Arch Med Vet **48, 247-253 (2016)**

COMMUNICATION

Flea and ticks species from dogs in urban and rural areas in four districts in Chile

Pulgas y garrapatas en perros urbanos y rurales en cuatro regiones en Chile

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ABSTRACT. Fleas and ticks frequently parasitise canines worldwide and their prevalence in dogs is influenced by many factors including climate and geography, among others. Different studies worldwide have shown no clear pattern of an urban or rural preference by different species of fleas and ticks infesting dogs. The aim of this study is to identify species of fleas and ticks present in urban and rural dogs from different ecoregions of Chile. A cross-sectional study was conducted in four urban-rural paired sites at four districts: Arica y Parinacota, Coquimbo, Metropolitana and Araucanía. A random and a convenience sampling of households in the urban and rural areas was carried out, collecting fleas and ticks from 112-114 dogs per locality. The frequency of fleas and ticks infestation between urban and rural areas was compared through Chi-square or Fisher's tests. A total of 921 dogs were examined, identifying four species of fleas (*Ctenocephalides canis, Ctenocephalides felis, Pulex irritans* and *Echidnophaga gallinacea*) and three species of ticks (*Rhipicephalus sanguineus*, *Amblyomma tigrinum* and *Amblyomma triste*). In general, a higher prevalence of dogs with fleas were observed in rural areas, being *C. canis* the most frequent species globally; there were significant variations in the distribution of different species according to district. *R. sanguineus* was the predominant tick in all the studied areas. *Amblyomma* species were found exclusively in rural areas; *A. triste* only in Arica y Parinacota and *A. tigrinum* in rural areas of Coquimbo and Araucanía districts.

Key words: ticks, fleas, dogs, urban and rural areas.

RESUMEN. Pulgas y garrapatas parasitan frecuentemente a caninos y su prevalencia se asocia a diversos factores incluyendo clima y geografía, entre otros. Diversos estudios han mostrado que no existe una clara preferencia entre sitios urbanos o rurales para las especies de pulgas y garrapatas que infectan perros. El objetivo de este estudio fue identificar especies de pulgas y garrapatas presentes en perros de zonas urbanas y rurales de diferentes ecorregiones de Chile. Se realizó un muestreo transversal en áreas urbano-rural de cuatro regiones de Chile: Arica y Parinacota, Coquimbo, Metropolitana y La Araucanía. En ciudades se realizó un muestreo estratificado y en zonas rurales un muestreo por conveniencia para muestrear pulgas y garrapatas de 112-114 perros por localidad. Se comparó la prevalencia de infestación entre urbano y rural por región mediante pruebas de Chi-cuadrado o Fisher. En total 921 perros fueron examinados, identificándose cuatro especies de pulgas (*Ctenocephalides canis, Ctenocephalides felis, Pulex irritans* y *Echidnophaga gallinacea*) y tres especies de garrapatas (*Rhipicephalus sanguineus*, *Amblyomma tigrinum* y *Amblyomma triste*). En general, se detectó un mayor número de perros con pulgas en zonas rurales, siendo *C. canis* la especie más frecuente; por otro lado, se detectó una diferencia en la distribución de las especies de acuerdo con el área estudiada. *R. sanguineus* fue la garrapata predominante en todas las áreas de estudio. Garrapatas del género *Amblyomma* se detectaron exclusivamente en áreas rurales; encontrándose *A. triste* solo en Arica y Parinacota y *A. tigrinum* en áreas rurales de las regiones de Coquimbo y La Araucanía.

Palabras clave: garrapatas, pulgas, perros, áreas urbanas y rurales.

INTRODUCTION

Ectoparasites of pets, as fleas and ticks, are nowadays recognised as able to host and transmit diverse infectious agents to both pets and humans (Hugh-Jones *et al* 2000, Shaw *et al* 2001). Fleas and ticks are widely distributed in the world, showing big adaptability and strong resistance to different climatic conditions (Durden and Hinkle 2009, Dantas-Torres 2010).

Fleas and ticks are the arthropods that most frequently parasitise canines worldwide, despite the increased availability of drugs to control them (Otranto and Wall 2008). There are several studies worldwide indicating that the presence of ectoparasites could be influenced by the environment in which they are found, for example species of fleas and ticks have been found mostly in rural areas where they can find adequate conditions for their maintenance (Beck *et al* 2006, Gracia *et al* 2008, Farkas *et al* 2009, Debárbora *et al* 2011). Other studies have reported that dogs inhabiting urban sites are more parasitised by fleas or ticks than those from rural sites (eg. Dantas-Torres 2010).

The flea species *Ctenocephalides canis, Ctenocephalides felis, Pulex irritans* and *Echidnophaga gallinacea* have been described in Chile as infesting dogs (Alcaíno and Gorman 1999). *C. felis* has been reported as the flea that most commonly parasitises canines in Santiago.

Accepted: 15.10.2015.

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On the other hand, tick species described in domestic dogs in the country are *Rhipicephalus sanguineus* (Tagle 1976), *Amblyomma tigrinum* (Tagle 1971) and recently *Amblyomma triste* (Abarca *et al* 2012). Unlike fleas, which can be found throughout Chile, ticks have a more specific geographic distribution. *A. tigrinum* has been reported in Pirque and Santiago (Metropolitan District) (Abarca *et al* 2013), Nahuelbuta National Park (Araucanía District), Arauco, Concepción (Biobío District), Valparaíso and Santo Domingo (Valparaíso District) (González-Acuña and Guglielmone 2005); *R. sanguineus* can be found from Viña del Mar (Valparaíso District) (Alcaíno 1985), to Concepción (Biobío District) (Muñoz and Casanueva 2002, González-Acuña and Guglielmone 2005) and Aysén District (Mastropaolo *et al* 2008); and *Amblyomma triste* was first found in rural areas near the city of Arica (Abarca *et al* 2012).

R. sanguineus is a tick found mainly in urban areas (Dantas-Torres 2010), whereas species of the genus *Amblyomma* are mainly found in rural areas because they have wildlife hosts, especially during their larval and nymphal stages, being found in dogs only during their adult stage (Debárbora *et al* 2011). On the other hand, *R. sanguineus* is more associated with urban areas, and it is expected that canids from rural areas close to them might be more likely to be infested by this tick. Moreover, if *A. tigrinum* is present mainly in rural areas, then a reverse pattern to that of *R. sanguineus* can be expected, i.e. the domestic dog populations inhabiting rural areas have higher probabilities to be infested with *A. tigrinum* rather than with *R. sanguineus*. In the case of fleas, a higher infestation has been found in rural areas, attributing this to low access to anti-flea products in these areas (Farkas *et al* 2009).

This study was part of a project aimed to detect zoonotic pathogens in ticks and fleas infecting dogs in urban and rural areas in regions with different temperature and climate conditions in Chile. This study shows results of the species of fleas and ticks currently present in dogs in Chile and their distribution in four districts with varying patterns of temperature and climate, from the arid further north to the moist southern region. Due to their vectorial potential regarding infectious diseases of great importance to both animals and people, a deeper knowledge of the species distribution is relevant to define more appropriate control measures in urban and rural areas of Chile.

MATERIAL AND METHODS

The study was conducted in four districts (figure 1), considering the following areas: the city of Arica (18º 28 'S, 70° 18' W) and surrounding rural areas in the Arica y Parinacota District, which is located in the far north of the country, with dry climate, extremely arid landscape and scarce vegetation. The city of Arica has 180,879 inhabitants (INE 2005) and an estimated dog population of 32,887. The city of Coquimbo (29º 57' S, 71° 20' W) and surrounding rural areas in Coquimbo region, with semi-arid climate, a population of 203,036 inhabitants (INE 2005) and an estimated dog population of 36,916. The municipality of Puente Alto (33º 37' S; 70° 34' W), in the Metropolitana District has 492,915 inhabitants (INE 2005) and an estimated population of 111,612 dogs, and in a nearby rural area, the municipality of Pirque, with mediterranean climate and extended dry season. The city of Angol (37º 48' S; 72° 43' W), 42,000 inhabitants (INE 2005) and an estimated dog population of 7,636, and rural areas between this city and the Nahuelbuta National Park in the Araucanía District, in southern Chile, with transitional climate from humid mild mediterranean to markedly rainy climate. Domestic dog population in urban areas were estimated using a human:dog ratio of 5:1 as reported in the literature (Acosta-Jamett *et al* 2010). Domestic dog population in rural areas was not estimated due to the difficulty in assessing the inhabitants in the areas to be surveyed.

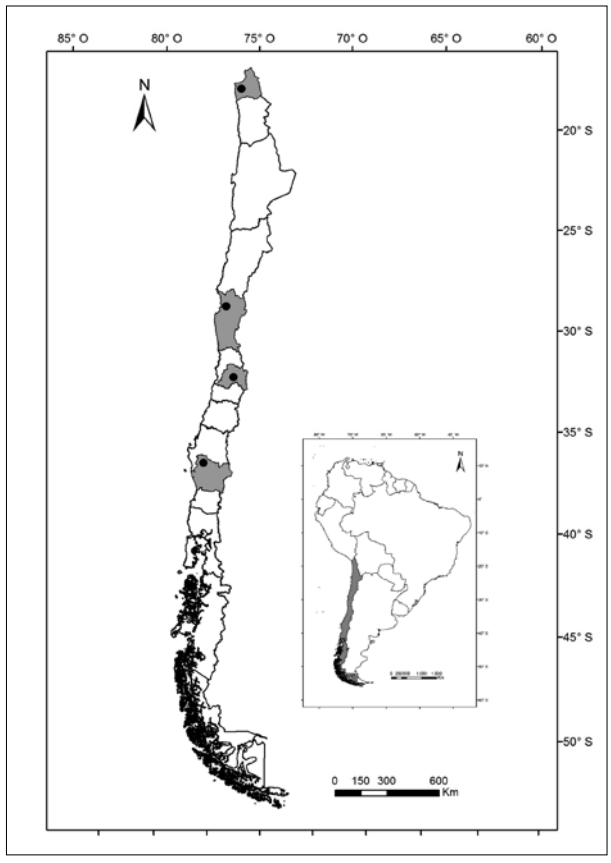

Figure 1. Map of Chile, showing selected areas assessed (black dots) in each studied region (highlighted with gray).

Mapa de Chile indicando las zonas seleccionadas que fueron evaluadas (círculos negros) en cada área de estudio (destacada en gris).

A cross-sectional study was conducted in the austral spring-summer seasons, when fleas and ticks are more prevalent (Durden and Hinkle 2009, Dantas-Torres 2010) in each study site, thus sampling was carried out

between September 2010 and January 2011 in Arica y Parinacota, and between October 2011 and February 2012 in the Metropolitana, Coquimbo and Araucanía districts. The sample size was at least 97 households per each area, where one dog per selected household was sampled in each urban and rural area. This sample size was estimated as part of a project aimed to determine the seroprevalence of *Anaplasma* spp. in Chile. In urban areas, a double stratified random sampling per building block and household was carried out while in rural areas a convenience sampling was performed until the number of pre-established households was completed. Thus a total of 921 dogs were examined, between 112 and 124 per area.

Owners and research assistants manually restrained dogs and during approximately 5 minutes a thorough examination of the whole body of each dog was carried out searching for the presence of adult fleas and ticks. A representative sample of these parasites were obtained. After manual extraction, ectoparasites were preserved in 70% alcohol until taxonomic analysis. The diagnosis of the species of fleas and ticks was performed by a professional trained in a reference laboratory at the University of São Paulo, Brazil, through observation with a stereoscopic microscope using published taxonomic keys (Pratt and Stojanovich 1966, Barros-Battesti *et al* 2006).

Presence of ectoparasites per each dog was recorded in Microsoft Excel and mixed infestations were registered. The association between flea and tick species, between cities, and the differences between urban and rural areas were analyzed using chi-square and Fisher exact tests with 95% confidence, using the software Epi Info 7 (Centers for Disease Control and Prevention, Atlanta).

RESULTS AND DISCUSSION

Four species of fleas were identified: *C. canis, C. felis, P. irritans* and *E. gallinacea,* with *C. canis* being the most abundant species. Neither *P. irritans* nor *E. gallinacea* were found in the Metropolitana region (table 1). In general, a higher number of dogs with fleas was observed in rural areas, varying according to the studied region, with *C. canis* being more abundant in rural areas of the Coquimbo (66%) and Metropolitana (29%) Districts, compared to urban areas

Table 1. Comparison between flea-infested dogs from urban and rural areas, by flea species and studied district.
Comparación de infestación por pulgas en perros de zonas urbanas y rurales, de acuerdo con la especie de pulga y la zona de estudio.

District	Urban			Rural			x^2	P
	pos	neg	%	pos	neg	%		
C. canis								
Arica y Parinacota	24	89	21	25	87	22	0.1	0.844
Coquimbo	29	85	25	74	38	66	37.6	<< 0.001
Metropolitana	6	111	5	34	83	29	23.6	<< 0.001
La Araucanía	21	103	17	26	86	23	1.5	0.227
C. felis								
Arica y Parinacota	42	71	37	25	87	22	5.9	0.015
Coquimbo	32	82	28	61	51	54	16.3	<< 0.001
Metropolitana	2	115	2	7	110	6	2.9	0.089
La Araucanía	4	120	3	3	109	3	0.1	0.804
P. irritans								
Arica y Parinacota	9	104	8	29	83	26	12.9	<< 0.001
Coquimbo	36	78	32	30	82	27	0.6	0.428
Metropolitana	0	117	0	0	117	0	NA	NA
La Araucanía	14	110	11	16	96	14	0.5	0.490
E. gallinacea								
Arica y Parinacota	0	113	0	1	111	1	*	0.498
Coquimbo	1	113	1	11	101	10	*	0.003
Metropolitana	0	117	0	0	117	0	NA	NA
La Araucanía	0	124	0	1	111	1	*	0.475

Asterisk (*) indicates that Fisher´s exact test was performed.

of the same regions (Coquimbo: 25% and Metropolitana 5%). *C. felis* was more abundant in rural areas of Coquimbo (54%) than in the city (28%), but the opposite was found in Arica y Parinacota (Rural: 22% and urban: 37%). Similarly, *P. irritans* infestation was also higher in rural (26%) than urban area (8%) in Arica y Parinacota region. In general, *E. gallinacea* was the flea that less affected dogs and was primarily associated with rural areas, however significant differences were only found in the Coquimbo District (table 1).

This study confirms that dogs in Chile are parasitised with the four species of fleas previously described in the country (Alcaíno *et al* 2002). The results of this study are consistent with those reported worldwide (Alcaíno *et al* 2002, González *et al* 2004, Durden *et al* 2005, Gracia *et al* 2008, Farkas *et al* 2009), where the predominant species infecting dogs are *C. felis* and *C. canis*. The predominance of *C. canis* or *C. felis* varies in the literature, with *C. canis* for instance most common in many towns of the Buenos Aires province in Argentina (González *et al* 2004) and in Osorno in Chile (Alcaíno *et al* 2002), while *C. felis* has prevailed in two cities in Chile, Santiago and Concepción (Alcaíno *et al* 2002).

The presence of *C. canis* and *C. felis* mostly in rural areas is consistent with the findings of other authors who point out that these species prevails in rural areas (Gracia *et al* 2008, Farkas *et al* 2009). The high level of flea infestation in rural areas has been previously attributed to the lower income of dog owners from these areas and also the low access to anti-flea products compared to urban areas (Farkas *et al* 2009). *P. irritans* was more frequently found in the rural region of Arica and Parinacota, with no differences among other regions. Whether this flea has an urban or rural predilection, it is not clear; for example, in a study in Hungary it was only found in rural areas (Farkas *et al* 2009), while Gracia *et al* (2008) reported in Spain that the flea was most commonly found in animals kept in apartments than houses. *E. gallinacea* is distributed globally and this fleas occur wherever chickens are presents and has been also occasionally reported on dogs and cats (Durden and Hinkle 2009), which is confirmed in this study, where we report its presence mainly in rural areas of three regions, suggesting a common infection among birds and dogs in these areas. Whether the presence of one dog infested with *E. gallinacea* in Coquimbo could indicate the likely contact of that dog with chickens within the property or in surrounding rural areas, remains unknown.

The results of this study confirm the presence of the three species of ticks previously described in the country (*R. sanguineus*, *A. tigrinum* and *A. triste*). *R. sanguineus* was the predominant species in all the studied areas, both rural and urban, being significantly more frequent in urban areas of all regions except in Coquimbo District. *Amblyomma* species were found exclusively in rural areas; *A. triste* was only found in Arica y Parinacota and *A. tigrinum* in rural areas of Coquimbo and Araucanía districts (table 2).

Table 2. Comparison between tick-infested dogs from urban and rural areas, according to tick species and studied district.
Comparación de infestación por garrapatas en perros de zonas urbanas y rurales, de acuerdo con la especie de garrapata y la zona de estudio.

District	Urban			Rural			x^2	P
	pos	neg	%	pos	neg	%		
R. sanguineus								
Arica y Parinacota	35	78	31	57	55	51	9.2	0.002
Coquimbo	57	57	50	52	60	46	0.3	0.591
Metropolitana	54	63	46	84	33	72	15.9	<<0.001
La Araucanía	64	60	52	18	94	16	32.8	<<0.001
A. tigrinum								
Arica y Parinacota	0	113	0	0	112	0	NA	NA
Coquimbo	0	114	0	12	100	11	*	<<0.001
Metropolitana	0	117	0	0	117	0	NA	NA
La Araucanía	0	124	0	8	104	7	*	<0.01
A. triste								
Arica y Parinacota	0	113	0	11	101	10	*	<<0.001
Coquimbo	0	114	0	0	112	0	NA	NA
Metropolitana	0	117	0	0	117	0	NA	NA
La Araucanía	0	124	0	0	112	0	NA	NA

Asterisk (*) indicates that Fisher's exact test was performed.

Table 3. Mixed species of ectoparasites infestation on dogs from urban and rural areas of four regions in Chile.
Infestación múltiple por ectoparásitos en perros de zonas urbanas y rurales en cuatro regiones de Chile.

Ectoparasite	Nº of infested dogs (%)								Total
	Arica y Parinacota		Coquimbo		Metropolitana		La Araucanía		
	Urban	Rural	Urban	Rural	Urban	Rural	Urban	Rural	
Five species	0	1 (1)	0	1 (1)	0	0	0	0	2 (1)
Rs+Cc+Cf+Pi+Eg	0	1	0	1	0	0	0	0	2
Four species	0	7 (6)	0	4 (3)	0	0	0	2 (2)	13 (1)
Rs+Ati+Cc+Cf	0	0	0	1	0	0	0	0	1
Rs+Ati+Cc+Pi	0	0	0	0	0	0	0	1	1
Rs+Ati+Cc+Eg	0	0	0	1	0	0	0	0	1
Rs+Ati+Pi+Eg	0	0	0	1	0	0	0	0	1
Rs+Atr+Cc+Cf	0	2	0	0	0	0	0	0	2
Rs+Cc+Cf+Pi	0	5	0	0	0	0	0	1	6
Rs+Cc+Pi+Eg	0	0	0	1	0	0	0	0	1
Three species	9 (8)	13 (12)	16 (14)	24 (21)	0	1 (1)	7 (6)	4 (3)	74 (8)
Rs+Cc+Cf	5	3	3	11	0	1	1	1	25
Rs+Cc+Pi	0	3	7	2	0	0	6	1	19
Rs+Ati+Cc	0	0	0	2	0	0	0	0	2
Rs+Cf+Pi	1	3	5	1	0	0	0	0	10
Ati+Cc+Cf	0	0	0	4	0	0	0	0	4
Ati+Cc+Pi	0	0	0	1	0	0	0	1	2
Ati+Cc+Eg	0	0	0	0	0	0	0	1	1
Ati+Cf+Pi	0	0	1	0	0	0	0	0	1
Cc+Cf+Pi	3	4	0	0	0	0	0	0	7
Cc+Cf+Eg	0	0	0	1	0	0	0	0	1
Cc+Pi+Eg	0	0	0	2	0	0	0	0	2
Two species	24 (21)	14 (12)	25 (22)	35 (32)	4 (3)	30 (26)	18 (14)	6 (5)	156 (17)
Rs+Cc	3	0	2	7	3	25	7	4	51
Rs+Cf	9	2	7	5	1	5	0	0	29
Rs+Pi	1	3	6	1	0	0	4	0	15
Ati+Cc	0	0	0	2	0	0	0	2	4
Ati+Pi	0	0	0	1	0	0	0	0	1
Atr+Pi	0	1	0	0	0	0	0	0	1
Cc+Cf	9	1	5	11	0	0	2	0	28
Cc+Pi	0	5	1	3	0	0	5	0	14
Cc+Eg	0	0	0	1	0	0	0	0	1
Cf+Pi	2	2	4	3	0	0	0	0	11
Cf+Eg	0	0	0	1	0	0	0	0	1
Total	33 (29)[a]	35 (31)[a]	41 (36)[a]	64 (57)[c]	4 (3)[d]	31 (27)[a]	25 (20)[a]	12 (10)[d]	245 (27)

Different letters indicate statistically significant differences ($P < 0.05$).
Rs: *Rhipicephalus sanguineus*, Ati: *Amblyomma* tigrinum, Atr: *Amblyomma triste*, Cc: *Ctenocephalides canis*, Cf: *Ctenocephalides felis*, Pi: *Pulex irritans,* Eg: *Echidnophaga gallinacea*.

The presence of *R. sanguineus* was not clearly higher in urban or rural areas; in fact this species predominated in rural areas of Arica y Parinacota and the Metropolitana districts and in Angol, Araucanía District. In scientific literature, *R. sanguineus* is depicted as preferring urban settlements (Dantas-Torres 2010). In fact, some authors have found higher prevalence of *R. sanguineus* in urban than rural areas in Mozambique (Neves *et al* 2004) and Japan (Shimada *et al* 2003). Additionally, a study found no dogs positive to *R. sanguineus* in a rural region of the Amazon far from a city (Labruna *et al* 2000). However, different patterns have been found depending on specific regions

between and within countries. For instance, Dantas-Torres *et al* (2009) found 49% of prevalence in a rural area of Pernambuco, in northeast Brazil. Also, Costa *et al* (2013) in Maranhão state, also in Brazil, found higher prevalence of *R. sanguineus* in rural than urban areas. In another study, conducted in Sao Paulo state in Brazil, dogs infected with *R. sanguineus* showed a prevalence of 28% (n = 102) and 18% (n = 42) in urban and rural areas, respectively (Szabo *et al* 2001); although numerically different, these prevalence between urban and rural sites did not differ statistically.

The presence of both *A. triste* and *A. tigrinum* was confirmed exclusively in rural areas, which is explained by their life cycle that includes immature stages feeding on wild rodents (*Cricetidae* and *Caviidae*) and ground forest feeding birds, which is a limitation to its maintenance in urban areas (González-Acuña *et al* 2004, Nava *et al* 2006, Mastropaolo *et al* 2008).The presence of *A. triste*, in rural areas near Arica had been previously reported (Abarca *et al* 2012). This species is distributed from southern Mexico to Argentina (Estrada-Peña *et al* 2005). Several studies in South America found that *A. triste* occurs in hot and humid habitats, and its immature stages are spent mostly on immature rodents and marsupials (Labruna *et al* 2003). Phylogenetically *A. triste* is 99.5% similar to that found in Uruguay, taxonomic and molecularly identical to that described in Perú (Abarca *et al* 2012). The latter suggests that it has been introduced from Perú and/or existed in this area from ancient times and they share intermediate wildlife hosts. The infestation of dogs with *A. triste* is important in terms of public health, because this tick is vector of *Rickettsia parkeri*, which has been reported in different South American countries such as Uruguay (Venzal *et al* 2008). Moreover, our group recently reported the presence of *Rickettsia andeanae* in specimens of *A. triste* we reported in this study in rural areas of the Arica y Parinacota District (Abarca *et al* 2012), and also in *A. tigrinum* ticks in dogs in rural areas of the Coquimbo and Araucanía districts (Abarca *et al* 2013). Although the zoonotic potential of this bacteria remains unknown, but many Rickettsial species that initially were considered as non-pathogenic, their impact on public health has been detected later (eg. Paddock *et al* 2004). Therefore, further studies are needed to identify the exact distribution of *A. triste* and *A. tigrinum* in Chile and their impact on public health.

Mixed infestation was recorded in all regions and ranged from two to five species. Rural area of the Coquimbo District showed higher mixed infestation while the urban area in Metropolitana region the lowest. On the other hand, rural areas of the Coquimbo and Metropolitana districts showed higher infestation than their corresponding rural site. On the contrary, dogs in Angol, Araucanía District, had higher infestation than dogs in their rural counterpart. Although in Arica y Parinacota a tendency to higher infestation in rural dogs was detected this difference was not statistically significant (table 3). The results of this study partially agree with Costa *et al* (2013), who detected higher prevalence of dogs with mixed infestations in rural than urban areas.

This study shows that fleas and ticks in domestic dogs are fairly abundant across Chile and that there is not a clear pattern of a dominance of one flea or tick species over the others, but there is local variation in the pattern of infestation. Former suggest that there are other factors implicated in the infestation of fleas and tick species beside than merely the place where dogs inhabit (i.e. anti-parasite treatment, dogs allowing to roam freely, etc.), which clearly need to be assessed, along with pathogens transmitted for these vectors.

ACKNOWLEDGEMENTS

This study was financially supported by FONDECYT Projects N°1100809, 1130817 and 11100303. We also thank Paulina Lepe for determining the taxonomy of fleas and ticks.

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