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A serosurvey for spotted fever group *Rickettsia* and *Coxiella burnetii* antibodies in rural dogs and foxes, Chile

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ABSTRACT

Antibodies against Spotted Fever Group (SFG) *Rickettsia* and *Coxiella burnetii*, investigated through indirect antibody immunofluorescence tests, were detected in serum samples from 3.1% and 0% of 358 rural dogs, respectively, and in none of 32 wild foxes tested. SFG *Rickettsia* seropositive dogs were only detected in the Mountain Desert (8%) and the Steppe-Mediterranean (9%) regions. Exposure in the Mountain Desert, where no ticks and fleas were found on any dog, could correspond to a new SFG *Rickettsia* sp. recently described in soft ticks or to a related agent. Our survey confirms low endemicity in the country of *C. burnetii*, as observed in recent serosurveys in humans.

1. Introduction

Rickettsia species are Gram-negative bacteria belonging to the class Alphaproteobacteria that are the causative agents of numerous diseases of humans. These comprise a group of vector-borne pathogens that live in close association with their arthropod hosts [1]. *Coxiella burnetii* is a Gammaproteobacteria and is the causative agent of Q fever that infects several vertebrate and invertebrate hosts [2]. It can persist for long periods in the environment and is assumed to have a sylvatic and a domestic transmission cycle. Livestock plays the most important role, with rodents suspected to act as a link between the sylvatic and domestic cycles. Vectors are not necessary for *C. burnetii* transmission, but they could play a role in the transmission among some vertebrates, especially in the sylvatic cycle [2].

In Chile, several rickettsial agents have been described. Among the Spotted Fever Group (SFG) rickettsiae, *Rickettsia felis* was detected in *Rhipicephalus sanguineus* ticks [3], fleas retrieved from dogs in Easter Island [4] and central Chile [5], and cats from Valdivia city [6]. A *Rickettsia* sp. showing only 97% identity with different published sequences of *R. felis* was found in a Darwin's fox [7]. *Candidatus* Rickettsia asemboensis and *Candidatus* Rickettsia senegalensis were also detected in dog fleas [4]. *Candidatus* Rickettsia andeanae has been identified in *Amblyomma* ticks from different parts of Chile [8–10] and another SFG *Rickettsia* sp. was detected in *Amblyomma parvitarsum*, which is associated to camelids from the Andean high plateau [11]. Recently, a further SFG *Rickettsia* sp. was detected in larvae of a soft tick in the Andean mountains [12]. In humans, a serological survey revealed antibodies against *Rickettsia* in 44 out of 294 patients using *R. honei*, *R. conorii* (both

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SFG), and *R. typhi* (typhus group) antigens [13]. In Chile is also present *Candidatus* Orientia chiloensis, one of the causal agents of scrub typhus [14]. A serological survey revealed antibodies against *Orientia* spp. in 20% of dogs from Chiloé island [15].

Information about *C. burnetii* is, in contrast, much scarcer and very recent [16]. Two large serosurveys in humans revealed low endemicity or absence of this pathogen among humans in four regions of Chile [17, 18]. Nevertheless, an outbreak took place in 1998 affecting workers of the Agriculture and Livestock Service, and the second one in 2017 affected dairy farmworkers in southern Chile [13]. A subsequent retrospective survey revealed that 23% of cases of atypical pneumonia met the serodiagnosis criteria for Q fever infection [13]. In animals, 9% of bats were positive by PCR [19], and 30 and 47 Darwin's fox (*Lycalopex fulvipes*) blood and serum samples were negative when evaluated, respectively, by PCR and a serological commercial kit [7,20].

Chile is a country over 4300 km long that includes a diversity of climates with precipitation and temperatures differing drastically between the different bioclimatic areas [21]. This variety of climatic conditions provide more or less suitable conditions for arthropod vectors to survive based on their biological requirements, and in consequence, more or less suitable conditions for the presence of vector-borne pathogens [22]. In Chile, there is a dog population estimate of over four million individuals [23]. Rural dogs in the country are commonly free-ranging and lack proper sanitary care [24]. Their free-ranging status makes them prone to come into contact with rodents, wildlife, carcasses, human leftovers, and livestock abortions, which may expose them to a range of pathogens. On the other hand, these dogs are often parasitized by heavy burdens of ticks and fleas [25]. All these facts make them ideal sentinels for the study of the distribution of zoonotic disease agents in Chile [22,26]. The present study aimed to determine the exposure of free-ranging dogs to two important zoonotic pathogens, SFG *Rickettsia* sp. and *Coxiella burnetii*, and to identify biotic and abiotic factors associated with the presence of antibodies against the mentioned agents.

2. Materials and methods

2.1. Study site and sampling

The sample size was calculated with WinEpi [28] to determine the prevalence of these pathogens as 350 dogs, for unknown population size, an expected seroprevalence of 35% [27] and with a 95% confidence level. Between 2006 and 2019, 358 rural dogs were sampled after the written consent of the owner in four different bioclimatic areas of Chile: Coastal Desert (n = 183), Mountain Desert (n = 90), Steppe-Mediterranean (n = 68), and Temperate Warm Rainy (TWR; n = 59) (Fig. 1, Table 1). The requisites to be included in the study included not being permanently confined and not having traveled to other localities. Serum was obtained by venipuncture of the cephalic vein and collected in clean tubes. Ectoparasites were collected through a 5-minute examination protocol and stored in 90% ethanol (see Di Cataldo et al., [22] for details in parasite identification and observed prevalences and abundances). Dogs were classified as juveniles (less than a year) or adults (older than a year) according to the information provided by the owner and confirmed by teeth eruption examination.

Serum samples from 32 foxes, obtained during captures as part of

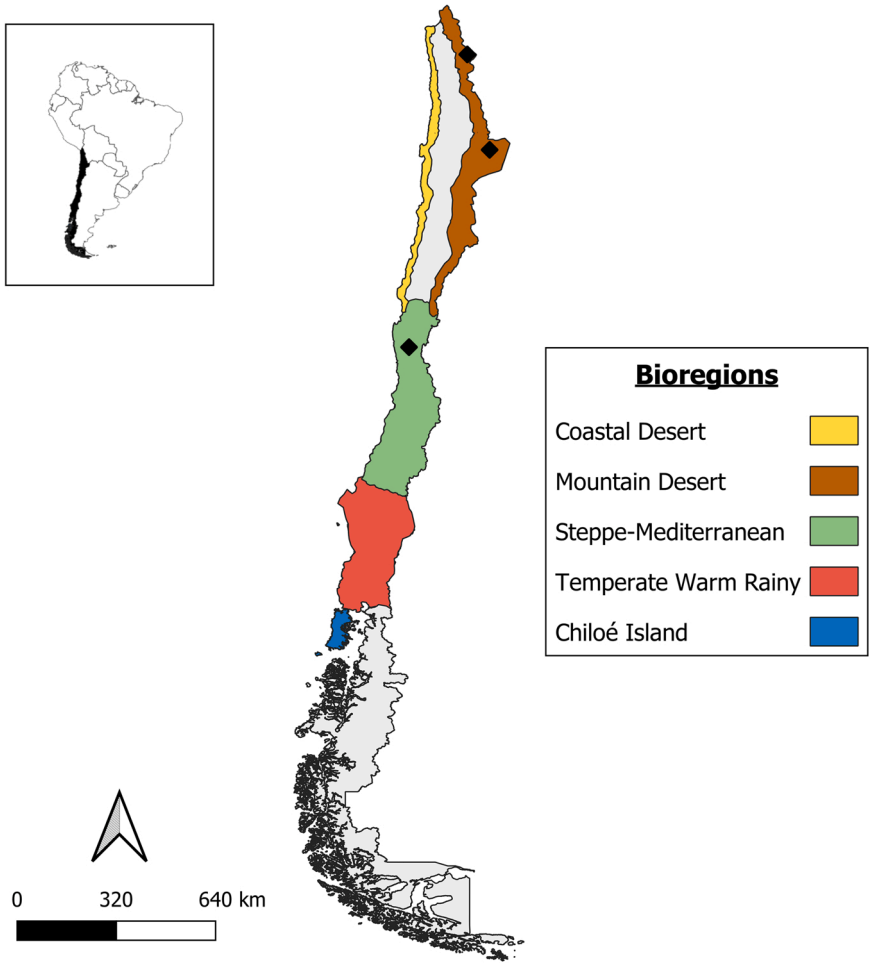

Fig. 1. Map of the study areas. The location of seropositive dogs for SFG *Rickettsia* is highlighted with diamonds.

Table 1
Detailed sample sizes and prevalence of antibodies against in SFG Rickettsia in rural dogs in Chile.

	Sample size	SFG *Rickettsia* seroprevalence (%)	95% C.I.
Bioregion			
Coastal Desert	183	0	0.0–2.0
Mountain Desert	86	8.1	3.3–16.5
Steppe-Mediterranean	44	9.1	2.5–21.7
Temperate Warm Rainy	45	0	0.0–7.9
Age and sex			
Juvenile Female	31	0	0.0–11.2
Adult Female	123	3.2	0.9–8.1
Juvenile Male	36	0	0.0–9.7
Adult Male	168	4.1	1.7–8.4

other studies, were also analyzed. Samples consisted of 12 South American grey foxes (*Lycalopex griseus*) and nine Andean foxes (*L. culpaeus*) from the Steppe-Mediterranean region (mostly captured around Santiago city), and 10 Darwin's foxes from the Chiloé Island and one from TWR. In these latter, exposure to *C. burnetii* was already studied by Hidalgo-Hermoso et al. (in press) through a commercial ELISA kit.

Indirect antibody immunofluorescence (IFA) test was performed on IFA slides specific for *R. conorii* and *C. burnetii* antigens (Fuller Laboratories Fullerton, California, USA). Blood sera were first diluted in phosphate-buffered saline (PBS, pH 7.2), 1:64 for *R. conorii* and 1:16 for *C. burnetii*, respectively, and then inoculated into individual slide wells and incubated to allow reaction of antibodies with the antigens. The resulting reactions were visualized using standard fluorescence microscopy.

Maximum possible prevalence was calculated using WinEpi [28]. Differences in the occurrence of antibodies between age and sex groups and regions were calculated using χ^2-square tests or Fisher's exact test.

3. Results and discussion

Eleven dogs had titers against *Rickettsia conorii* above the cut-off values, for an observed prevalence of 3.1% (95% Confidence Intervals= 1.5%−5.4%; Table 1). All seropositive dogs were adults, but differences between age classes were not significant (3.8% vs 0%, Fisher's $p = 0.23$), probably owing to low sample size in juveniles. Seroprevalence in adult females and adult males was similar (Table 1). Seropositive dogs were only detected in the Mountain Desert and the Steppe-Mediterranean regions. Seroprevalence was significantly higher in these areas than in the Coastal Desert region (Fisher's $p = 0.0003$ and $p = 0.0013$, respectively). Unfortunately, the low seroprevalence prevented us from making statistical inferences regarding risk factors for exposure to *Rickettsia*. All sera turned out below the cut-off level for *C. burnetii* and were considered negative (0%, 95%C.I.=0.0%−1.0%), for a maximum possible prevalence (with 95% confidence) of 0.75%.

Our extensive survey revealed a low degree of exposure of the sampled dog population with the studied pathogens. The only previous serological survey in dogs against SFG *Rickettsia* reported a prevalence of 35% and was carried out only in urban dogs of Santiago [27]. This is markedly higher when compared with the subpopulation of our dogs from the same bioregion and is probably explained by the fact that the dogs from the study by López et al. [27] were actively selected by the authors due to a history of tick infestation, biasing the actual prevalence.

For the detection of antibodies against SFG *Rickettsia*, we used a *R. conorii* antigen. It is known that, by IFA testing, different members of the SFG show serological cross-reactivity. Since *R. conorii* is not present in South America, the detected antibodies could represent previous exposure of dogs to other members of the SFG, such as *R. felis* or C. *R. andeanae*. The presence of *R. felis* has been demonstrated in different parts of the country in dog and/or cat fleas and ticks in different parts of the country [3–5], whereas C. *R. andeanae* is associated with *Amblyomma* ticks [8,9]. This could be the case in those dogs from the Steppe-Mediterranean areas, where the sampled dogs hosted ticks (mostly *Rhipicephalus sanguineus*) and fleas (mostly *Ctenocephalides felis*), but not in the Mountain Desert, where dogs did not host any tick or flea [22]. In consequence, we hypothesize that those dogs might have been exposed to the *Rickettsia* sp. associated with plateau camelid ticks [11] or, more likely, with the new *Rickettsia* sp. Cachapoal recently reported by Muñoz-Leal et al. [11]. *Rickettsia* sp. Cachapoal is closely related to *R. amblyommatis*, another SPG rickettsiae. Muñoz-Leal et al. [11] identified this sequence in an *Ornithodoros atacamensis* larvae retrieved from a Darwin's leaf-eared mouse (*Phyllotis darwini*) in the Andes region of central Chile. *Ornithodoros atacamensis* is a parasite of lizards in the Atacama Desert, which precisely corresponds to the Mountain Desert of the present study. Therefore, we consider a reasonable hypothesis that our dogs were exposed to one of these bacteria or a closely related one, which calls for specific studies for this potentially zoonotic pathogen in the study areas.

All dog samples resulted negative for antibodies against *C. burnetii*. Seropositivity in rural dogs is frequent in areas where this bacterium is endemic [29–31]. Although in Chile there have been isolated outbreaks [18], it seems to be a country of low endemicity of this agent, as showed by serosurvey in humans [17,18] and confirmed by the present serosurvey in dogs.

All fox samples turned out negative for antibodies against both agents. Our opportunistic sample was by no means representative and, in addition, most foxes were captured in areas where few or no seropositive dogs were found. Nevertheless, this is the first time that wild foxes are tested for antibodies against SFG *Rickettsia* in Chile, and we believe these results are worth reporting. Finally, it is worth mentioning that the IFI applied for *C. burnetii* antibody detection in the Darwin's fox was in concordance with previous analyses by Hidalgo-Hermoso et al. [20], which did not detect antibodies in any Darwin's fox using a commercial ELISA kit.

In conclusion, exposure of rural dogs against the studied pathogens was low, but the detection of antibodies against SFG rickettsiae in the Andean high plateau calls for further investigation and highlights the use of rural dogs as a sentinel for zoonotic pathogens.

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CRediT authorship contribution statement

S. Di Cataldo: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Visualization, Writing – original draft, Writing – review & editing. **A. Cevidanes:** Resources, Writing – review & editing. **C. Ulloa-Contreras:** Resources, Writing – review & editing. **E. Hidalgo-Hermoso:** Resources, Writing – review & editing. **V. Gargano:** Resources. **J. Cabello:** Resources, Writing – review & editing. **I. Sacristán:** Writing – review & editing. **C. Napolitano:** Funding acquisition, Resources, Writing – review & editing. **D. Gambino:** Resources, Writing – review & editing. **D. Vicari:** Funding acquisition, Resources, Writing – review & editing. **J. Millán:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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