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Original article

Human seroepidemiology of *Rickettsia* and *Orientia* species in Chile – A cross-sectional study in five regions

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ABSTRACT

In recent years, the spectrum and epidemiology of human rickettsioses has become an emerging topic in Chile. This survey aimed to assess the seroprevalence of spotted fever group rickettsiae (SFGR), typhus group rickettsiae (TGR), and scrub typhus group orientiae (STGO) in northern, central, and southern Chile. We performed a cross-sectional study of healthy adults in rural and urban settings of five regions. Participants were chosen by double stratified random sampling in urban and by convenience in rural locations (n = 1302). Serum specimens were analyzed for group-specific IgG antibodies against SFGR, TGR, and STGO by enzyme-linked immunosorbent assays (ELISAs). Overall seroprevalences to SFGR, TGR, and STGO were 5.3 %, 1.2 %, and 0.4 %, respectively. Prevalences showed geographical differences. Statistical analyses revealed an association of older age with seropositivity to SFGR and to TGR and of rural setting and male gender with seropositivity to SFGR. The study indicates that SFGR, TGR, and STGO are endemic in Chile. The very low STGO seroprevalence might indicate an insufficient sensitivity of serological tests using Asian *O. tsutsugamushi* strains as ELISA antigens for the detection of antibodies against Chilean *Orientia* species.

1. Introduction

Rickettsiales are obligate intracellular bacteria transmitted by arthropod vectors. Rickettsial diseases are rapidly emerging and are now found in many regions, where they had not been identified before (Blanton, 2019). The main reasons for this growing spectrum are new molecular tools together with advanced cell culture systems and increasing clinical awareness (Merhej et al., 2014). Frequently, new *Rickettsia* species were firstly identified in their vectors and only recognized decades later as human pathogens (Paddock et al., 2004).

In Chile, members of the Rickettsiales have just been discovered recently. The most important finding was the identification of *Orientia* species, causing South American scrub typhus, which was identified in patients in southern Chile (Balcells et al., 2011; Weitzel et al., 2016a). Other emerging species include *Rickettsia felis,* found in fleas and hard ticks (Abarca et al., 2013a; Labruna et al., 2007), and the veterinary pathogens *Anaplasma platys* and *Ehrlichia canis*, isolated from symptomatic dogs (Abarca et al., 2007; López et al., 2012). Furthermore, species of unknown pathogenicity, *Candidatus* Rickettsia andeanae and other unidentified spotted fever group rickettsia (SFGR), have been found in hard and in soft ticks (Abarca et al., 2012, 2013b; Ogrzewalska et al., 2016; Muñoz-Leal et al., 2019). Up to now, our knowledge of the distribution, prevalence, and clinical relevance, as well as spectrum of rickettsial species in Chile is incomplete. Here we present a cross-

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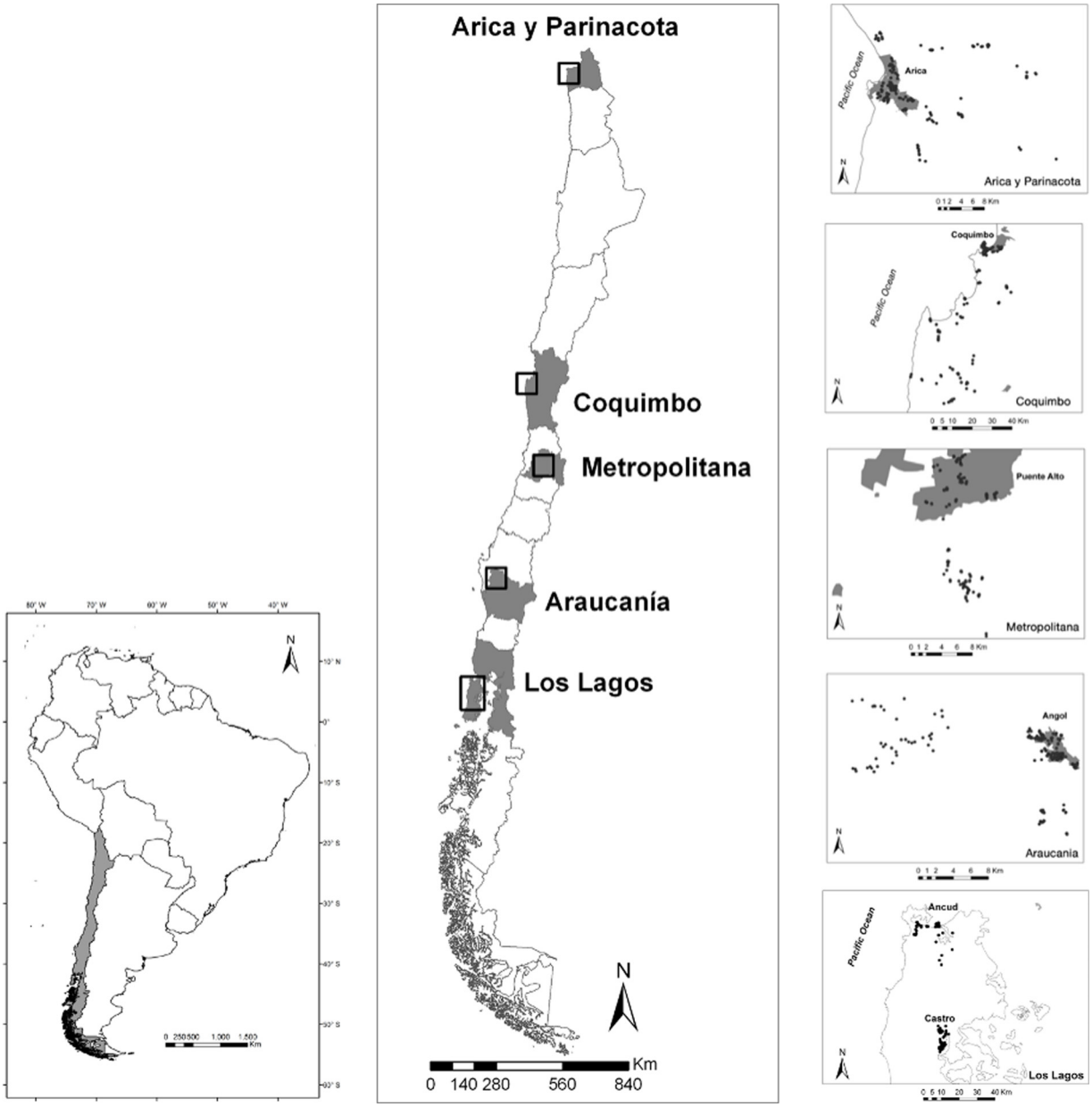

Fig. 1. Geographical distribution of study sites. The left panel shows Chile (grey shading) within South America. The central panel marks the locations of the study sites (black squares) within the five administrative regions of Chile (grey shading). The panels on the right indicate the locations of the sampled participants (black dots); urban areas are marked in grey.

sectional serosurvey, assessing the prevalence of SFGR, typhus group rickettsiae (TGR), and scrub typhus group orientiae (STGO) in healthy adults of rural and urban sites in five regions in Chile.

2. Materials & methods

2.1. Specimen collection

The serum samples of this study derived from an epidemiological project studying various vector-borne zoonotic infections among humans, pets, and vectors in Chile (Weitzel et al., 2016b, 2018; Acosta-Jamett et al., 2020b). The cross-sectional study was performed in urban sites and surrounding rural areas of five administrative regions in Chile (Fig. 1), stretching over a distance of more than 2,600 km: 1) the city of Arica (18°28′S,70°18′W) in the Arica y Parinacota Region in the extreme north, 2) the city of Coquimbo (29°57′S,71°20′W) in the Coquimbo Region in northern Chile, 3) the municipality of Puente Alto (33°37′S,70°34′W) in the city of Santiago, 4) the city of Angol (37°48′S,72°43′W) in the Araucanía Region in southern Chile; and 5) the cities of Ancud (41°52′S,73°49′W) and Castro 42°28′S,73°46′W) on Chiloé Island in the Los Lagos Region. The survey was performed between September 2010 and January 2011 in the Arica y Parinacota and Metropolitan regions, between October 2011 and February 2012 in the Coquimbo and Araucania regions, and in January 2016 in the Los Lagos Region. Further details regarding study sites and study design can be found elsewhere (Weitzel et al., 2016b, 2018; Acosta-Jamett et al., 2020b). In brief, households owning at least one dog were chosen by double stratified random sampling per building block and household for urban regions and by convenience sampling in rural areas. Household locations were recorded using a GPS device. Individuals were informed about the study and after written consent was obtained, whole blood was drawn from up to two adult members per household. Blood specimens were centrifuged on the same day; serum was separated, aliquoted, and kept at −20 °C until shipment.

2.2. Antibody testing

All samples were tested in the Naval Medical Research Center, Silver Spring, MD, USA, in a blinded manner for group-specific IgG antibodies against SFGR, TGR, and STGO utilizing in-house enzyme-linked immunosorbent assays (ELISAs) based on whole-cell antigen preparations from *Rickettsia conorii* (Malish 7 strain), *Rickettsia typhi* (Wilmington strain), and *Orientia tsutsugamushi* (Karp, Kato, and Gilliam strains), respectively, as previously described (Jiang et al., 2015). Briefly, samples were assessed at 1:100, 1:400, 1:1600, and 1:6400 dilutions in microtiter plates coated with ELISA antigens. Detection of group-specific antibodies against the rickettsial antigens with goat anti-human IgG conjugated with horseradish peroxidase enzyme (SeraCare, Gaithersburg, MD, USA) followed by addition of the ABTS substrate (SeraCare). The color reaction was measured by an ELISA reader (SpectraMax, Molecular Devices LLC, San Jose, CA, USA) and the absorbance of the sera in wells without ELISA antigen was subtracted from absorbance of the same sera with ELISA antigen resulting in a net absorbance value. The net absorbance for each dilution was added together and those serum samples with a total net absorbance ≥ 1.000 were considered positive; the titer for such samples was defined as the inverse of the highest serum dilution with a net absorbance of ≥ 0.2, as described previously (Supplementary Material). In addition, all ELISA procedures contained three negative human control samples that had total net absorbance values of less than 0.200 (Jiang et al., 2015). The principle of using a total net absorbance ≥ 1.000 as cut-off instead of a single dilution cut-off has been adapted for rickettsial serologies in the Naval Medicinal Research Center for the last 10 years and has since then been used for more than 12 studies in multiple locations around the world with consistent results (for additional information, see Supplementary Material).

2.3. Statistical analyses

Sample size calculation was performed by Epi Info™ 7. A sample size of 114 people per area was calculated using an estimated *Rickettsia* prevalence of 5 %, a confidence interval of 90 %, and an error of 4%. Generalized Lineal Models with binomial errors were fitted to assess the effect of age, gender and setting (urban/rural) on the seropositivity to SFGR, TGR, and STGO. Age was used as a discrete variable in order to improve model fitting. Additionally, Fisher Exact Tests were used to assess the effect of gender and age stratum (*i.e.* 18 − 24, 25 − 44, 45 − 65 and > 65 years) and the frequency of positive samples with titers = 400 or > 400.

2.4. Ethical approval

The study protocol was reviewed and approved by the Comité Ético Científico of the Faculty of Medicine, Pontificia Universidad Católica de Chile in Santiago, Chile (Aproval N°12 − 170) and the Naval Medical Research Center, Silver Spring, Maryland, USA (PJT-16 − 24), and by the respective health authorities of the five study regions.

3. Results

The study included a total of 1302 individuals, 216–289 persons per region. Among those, 648 (49.8 %) were from urban and 654 (50.2 %) from rural areas; the average age was 45.2 years and 63 % were female (Table 1). As shown in Table 2, the overall seroprevalence for SFGR, TGR, and STGO were 5.3 %, 1.2 %, and 0.4 %, respectively. SFGR seroprevalences varied regionally from 1.3 % to 8.3 %, whereas TGR prevalence ranged from 0.4 % to 2.1 %; STGO rates were very low in all study areas (0.4 % to 0.8 %), including the known endemic regions in southern Chile. Most of the seroreactive samples (76.4 %) displayed a titer of 400 (Table 3). Among the 69 positive SFRG samples, 18 (26.1 %) had titers of ≥1,600. Three of 12 (25 %) TGR positive samples had a titer of 1600, while all five STGO positive specimens had a titer of 400. In the Araucanía Region seven of the 18 (38.9 %) SFGR positive samples had titers ≥1600 (Table 3). Overall, 18 of 21 samples (85.7 %) with titers ≥1600 to rickettsiae were from rural areas (Table 3). The sample, which had a titer of $\geq$6400 to SFGR, was also positive for TGR (titer 400); one sample was low titer positive for SFGR and for STGO (Table 3).

Assessing the factors associated with SFGR seroreactivity revealed that individuals from rural settings had 2.21 more chances to be positive than inhabitants of urban sites and that seropositivity was associated with increased age (Table 4). The latter association was confirmed by an analysis of the distribution of samples with titers > 400, which increased significantly with age (18 − 24 years, 0 %; 25 − 44 years, 20 %; 45 − 65 years, 23 %; and > 65 years, 38 %; Fisher Exact Test, $p = 0.02$). Among samples with higher titers, there was a slight predominance of male participants (male/female: titer 400, 21/30; titer > 400, 11/6; Fisher Exact Test, $p = 0.06$). Antibodies to TGR did not show differences between urban or rural settings, but also increased with age (Table 4). The age strata analysis showed a trend towards higher titers in advance age groups (18 − 24 years, 0%; 25 − 44 years, 0%; 45 − 65 years, 14 %; and > 65 years, 33 %), although the difference did not reach statistical significance (Fisher Exact Test, $p > 0.05$). Gender differences were not observed (male/female: titer 400, 4/8; titer > 400, 2/1; Fisher Exact Test, $p > 0.05$). Risk factor analyses for STGO did not reveal relevant associations, most probably due to the small number of positive samples.

4. Discussion

The epidemiology of rickettsial pathogens in South America has undergone important changes in the last two decades. Formerly, *Rickettsia rickettsii* was the only known endemic species; but today several other human pathogenic species including *Rickettsia parkeri*, *Rickettsia massiliae*, and *R. typhi* have been identified, together with *R. felis* and other *Rickettsia* species of undetermined pathogenicity (Labruna et al., 2011). However, due to their unspecific clinical presentation, the lack of diagnostic tools in most settings, and low level of clinical awareness, rickettsioses are known to be chronically underdiagnosed and underreported (Blanton, 2019).

In Chile, the first clinically relevant Rickettsiales have only recently been identified. In 2007, a seroprevalence study showed evidence for canine infections with SFGR and *Anaplasma* spp. (López et al., 2007). Shortly after, *Anaplasma platys* and *Ehrlichia canis* were molecularly diagnosed in sick dogs (Abarca et al., 2007; López et al., 2012). At the same time, *R. felis* was identified in fleas (*Ctenocephalides felis*) and ticks (*Rhipicephalus sanguineus* sensu lato) (Labruna et al., 2007; Abarca et al., 2013a). In addition, *Ca.* R. andeanae and another unknown SFGR have recently been detected in different tick species (Abarca et al., 2012, 2013b; Ogrzewalska et al., 2016). The most important finding, however, was the discovery of human scrub typhus cases, caused by *Orientia* species, on Chiloé Island and different parts of continental Chile (Weitzel et al., 2016a, 2019). Apart from scrub typhus, human rickettsial infections have not been detected in Chile to date.

The presented data revealed infection with SFGR over the five study regions, which displayed significant geographical variations ranging from 1.3%–8.3% and was higher in rural areas and older participants. Higher prevalences have been reported from countries neighboring Chile. In Peru, they ranged from 6% to 14 % in four distinct regions (Salmon-Mulanovich et al., 2019); however, a much higher exposure rate (44 %) was found in a survey from Iquitos in the Amazon Basin (Forshey et al., 2010). A SFGR prevalence of 28 % was reported from a serosurvey in Argentina (Cicuttin et al., 2015). The causative species of the seroreactivity remain uncertain in Chile, where none of the pathogenic SFGR endemic in South America such as *R. rickettsii*, *R. parkeri*, and *R. massiliae* have been reported yet; however, studies exploring rickettsial infections of ticks and humans are scarce. The detected

Table 1
Age and gender composition of people sampled from urban and rural settings of five regions in Chile.

Region/setting	n	Median age (years)[a]	Age strata (n)[a]				Male gender[b]		
			18 – 24 years	25 – 44 years	45 – 65 years	> 65 years	n	%	CI90 %
Arica y Parinacota	216	51.4	21	73	83	31	80	37	32–43
Coquimbo	289	46.1	33	119	110	27	102	35	31–40
Metropolitana	289	43.9	49	93	105	37	85	30	26–35
Araucanía	282	40.8	19	87	109	66	105	37	33–42
Los Lagos	226	44.2	46	84	75	20	103	46	40–51
Urban	648	44.6	93	222	244	78	218	34	31–37
Rural	654	46.2	75	234	238	103	257	39	36–43
TOTAL	1302	45.4	168	456	494	169	475	37	35–39

CI 90 %, confidence interval 90 %.
[a] Missing data n = 15.
[b] Missing data n = 7.

higher risk in rural regions is compatible with *Amblyomma*-associated rickettsiae, since these ticks are predominantly found in rural settings in Chile (Abarca et al., 2013b). The trend to higher titers in males might be associated to activities with higher tick exposure, as reported from other studies (Tay et al., 2000; Jensenius et al., 2009; Salmon-Mulanovich et al., 2019); furthermore, males might be more susceptible to rickettsial infections (Walker, 2007). A possible candidate is *Ca.* R. andeanae, a species with unknown human pathogenic potential, which has been reported in *Amblyomma triste* and *Amblyomma tigrinum* over a wide geographical range in Chile (Abarca et al., 2012, 2013b). Furthermore, in 2016 a novel SFGR was reported in *Amblyomma parvitarsum* ticks in Chile (Ogrzewalska et al., 2016; Munoz-Leal et al., 2019). The existence of *R. parkeri* and *Rickettsia bellii* might also be possible, since they are found in neighboring countries (Labruna et al., 2011), and suitable vector species such as *A. triste* and *A. tigrinum* are endemic in Chile (Abarca et al., 2012). *Rickettsia amblyommatis*, a widespread SFGR in North, Central and South America including Argentina, is transmitted by a variety of *Amblyomma* species and could also be present (Karpathy et al., 2016), although the vector capacity of the three endemic species in Chile is uncertain. Another possible cause of the SFGR seroreactivity might be *R. felis*, which has been detected in cat fleas and *R. sanguineus* sensu lato ticks in Chile (Abarca et al., 2013a; Labruna et al., 2007). Although this species is grouped by most experts within the transitional *Rickettsia* group, it serologically cross-reacts with SFGR antigens (Blanton and Walker, 2017). Other *R. sanguineus*-associated SFGR, such as *R. massiliae*, which has been found in Argentina (Labruna et al., 2011), might also exist in Chile. Although this tick species complex only occasionally parasitizes humans, it is abundant in large parts of Chile and, as suggested by a recent *Anaplasma* seroprevalence study, human exposure in Chile might occur regularly (Acosta-Jamett et al., 2020b). *Rickettsia rickettsii*, which is transmitted in Latin America by *R. sanguineus* sensu lato and various *Amblyomma species*, has never been reported in Chile or neighboring countries (Labruna et al., 2011).

Our study provides the first evidence for the existence of TGR such as *R. typhi* in Chile, suggesting a low level of endemicity in all studied regions, including rural areas. Seroepidemiological studies of TGR in South America are scant. The prevalence level detected in our study (1.2 %) is compatible with data from Argentina (1.0 %), Peru (1.0 %), and Brazil (1.1 %) (Ripoll et al., 1999; Kocher et al., 2016; da Costa et al., 2005). A markedly higher prevalence (25.2 %) has been reported from the Caldas Region in Colombia (Hidalgo et al., 2013). The classical life cycle of *R. typhi*, causing murine typhus, involves the transmission by the Oriental rat flea (*Xenopsylla cheopis*), but the pathogen has also been found in the cat flea, *C. felis* (Civen and Ngo, 2008), which is highly endemic in Chile (Abarca et al., 2016). Interestingly, we did not detect an association of seroreactivity to urban settings as in other reports (Vallée et al., 2010). Murine typhus is known to be notoriously underdiagnosed due to its unspecific clinical presentation (Blanton, 2019). Together with the lack of clinical attention and absence of diagnostic tools for routine care, this might explain that autochthonous human cases have not been diagnosed in Chile so far. Undetected infections with *Rickettsia prowazekii* due to recurrence (Brill-Zinser disease) or low endemicity are less probable, since the last Chilean clusters of epidemic typhus were reported in 1939 (Laval, 2013). Further studies are warranted to confirm and clarify the endemicity of TGR in Chile; those should include the search of *R. typhi* in fleas of rodents and pet animals as well as serological and molecular diagnostic studies for TGR in patients with undifferentiated febrile diseases and in para-domestic rodents.

Scrub typhus is a mite-borne rickettsial disease, which causes up to one million cases per year in the Asia Pacific region (Paris et al., 2013). Scrub typhus was up until recently considered to be confined to the so called 'tsutsugamushi triangle' in the Asia Pacific region. Surprisingly, the disease was recently discovered to be endemic in Chile (Balcells et al., 2011; Weitzel et al., 2016a). Trombiculid mites of the genus *Herpetacarus*, infected with *Orientia* species, were identified as the probable vector in endemic regions in Chile (Acosta-Jamett et al.,

Table 2
Seroprevalence to group-specific *Rickettsia* and *Orientia* ELISA antigens in urban and rural settings of five regions in Chile.

Region/setting	n	Spotted fever group rickettsiae			Typhus group rickettsiae			Scrub typhus group orientiae		
		Pos	%	90 % CI	Pos	%	90 % CI	Pos	%	CI 90 %
Arica y Parinacota	216	13	6.0	3.9 – 9.4	2	0.9	0.4 – 2.9	1	0.4	0.0 – 2.2
Coquimbo	289	24	8.3	6.1 – 11.4	4	1.4	0.7 – 3.1	1	0.4	0.1 – 1.6
Metropolitana	289	12	4.2	2.7 – 6.6	2	0.7	0.3 – 2.2	2	0.7	0.3 – 2.2
Araucanía	282	17	6.0	4.2 – 8.9	6	2.1	1.2 – 4.1	1	0.4	0.1 – 1.7
Los Lagos	226	3	1.3	0.1 – 3.4	1	0.4	0.2 – 2.1	0	0.0	0.0 – 1.3
Urban	648	21	3.2	2.3 – 4.6	4	0.6	0.3 – 1.4	1	0.2	0.0 – 0.7
Rural	654	48	7.3	5.9 – 9.2	11	1.7	1.1 – 2.8	4	0.6	0.3 – 1.4
TOTAL	1302	69	5.3	4.4 – 6.4	15	1.2	0.8 – 1.8	5	0.4	0.2 – 0.8

Pos, positive; CI 90 %, confidence interval 90 %.

Table 3
Titer of seroreactivity to group-specific *Rickettsia* and *Orientia* ELISA antigens from urban and rural areas of five regions in Chile.

Region/setting	n	Spotted fever group rickettsiae titer				Typhus group rickettsiae titer			Scrub typhus group orientiae titer	
		Neg.	400	1600	≥6400	Neg.	400	1600	Neg.	400
Arica y Parinacota	216	203	10	3	0	214	1	1	215	1
Coquimbo	289	265	20	4	0	285	4	0	288	1
Metropolitana	289	277	9	3	0	287	2	0	287	2
Araucanía[a]	282	265	10	6	1	276	4	2	281	1
Los Lagos	226	223	2	1	0	225	1	0	226	0
Urban	648	627	19	2	0	644	3	1	647	1
Rural[a]	654	606	32	15	1	643	9	2	650	4
TOTAL	1302	1233	51	17	1	1287	12	3	1297	5

[a] 1 sample was seroreactive to SFGR (≥6400) and to TGR (400), and 1 sample was seroreactive to SFGR (400) and to STGO (400).

Table 4
Multivariable analysis with binomial errors of factors associated with *Rickettsia* seropositivity in humans.

Risk Factor	OR	CI 90 %	*p*
Spotted Fever Group			
Setting			
Urban	1.00		
Rural	2.21	1.43–3.48	0.003
Age	1.03	1.02–1.04	< 0.001
Typhus Group			
Age	1.06	1.03–1.09	< 0.001

OR, odd ratio; CI 90 %, confidence interval 90 %.

2020a). The epidemiological range of this emerging infection is still uncertain, but cases have been reported over a range of > 1100 km in southern Chile (Weitzel et al., 2019). Moreover, serologic evidence of scrub typhus has been reported from Peru (Kocher et al., 2017). Among the study regions of this study, the most southern site (Chiloé Island, Los Lagos Region) is known as highly endemic for scrub typhus (Abarca et al., 2018). Angol, the study site in the Araucanía Region, is at the same latitude as the most northern scrub typhus cases reported so far in Chile (Weitzel et al., 2019). In our study, the STGO seroprevalences were consistently low in all regions, including Chiloé Island. Nevertheless, four of the five positive participants were from rural regions, where there is a higher risk of exposure to trombiculid mites. The overall very low prevalence might be related to the ELISA antigens used, which utilized the three Asian *O. tsutsugamushi* strains Karp, Kato and Gilliam. Low cross-reactivity of IgG antibodies of Chilean patients to such antigens has been reported before, suggesting relevant antigenic difference of the Chilean *Orientia* species/strain to those from Asia Pacific (Weitzel et al., 2019). On the other hand, the presence of two positive cases in the northern study sites (Arica y Parinacota and Coquimbo) might indicate that scrub typhus is also endemic in these regions. To our opinion, future seroepidemiological studies using antigens from Chilean *Orientia* strains are necessary to understand the real prevalence of exposure to STGO in Chile.

The ELISA technique applied in this study is less operator-dependent and permits a higher sample throughput than the traditional immunofluorescence assay (IFA) (Paris and Dumler, 2016). A limitation of all rickettsial serological studies is that antigens are group-specific and do not permit one to distinguish between individual species; cross-reactions among different antigen groups are also possible, but less frequent (Paris and Dumler, 2016). Nevertheless, since homologous antigens often produce higher titers than heterologous antigens, a species approximation is serologically possible, if broader antigen panels are used and combined with cross-absorption (Brouqui et al., 2004). These techniques are currently not established in Chile, but might be useful for future research.

In conclusion, this cross-sectional study firstly reports seroepidemiological data of infection and/or exposure to SFGR, TGR, and STGO over a wide geographical range in Chile. Our results indicate that the spectrum of rickettsial infections in Chile might be wider than currently known and that SFGR and TGR affecting humans are endemic in Chile. Further molecular and serological studies of vectors and hosts are necessary to understand the epidemiological and clinical relevance of these findings. Moreover, seroprevalence studies on scrub typhus in Chile require using more sensitive antigens based on Chilean *Orientia* species. Clinicians in Chile should include rickettsial infections into their differential diagnosis of patients with undifferentiated fever or fever of unknown origin and laboratories should implement tests for diagnostic routine of such patients.

Disclaimers

The views expressed in this article reflect the results of research conducted by the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, or the United States Government. CMF is a federal employee of the United States government. This work was prepared as part of her official duties. Title 17 U.S.C. 105 provides that “copyright protection under this title is not available for any work of the United States Government.” Title 17 U.S.C. 101 defines a U.S. Government work as work prepared by a military service member or employee of the U.S. Government as part of that person’s official duties.

CRediT authorship contribution statement

Thomas Weitzel: Conceptualization, Resources, Data curation, Writing - original draft, Supervision, Writing - review & editing. **Gerardo Acosta-Jamett:** Conceptualization, Methodology, Formal analysis, Resources, Data curation, Writing - original draft, Visualization, Writing - review & editing. **Ju Jiang:** Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - review & editing. **Constanza Martínez-Valdebenito:** Methodology, Investigation, Data curation, Writing - review & editing. **Christina M. Farris:** Resources, Funding acquisition, Writing - review & editing. **Allen L. Richards:** Validation, Data curation, Supervision, Funding acquisition, Writing - review & editing. **Katia Abarca:** Conceptualization, Validation, Resources, Data curation, Supervision, Project administration, Funding acquisition, Writing - review & editing.

Declaration of Competing Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Appendix A. Supplementary data

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