. Makundi R, Oguge N, Mwanjabe P, 1999. Rodent pest management in East Africa—an ecological approach. Singleton GR, Hinds L, Leirs H, Zhang Z, eds. *Ecologically-Based Rodent Management*. Canberra, Australia: ACIAR, 460–476.
7. Glass GE, Johnson JS, Hodenbach GA, Disalvo CL, Peters CJ, Childs JE, Mills JN, 1997. Experimental evaluation of rodent exclusion methods to reduce hantavirus transmission to humans in rural housing. *Am J Trop Med Hyg 56:* 359–364.
8. Yin JX, Geater A, Chongsuvivatwong V, Dong XQ, Du CH, Zhong YH, McNeil E, 2008. Predictors for presence and abundance of small mammals in households of villages endemic for commensal rodent plague in Yunnan province, China. *BMC Ecol 8:* 18.
9. Bonner PC, Schmidt WP, Belmain SR, Oshin B, Baglole D, Borchert M, 2007. Poor housing quality increases risk of rodent infestation and Lassa fever in refugee camps of Sierra Leone. *Am J Trop Med Hyg 77:* 169–175.
10. Meerburg BG, Singleton GR, Kijlstra A, 2009. Rodent-borne diseases and their risks for public health. *Crit Rev Microbiol 35:* 221–270.
11. Alexander AD, 1960. The distribution of leptospirosis in Latin America. *Bull World Health Organ 23:* 113–125.
12. Arias HP, Nunez MG, Valenzuela IG, Olivares AM, 2003. Brote epidémico de leptospirosis en niños de Linares. *Rev Chil Pediatr 74:* 405–410.
13. Martinez P, Ortega D, Salinas K, 2012. Evolucion de la leptospirosis segun el Sistema de Vigilancia Epidemiologica Nacional, Chile 2003–2009. *Rev Chilena Infectol 29:* 648–654.
14. Zamora J, Riedemann S, Montecinos MI, Cabezas X, 1990. Serological survey of human leptospirosis in a high risk population in Chile. *Rev Med Chil 118:* 247–252.
15. Zamora J, Riedemann S, Tadich N, 1999. A serological survey of leptospirosis in sheep in Chile. *Rev Latinoam Microbiol 41:* 73–76.
16. Silva RF, Riedemann S, 2007. Frequency of canine leptospirosis in dogs attending veterinary practices determined through microscopic agglutination and comparison with isolation and immunofluorescence techniques. *Arch Med Vet 39:* 269–274.
17. Zamora J, Riedemann S, 1999. Wild animals as reservoirs of leptospirosis in Chile. Revision of studies in the country. *Arch Med Vet 31:* 151–156.
18. Iriarte A, 2009. *Field Guide to the Mammals of Chile*. Santiago, Chile: Ediciones Flora y Fauna Chile.
19. Stoddard RA, Gee JE, Wilkins PP, McCaustland K, Hoffmaster AR, 2009. Detection of pathogenic *Leptospira* spp. through TaqMan polymerase chain reaction targeting the LipL32 gene. *Diagn Microbiol Infect Dis 64:* 247–255.
20. Murgia R, Riquelme N, Baranton G, Cinco M, 1997. Oligonucleotides specific for pathogenic and saprophytic leptospira occurring in water. *FEMS Microbiol Lett 148:* 27–34.
21. Atkins DC, Gallop RJ, 2007. Rethinking how family researchers model infrequent outcomes: a tutorial on count regression and zero-inflated models. *J Fam Psychol 21:* 726–735.
22. Linder JM, Lawler RR, 2012. Model selection, zero-inflated models, and predictors of primate abundance in Korup national park, Cameroon. *Am J Phys Anthropol 149:* 417–425.
23. Zeileis A, Kleiber C, Jackman S, 2008. *Regression Models for Count Data in R. Journal of Statistical Software 27:* 11/28/12. Available at: http://www.jstatsoft.org/v27/i08/. Accessed December 7, 2013.
24. Harrell FE Jr, 2012. *Regression Modeling Strategies*. Department of Biostatistics, *Vanderbilt University School of Medicine*. Available at: http://biostat.mc.vanderbilt.edu/rms. Accessed December 7, 2013.
25. Bates D, Maechler M, Bolker B, 2011. *lme4: Linear Mixed-Effects Models Using S4 Classes*. *R package version 0.999375-42*. Available at: http://lme4.r-forge.r-project.org. Accessed December 7, 2013.

26. R Development Core Team, 2012. *R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria*. Available at: http://www.R-project.org/. Accessed December 7, 2013.
27. Ortiz J, Venegas W, Sandoval J, Chandía P, Torres-pérez F, 2004. Hantavirus in rodents of the VIII region of Chile. *Rev Chil Hist Nat 77:* 251–256.
28. Mills JN, 2005. Regulation of rodent-borne viruses in the natural host: implications for human disease. *Arch Virol Suppl 19:* 45–57.
29. Spotorno AE, Palma RE, Valladares JP, 2000. Biology of rodent reservoirs of hantavirus in Chile. *Rev Chilena Infectol 17:* 197–210.
30. Dickman CR, Mahon PS, Masters P, Gibson DF, 1999. Long-term dynamics of rodent populations in arid Australia: the influence of rainfall. *Wildl Res 26:* 389–403.
31. Calhoun JB, 1963. *The Ecology and Sociology of the Norway Rat*. Bethesda, MD: US Department of Health, Education, and Welfare, Public Health Service.
32. Mutze GJ, Veitch LG, Miller RB, 1990. Mouse plagues in south Australian cereal-growing areas. II. An empirical model for prediction of plagues. *Aust Wildl Res 17:* 313–324.
33. Leirs H, Verhagen R, Verheyen W, Mwanjabe P, Mbise T, 1996. Forecasting rodent oubreaks in Africa: an ecological basis for mastomys control in Tanzania. *J Appl Ecol 33:* 937–943.
34. Boonstra R, Redhead TD, 1994. Population dynamics of an outbreak population of house mice (*Mus domesticus*) in the irrigated rice-growing area of Australia. *Wildl Res 21:* 583–598.
35. Dowler RC, Katz HM, Katz AH, 1985. Comparison of live trapping methods for surveying small mammal populations. *Northeast Environ Sci 4:* 165–171.
36. Stokes MK, Slade NA, Blair SM, 2001. Influences of weather and moonlight on activity patterns of small mammals: a biogeographical perspective. *Can J Zool 79:* 966–972.
37. Calderon G, Pini N, Bolpe J, Levis S, Mills J, Segura E, Guthmann N, Cantoni G, Becker J, Fonollat A, Ripoll C, Bortman M, Benedetti R, Enria D, 1999. Hantavirus reservoir hosts associated with peridomestic habitats in Argentina. *Emerg Infect Dis 5:* 792–797.
38. Maciel EA, de Carvalho AL, Nascimento SF, de Matos RB, Gouveia EL, Reis MG, Ko AI, 2008. Household transmission of *Leptospira* infection in urban slum communities. *PLoS Negl Trop Dis 2:* e154.
39. de Faria MT, Calderwood MS, Athanazio DA, McBride AJ, Hartskeerl RA, Pereira MM, Ko AI, Reis MG, 2008. Carriage of *Leptospira interrogans* among domestic rats from an urban setting highly endemic for leptospirosis in Brazil. *Acta Trop 108:* 1–5.
40. Malcolm I, 2006. *Socio-Economic Factors Influencing the Transmission of Rodent-Borne Diseases in Southern Africa*. London, UK: Natural Resources Institute, University of Greenwich.
41. Mgode GF, Mhamphi G, Katakweba A, Paemelaere E, Willekens N, Leirs H, Machang'u RS, Hartskeerl RA, 2005. PCR detection of *Leptospira* DNA in rodents and insectivores from Tanzania. *Belg J Zool 135:* 17–19.
42. Foronda P, Martin-Alonso A, Del Castillo-Figueruelo B, Feliu C, Gil H, Valladares B, 2011. Pathogenic *Leptospira* spp. in wild rodents, Canary Islands, Spain. *Emerg Infect Dis 17:* 1781–1782.
43. Perez J, Brescia F, Becam J, Mauron C, Goarant C, 2011. Rodent abundance dynamics and leptospirosis carriage in an area of hyper-endemicity in New Caledonia. *PLoS Negl Trop Dis 5:* e1361.
44. Desvars A, Naze F, Vourc'h G, Cardinale E, Picardeau M, Michault A, Bourhy P, 2012. Similarities in *Leptospira* serogroup and species distribution in animals and humans in the Indian Ocean Island of Mayotte. *Am J Trop Med Hyg 87:* 134–140.
45. Villanueva SY, Ezoe H, Baterna RA, Yanagihara Y, Muto M, Koizumi N, Fukui T, Okamoto Y, Masuzawa T, Cavinta LL, Gloriani NG, Yoshida S, 2010. Serologic and molecular studies of *Leptospira* and leptospirosis among rats in the Philippines. *Am J Trop Med Hyg 82:* 889–898.
46. Vinetz JM, Glass GE, Flexner CE, Mueller P, Kaslow DC, 1996. Sporadic Urban Leptospirosis. *Ann Intern Med 125:* 794–798.
47. Desvars A, Naze F, Benneveau A, Cardinale E, Michault A, 2012. Endemicity of leptospirosis in domestic and wild animal species from Reunion Island (Indian Ocean). *Epidemiol Infect 141:* 1154–1165.
48. Rahelinirina S, Leon A, Harstskeerl RA, Sertour N, Ahmed A, Raharimanana C, Ferquel E, Garnier M, Chartier L, Duplantier JM, Rahalison L, Cornet M, 2010. First isolation and direct evidence for the existence of large small-mammal reservoirs of *Leptospira* sp. in Madagascar. *PLoS One 5:* e14111.
49. Webster JP, Ellis WA, Macdonald DW, 1995. Prevalence of *Leptospira* spp. in wild brown rats (*Rattus norvegicus*) on UK farms. *Epidemiol Infect 114:* 195–201.
50. Krojgaard LH, Villumsen S, Markussen MD, Jensen JS, Leirs H, Heiberg AC, 2009. High prevalence of *Leptospira* spp. in sewer rats (*Rattus norvegicus*). *Epidemiol Infect 137:* 1586–1592.
51. Madhav NK, Wagoner KD, Douglass RJ, Mills JN, 2007. Delayed density-dependent prevalence of Sin Nombre Virus antibody in Montana deer mice (*Peromyscus maniculatus*) and implications for human disease risk. *Vector Borne Zoonotic Dis 7:* 353–364.
52. Holt J, Davis S, Leirs H, 2006. A model of leptospirosis infection in an African rodent to determine risk to humans: seasonal fluctuations and the impact of rodent control. *Acta Trop 99:* 218–225.
53. Turk N, Milas Z, Margaletic J, Staresina V, Slavica A, Riquelme-Sertour N, Bellenger E, Baranton G, Postic D, 2003. Molecular characterization of *Leptospira* spp. strains isolated from small rodents in Croatia. *Epidemiol Infect 130:* 159–166.