

## PERIDOMESTIC SMALL MAMMALS ASSOCIATED WITH CONFIRMED CASES OF HUMAN HANTAVIRUS DISEASE IN SOUTHCENTRAL CHILE

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**Abstract.** Cases of human hantavirus disease have been reported in Chile since 1995, most of them in people living in rural and periurban areas. We conducted a peridomestic study of small mammals to evaluate the relationships between the presence of rodents with antibodies to Andes virus confirmed human cases of hantavirus pulmonary syndrome in southcentral Chile. The results of 20 sampled sites, which involved the capture of 272 mice over an 18-month period, showed the occurrence of 10 small mammal species, of which *Oligoryzomys longicaudatus* was the only seropositive species for hantavirus, with an intra-specific serologic rate of 10.4%.

### INTRODUCTION

Human diseases caused by hantaviruses (genus *Hantavirus*, family Bunyaviridae) have been reported in several countries.<sup>1,2</sup> In the Americas, this disease is known as hantavirus pulmonary syndrome (HPS)<sup>3</sup> and is one of the most dangerous emerging infectious diseases.<sup>4</sup> Several rodent species in the Americas constitute the reservoir for distinctive strains of *Hantavirus* with high levels of species-specificity among them.<sup>1,5</sup> The disease is transmitted to humans by inhalation of virus-contaminated aerosols of rodent excreta<sup>6</sup> and secretions.<sup>7</sup>

The first case of HPS reported in Chile was in 1995, although it has been retrospectively reported in 1975.<sup>8</sup> To date, five rodent species of the subfamily Sigmodontinae (Muridae) have been identified to be serologically positive for hantavirus in Chile.<sup>9,10</sup> Among them, the species *Oligoryzomys longicaudatus*, the colilargo (tribe Oryzomyini), is one of the major reservoirs for the virus.<sup>11</sup> Other sigmodontines that have been found to be positive for the virus in Chile (although at a considerably lower rate) are *Abrothrix longipilis* (ratón de pelo largo), *Abrothrix olivaceus* (ratón oliváceo), *Phyllotis darwini* (ratón orejudo), and *Loxodontomys micropus* (pericote del sur).<sup>9</sup> All of these species have an extensive range of distribution in Chile.<sup>12</sup> In fact, *O. longicaudatus* ranges from Copiapó (28°S) southward to Aysén (48°S), as well as to the southern Patagonian forests of Argentina.<sup>13</sup> This species is mainly restricted to forests and bushy areas, showing a preference for humid habitats near rivers and other water sources.<sup>14,15</sup> A second species, *O. magellanicus*, inhabits the forests of region XII.<sup>13</sup> However, neither human cases nor seropositive *O. magellanicus* have been reported in region XII. The other taxa, *A. longipilis* and *A. olivaceus*, coexist with *O. longicaudatus* along most of its distributional range.

The first confirmed human case of HPS reported in Chile was in Llanquihue Province (X region) in 1995. Since then, several other cases have been confirmed along the geographic range of *O. longicaudatus*. One possible explanation for this emerging disease has been the settlement of human populations in periurban and rural areas near the habitats where wild rodents occur, which would facilitate the exposure of human populations.<sup>16</sup> For this reason, rural (inhabiting in the countryside outside cities and towns) and periurban (around

villages and towns) human populations are usually considered to be at higher risk of acquiring the disease than people living in cities. In addition, the large home range (320–4,800 m<sup>2</sup>) and high mobility reported for *O. longicaudatus* in populations of southern Chile<sup>14</sup> increase the probability of contact between wild rodents and people, particularly in rural areas. However, a more restricted home range and mobility has been reported for colilargo populations in central Chile.<sup>17</sup>

To evaluate the relationships between confirmed cases of HPS and the occurrence of seropositive rodents, we conducted a survey in periurban and rural areas of southcentral Chile to determine which species are involved in the disease. To date, *O. longicaudatus* has been reported as the sole reservoir for Andes virus in Chile, and no information has been obtained about the participation of others sigmodontine species in the transmission of the disease. In addition, we report the type of human activity that people were conducting at the moment of contracting the disease, as well as the site category and the type of habitat where the cases were confirmed.

### MATERIALS AND METHODS

**Study area.** Peridomestic trapping was conducted in collaboration with the Ministry of Health. Immediately after serologic confirmation of HPS, Ministry of Health staff interviewed the index case and family members to identify all potential exposure sites where the HPS patient had been in the 45 days prior to symptom onset. In each case, we contacted the local Health Service representative to determine the exact location for small mammal sampling. Trapping was then initiated at all potential exposure sites. Most of the sites sampled were located between regions V and X in Chile (Figure 1) where two ecogeographic zones meet (the Mediterranean and the Temperate Forests regions), and where approximately two-thirds of the Chilean population is concentrated. Infectious sites were defined as periurban and rural as previously described,<sup>18</sup> with modifications.

We defined periurban as those areas located between 500 and 3,000 meters from a small town or city (5,000 or more inhabitants). Rural sites are defined as areas that might be close to (approximately 500–1,000 meters) a small village (up to 5,000 inhabitants) or in the countryside. Periurban sites in-

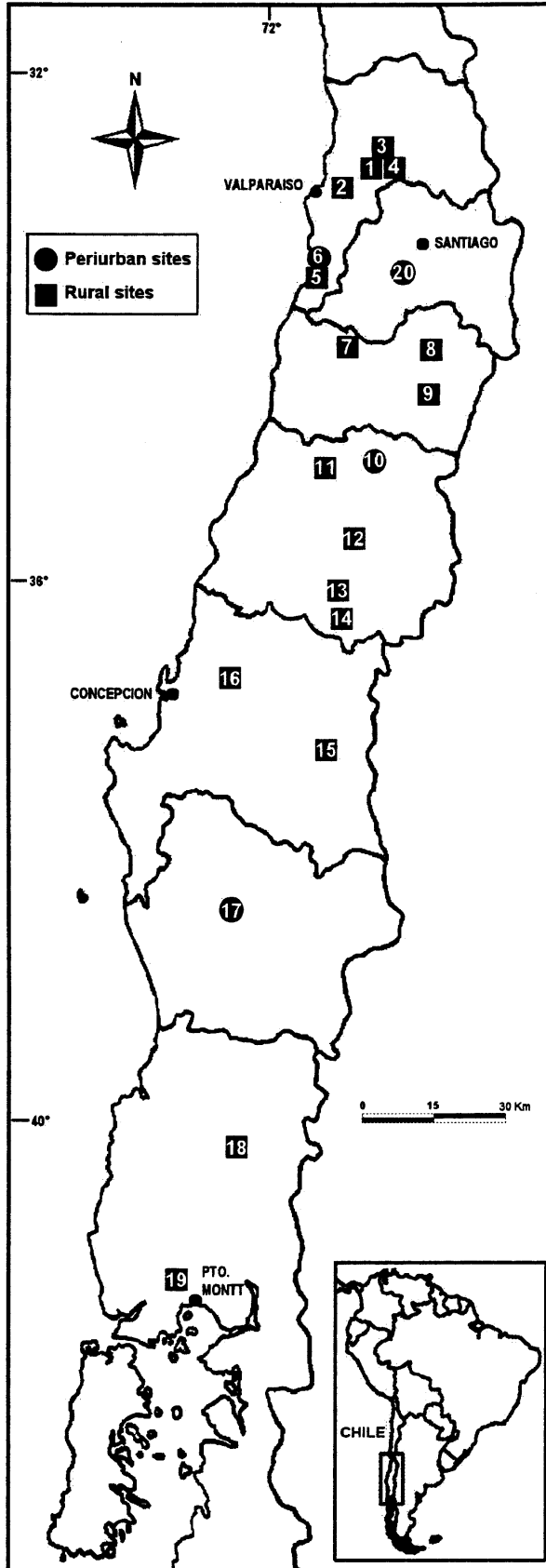


FIGURE 1. Sampling sites along the study area in Chile (numbers are as in Table 1).

cluded yards, adjoining lands, outbuildings, weeds, ponds, modified lands, natural understories, cultivated areas, and fence lines. Rural sites included the countryside such as cultivated areas, ponds, natural or planted forests, modified lands, natural understories, bamboo (e.g., *Chusquea* sp.), ravines, camping sites, outbuildings, and weeds (Table 1). We also described the most probable kind of human activity that people were conducting when exposed to the virus. We classified these as farmers, resident people (people that live in the area of exposure), and visitors (persons who do not live or work in the area of exposure; e.g., camping, trekking, etc).

**Trapping.** Between July 2001 and December 2002 we visited 20 sites (Table 1) where confirmed cases of hantavirus disease were reported. All sampled sites were located between regions V and X (32–42°S) classified in four periurban and 16 rural sites (Figure 1 and Table 1). Trapping was conducted using Sherman traps (8 × 9 × 23 cm; H. B. Sherman Traps, Inc., Tallahassee, FL) for three nights at each site (trappings varied between 225 and 771 traps/night depending on the availability of sites in the area) and the bait used was a mixture of oats and tinned fish. When trapping locations included human buildings (e.g., houses, sheds), traps were set around and below them (when possible). In 11 of the 20 sites studied traps were set around houses and immediate vicinities since not all sites included human constructions. Immediate vicinities are defined as grassy areas, timber, and/or log piles, crops, etc. The number of traps set around houses varied depending on the area of the same, but the numbers varied between 10 and 15 traps. Additional trapping places involved sites far from human buildings where local people previously detected rodents. We followed established safety guidelines for rodent captures and processing according to the Center for Disease Control and Prevention (Atlanta, GA) protocols.<sup>19</sup> Animals in the field were anesthetized and blood was drawn from the retro-orbital sinus for further serologic analyses. Blood samples were preserved in liquid nitrogen and brought to the laboratory for analysis. Specimens were identified in the field with further taxonomic confirmation in the laboratory at the species level. In addition, for each specimen the heart, kidney, spleen, liver, and lung were extracted, and stored in liquid nitrogen for further virus detection. Voucher specimens were preserved in 96% ethanol, and deposited in the Colección de Flora y Fauna, Professor Patricio Sánchez Reyes, Pontificia Universidad Católica de Chile (Santiago, Chile) and in the Division of Mammals of the Museum of Southwestern Biology, University of New Mexico (Albuquerque, NM). We evaluated small mammals captures by calculating the relative density, calculated as the number of captures/number of total traps × 100.

**Serologic test.** Serologic analyses were performed on all rodent samples. All analyses were performed in the Centro de Investigaciones Médicas, Pontificia Universidad Católica de Chile (Santiago, Chile). Antibodies against Andes virus were detected in blood samples using the strip immunoassay vacuum-blot test.<sup>20</sup> Briefly, 5 µL of rodent blood was used to probe a 1.6 × 5-cm nitrocellulose membrane bearing a horizontal ~0.5-mm × 1.6-mm band containing approximately 300 ng of affinity-purified recombinant Andes virus N antigen overnight at room temperature in a volume of 1 mL. After washes in detergent buffer, any bound antibodies to N antigen were detected by incubating the membrane with a 1:1,000

TABLE 1

Sampled sites, location, category of sites, habitat, human activity of affected, and relative density of rodent species captured in the sampling period (July 2001–December 2002)\*

Sampled site (Georeference)	Habitat category	Habitat	Human activity of affected	Night traps	Relative density† of species captured										
					Al	Ao	As	Lm	Ol	Pd	Mm	Rn	Rr	Te	
1. Llai Llay V region (32° 51' S; 70° 55' W)	R	Cultivated area, weeds	Farm worker	237	0	0.4	0	0	0	0	0	2.5	0.4	0	0
2. Villa Alemana V region (33° 4' S; 70° 21' W)	R	Cultivated area, weeds, natural understory	Visitor	300	0	4.7	0	0	0	0	0	1.0	0	0.3	0
3. Hijuélas I V region (32° 48' S; 70° 08' W)	R	Cultivated area, weeds	Farm worker	210	0	1.0	0	0	0	0	0	1.0	0.5	0	0
4. Hijuélas II V region (32° 49' S; 71° 6' W)	R	Cultivated area, natural understory, ravines	Farm worker	270	0	0	0	0	0.4	0	0	0	1.1	0	1.5
5. Santo Domingo V region (33° 44' S; 71° 39' W)	R	Cultivated area, ponds, weeds	Visitor	300	4.0	6.7	0	0	0	0	0	0	0.7	0	0
6. San Antonio V region (33° 34' S; 71° 37' W)	P	Yards, outbuildings, adjoining lands, fence lines, weeds	Resident	225	0	0	0	0	0.4	0	0	1.3	0	0.9	0
7. Las Cabras VI region (34° 4' S; 71° 17' W)	R	Ponds, modified land, weeds	Resident	300	0.3	1.0	0	0	2.7	0	0	0	0.7	0	0
8. Coya VI region (34° 11' S; 70° 32' W)	R	Outbuildings, modified land, weeds, ravines	Resident	145	0	0.7	0	0	0.7	1.4	0	0	3.4	0	0
9. San Fernando VI region (34° 46' S; 70° 46' W)	R	Ponds, modified land, weeds	Resident	360	0	0.6	0	0	0.3	0	0	0	0	0	0.3
10. Romeral VII region (34° 58' S; 71° 7' W)	P	Ponds, modified land, natural understory, weeds	Resident	300	0.7	3.0	0	0	0.3	0	0	0	0	0.7	0
11. Rauco VII region (35° 1' S; 71° 32' W)	R	Cultivated area, weeds	Farm worker	270	0	0.7	0	0	0.4	0	0	0	0.4	0	0
12. San Clemente VII region (35° 29' S; 71° 13' W)	R	Cultivated area, weeds	Farm worker	300	0	0.7	0	0	0	0	0	0	0	0.7	0
13. Longaví VII region (36° 1' S; 71° 30' W)	R	Cultivated area, ponds, weeds, ravines	Farm worker	375	0.3	1.9	0	0	0.8	0	0	0	0	0	0
14. Bullileo VII region (36° 17' S; 71° 24' W)	R	Natural and planted forest, weeds, camping sites	Farm worker, resident and visitors	240	0	0.4	0	0	1.3	0	0	0	0.4	1.3	0
15. Tucapel VIII region (37° 14' S; 71° 47' W)	R	Natural forest, weeds, camping sites, bamboo	Visitor	300	1.3	7.0	0	0	3.0	0	0	0	0	0.3	0
16. Quillón VIII region (36° 43' S; 72° 33' W)	R	Cultivated area, ponds, weeds	Farm worker	288	0	1.4	0	0	2.1	0	0	0	1.0	1.0	0
17. Lautaro IX region (38° 31' S; 72° 25' W)	P	Yards, outbuildings, adjoining lands, weeds	Visitor	300	0	2.0	0	0	0.3	0	0	0.7	0	0	0
18. Lago Ranco X region (40° 21' S; 72° 18' W)	R	Natural forest, bamboo	Farm worker	273	3.6	1.8	0.7	0	3.3	0	0	0	0	0	0
19. Puerto Montt X region (41° 25' S; 73° 11' W)	R	Natural forest, modified land, weeds, bamboo	Resident	771	0.3	1.3	0	0.1	0.4	0	0	0	0	0.1	0
20. Talagante Metropolitan region (33° 39' S; 70° 52' W)	P	Cultivated area, outbuildings, weeds	Farm worker	240	0	6.7	0	0	0	0	0	0.4	2.1	0	0

\* Al = *Abrothrix longipilis*; Ao = *Abrothrix olivaceus*; As = *Abrothrix sanborni*; Lm = *Loxodontomys micropus*; Ol = *Oligoryzomys longicaudatus*; Pd = *Phyllotis darwini*; Mm = *Mus musculus*; Rn = *Rattus norvegicus*; Rr = *Rattus rattus*; Te = *Thylamys elegans*; R = rural; P = periurban.

† Assessed as trap success, indicated as the number of captures/number of total traps × 100.

dilution of alkaline phosphatase–conjugated deer mouse anti-*Peromyscus leucopus* IgG and revealed with the 5-bromo, 4-chloro, 3-indolylphosphate/nitroblue tetrazolium (BCIP/NBT) phosphatase substrate system (Kirkegaard and Perry Laboratories, Gaithersburg, MD).

## RESULTS

In the 18-month study period, 82 human cases were confirmed as positive for HPS in the regions mentioned earlier (information available from the web page of Ministerio de

Salud de Chile, <http://epi.minsal.cl>). Sampling was conducted in the southcentral area of Chile as human cases were arising. However, in several cases human disease reports occurred almost simultaneously, making it difficult to sample all localities at the same time by a single field team. When more than one case was reported simultaneously, we chose that site that involved more people with the disease. Additionally, we selected localities that were not sampled previously. Of the 20 selected sites, six were located in region V, three in region VI, five in region VII, two in region VIII, one in region IX, two in region X, and one in the Metropolitan region (Santiago). We characterized 16 rural and 4 periurban sites (Figure 1 and Table 1). *Oligoryzomys longicaudatus* was captured both in periurban and rural sites. Fourteen habitat types were characterized in the sampled areas, including natural forests, cultivated areas, and weeds. The latter was the most prevalent type of habitat, while fence lines and planted forests were the least represented (Table 1). Of the three types of human activities described earlier, 10 cases were farm workers, seven were residents, and five were visitors.

We trapped nine species of rodents and one mouse-opossum of the following species: Sigmodontinae: *O. longicaudatus*, *A. olivaceus*, *A. longipilis*, *Abrothrix sanborni*, *P. darwini*, *L. micropus*; Murinae: *Rattus norvegicus*, *Rattus rattus*, *Mus musculus*; Didelphimorphia: *Thylamys elegans*. All localities sampled had at least one species that had been reported serologically positive for hantavirus either in this or other studies.<sup>9,10</sup> We captured 272 specimens of these 10 species. Nine of the 11 sites where traps were set around houses recorded sigmodontine and murine mice. The species trapped around human buildings were *O. longicaudatus*, *A. olivaceus*, and the three murine rodents. Forty-five mice were trapped around houses (of a whole total of 272 specimens), of which 21 were murines, 21 *A. olivaceus*, and 4 *O. longicaudatus*. Of these, only one specimen was seropositive for Andes virus (*O. longicaudatus*); this was from the locality of Longaví (region VII).

*Abrothrix olivaceus* was the species most frequently trapped (127 individuals, 46.7%), being collected in 18 of the 20 sites, and absent only in the localities of Hijuelas II and San Antonio (both in the V region). The colilargo *O. longicaudatus*, was found in 14 localities and was the second most frequently captured species (48 individuals, 17.65%). The sigmodontine *A. sanborni* was the least captured species, being trapped only in Lago Ranco (X Region). The sites of Quillón and Puerto Montt showed the highest species richness (five species), while Lago Ranco and Puerto Montt showed the highest abundance of sigmodontine mice with four species.

Relative density (Table 1) of sigmodontine rodents ranged between 0.1 (*L. micropus*) to 7.0 (*A. olivaceus*). Among murine rodents these values ranged between 0.1 (*R. rattus*) and 2.5 (*M. musculus*). The highest relative density for *O. longicaudatus* was in Lago Ranco (3.3). Among sites, the highest mean relative densities were in Tucapel (11.7) and Santo Domingo (11.3), while the lowest density was in San Fernando (1.12).

Five *O. longicaudatus* were positive for antibodies to Andes virus (seroprevalence rate of 1.87%). All seropositive colilargos were adult males. There was one seropositive mouse in Coya (region VI), one in San Fernando (region VI), and one in Longaví (region VII), and there were two seropositive mice in Tucapel (region VIII).

## DISCUSSION

The seroprevalence rate of 1.87% in our study contrasts with other serologic reports in South America, which have shown higher rates (3–7%).<sup>10,18,21</sup> Indeed, when these studies considered only sigmodontine rodents, seropositive rates increased to between 3% and 11%.<sup>10,18,22–24</sup> When we considered only sigmodontine mice, our rate increased to 2.36%. This rate is similar to that of 2.28% obtained in an ongoing latitudinal study in Chile, which involves a great variety of wild and semi-undisturbed areas, as well as a high trapping density (Palma RE and others, unpublished data). A probable explanation for the lower rate obtained in Chile compared with that of Argentina may be the higher diversity of sigmodontine mice recognized in Argentina.<sup>25</sup> The latter rate may increase the probability of potential reservoirs. In fact, several genetic variants<sup>26,27</sup> or hantavirus strains<sup>28</sup> have been described in Argentina in five species of rodents, with four pathogenic for humans.

Trapping mice success (and serologic results) in our study was related to the type of areas sampled. In fact, there was a high degree of human intervention at many of the sites, particularly those in periurban areas, including poisoning of rodents prior to trapping, manipulation of traps by local residents, and loss of traps. However, the low number of rodents captured and the relative frequencies of trap success were similar to that in other studies that have involved peridomestic studies.<sup>18</sup>

Seropositive rodents were found mostly in rural sites, which were areas of highest risk in which people could get the disease, thus supporting results of previous studies.<sup>10</sup> Four of the 20 sampling sites had *O. longicaudatus* positive for Andes virus, representing a 20% success rate in detecting seropositive mice in areas where transmission presumptively occurred. In addition, mice were most likely to be found in areas with the presence of weeds (18 sites), cultivated areas (10 sites), and natural understory or forests (7 sites), which are widely developed in the Mediterranean region in Chile. Houses and other human buildings also constituted areas of high risk since a significant number of sigmodontine mice were trapped around them and/or in their vicinities. Interestingly, of the four colilargos trapped in this kind of area, one was seropositive for hantavirus. With respect to human infection, farm workers were a higher risk category. Risk of infection was also high in rural environments for both farmers and residents, as well as for visitors during the spring and summer.<sup>29</sup>

To date, we have captured the five species reported as seropositive for hantavirus in Chile.<sup>9</sup> It is significant that five *O. longicaudatus* were seropositive since this supports previous publications that suggest that *O. longicaudatus* is the major reservoir of the virus.<sup>11,27,28</sup> The species was captured both in periurban and rural sites (21.4% and 78.6%, respectively), with the highest abundance found in natural (Lago Ranco) and little disturbed areas (e.g., Tucapel). The presence of this species in periurban zones is an indication of high risk for exposure to hantavirus, since considerable numbers of people are moving to periurban areas.<sup>30</sup> In addition, at the intra-specific level, we have obtained one of the higher serologic rates for *O. longicaudatus* (10.4%) compared with other studies in the southern cone of South America. Finally, it is important to mention that at least in this study, *O. longicaudatus*

*datu* was the only species found positive for Andes virus even though the most frequently trapped species was *A. olivaceus* (more than two-fold higher).

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Small mammals present in areas where hantavirus cardiopulmonary syndrome (HCPS) cases had occurred in central and southern Chile were captured and analyzed to evaluate the abundance of rodents and seroprevalence rates of antibodies to Andes orthohantavirus (ANDV). Sampling areas ranged from the Coquimbo to Ays n regions (30 45  S approx.) regions. Ninety-two sites in peridomestic and countryside areas were evaluated in 19 years of sampling. An antibody against ANDV was detected by strip immunoassay in 58 of 1847 specimens captured using Sherman traps. Of the eleven species of rodents sampled, *Abrothrix olivacea*, *Oligoryzomys longicaudatus* and *Abrothrix hirta* were the most frequently trapped. Small mammals present in areas where hantavirus cardiopulmonary syndrome (HCPS) cases had occurred in central and southern Chile were captured and analyzed to evaluate the abundance of rodents and seroprevalence rates of antibodies to Andes orthohantavirus (ANDV). Sampling areas ranged from the Coquimbo to Ays n regions (30 45  S approx.) regions. Ninety-two sites in peridomestic and countryside areas were evaluated in 19 years of sampling. Climate Clinics and Practice Clocks & Sleep Coatings Colloids and Interfaces Compounds Computation Computers Condensed Matter Conservation Construction Materials Corrosion and Materials Degradation (CMD) Cosmetics COVID Crops Cryptography... This chapter focuses on hantaviruses of known human health importance. In humans, New World hantaviruses cause hantavirus pulmonary syndrome (HPS) and Old World hantaviruses cause hemorrhagic fever with renal syndrome (HFRS). Hantaviruses are distributed world-wide and are maintained primarily in rodent hosts. Torres-P rez F, Navarrete-Droguett J, Aldunate R, Yates TL, Mertz GJ, Vial PA, Ferres M, Marquet PA, Palma RE (2004) Peridomestic small mammals associated with confirmed cases of human hantavirus disease on southcentral Chile. *Am J Trop Med Hyg* 70:305 309PubMedGoogle Scholar.