

Canine vector-borne pathogens in rural dogs in Chile: molecular survey and co-infection patterns

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Abstract

Background: Canine vector-borne pathogens (CVBP) comprise a relevant and globally distributed group of disease agents. The aim of this study is to determine the co-occurrence of the most relevant CVBP of veterinary and zoonotic interest, in free-ranging, owned, rural dogs of central Chile, and to evaluate risk factors and potential non-apparent hematological alterations associated to pathogen co-infection by two or more agents.

Methods: Nine groups of canine vector-borne pathogens (CVBP) were molecularly investigated in 111 free-ranging, owned rural dogs in the Metropolitan Region of Chile.

Results: At least one pathogen was detected in 75% of the dogs. The most prevalent agent was *Anaplasma platys* (36%), followed by *Candidatus Mycoplasma haematoparvum* (CMhp; 31%), *Mycoplasma haemocanis* (Mhc; 28%), *Trypanosoma cruzi* (17%), *Leishmania* spp. (4.5%) and *Acanthocheilonema reconditum* (1%). DNA of *Ehrlichia* spp., *Rickettsia* spp., *Bartonella* spp., Piroplasmida and *Hepatozoon* spp. was not detected. Thirty-eight dogs (34%) were coinfecting, either by two (n=20), three (n=7), or four agents (n=1). The most common co-infection pattern was CMhp – Mhc (n=14). CMhp was involved in 71%, Mhc in 58%, and *A. platys* in 50% of the co-infections. Prevalence of *A. platys* was higher in juvenile than in adult dogs, whereas the opposite was found for CMhp and Mhc. Adult dogs had five times more probabilities of being coinfecting than young animals. Dogs positive for *A. platys* were infested by a larger number of *Rhipicephalus sanguineus* sensu lato ticks than uninfected individuals. At clinical evaluation, most of the animals were considered healthy, with only eight dogs (7%) presenting pale mucous membranes. Co-infecting animals showed higher white blood cell count, segmented neutrophil count and GGT levels than non-co-infecting dogs.

Conclusions: Widespread occurrence of and co-infection by CVBP was found in rural free-ranging dogs of central Chile. This study represents the first report of *Leishmania* spp. in Chile. Clinically healthy but infected dogs as those studied here may act as reservoirs of CVBP, potentially contributing to the spread of these pathogens to other tick-exposed dogs as well as human beings or wild carnivores.

Background

Canine vector-borne pathogens (CVBP) comprise a relevant and globally distributed group of disease agents (i.e., viruses, bacteria, protozoa and helminths) transmitted by hematophagous arthropods such as ticks, fleas, lice, triatomines, mosquitoes, and sand flies [1,2]. The distribution of some vector and the pathogens they transmit is changing and the transmission risk is increasing due, among other factors, to climate change [3–5]. The increased mobility and worldwide distribution of domestic dogs and cats have also contributed to the rapid extension of some vector arthropods and CVBP [6]. Furthermore, the importation of dogs from endemic areas has resulted in an overall increasing number of diagnoses of canine vector-borne diseases (CVBD) in previously non-endemic areas [7]. In addition to canine welfare, CVBD are attracting a growing medical interest due to the zoonotic nature of some of those pathogens [8]. An extended range of clinical manifestations characterizes the outcomes of CVBDs, according to host

individual factors, as well as of the occurrence of co-infections with more than one agent [9]. Hematological and biochemical abnormalities induced by CVBP are often unpredictable, especially when the dog has become co-infected by two or more organisms [8].

Little is known about the presence and the impact of CVBP in Chile, and no study in dogs has ever evaluated more than a single pathogen. *Anaplasma platys* was detected in six of 30 sick dogs analyzed in Santiago de Chile [10] but few studies have been conducted since then. The first molecular detection of *Ehrlichia canis* occurred in a dog from the northernmost part of the country [11] where the infection was believed to be confined, coinciding with the tropical lineage of *Rhipicephalus sanguineus* sensu lato [12]. DNA of *Rickettsia* spp. was previously detected in a few *Ctenocephalides* spp. fleas and *R. sanguineus* from dogs in the Metropolitan Region [13,14]. Accordingly, exposure to spotted fever rickettsiae in 35% of dogs from Santiago city was confirmed by immunofluorescence [15]. In other parts of Chile, *Rickettsia* DNA was also found in Darwin's foxes (*Lycalopex fulvipes*) [16] and also in *Ctenocephalides felis* [17] and ticks [14,18] retrieved from dogs. *Bartonella vinsonii* subsp. *berkhoffii* and *Bartonella henselae* was detected in dogs in southern Chile [19], and *Bartonella rochalimae* in *Pulex irritans* fleas retrieved from dogs [20]. Hemotropic mycoplasmas, aka hemoplasmas, were molecularly detected in dogs in southern Chile [21] and *Mycoplasma haemocanis* was also found to be prevalent in Darwin's foxes also in the south of the country [16]. The Metropolitan Region of Chile is an endemic area for Chagas' disease, with 1507 human cases notified in 2017 [22]. Since 1999, Chile has been recognized as a country that eliminated the domestic cycle of *Trypanosoma cruzi* [22]. Antibodies against *T. cruzi* were detected in 7% of periurban dogs in the Metropolitan Region [23] though no molecular evidence has been provided. While the main vector of the domestic cycle, *Triatoma infestans* has been controlled, the role of the sylvatic vector, the endemic triatomine *Mepraia spinolai* (syn. *Triatoma spinolai*), is becoming increasingly important in periurban areas of Santiago [24,25]. *Acanthocheilonema* spp. was firstly detected in a dog [26], and later on reported with a prevalence of 17% with the Knott test in the Metropolitan Region [27]. Circulating microfilariae of *Dirofilaria repens*, *Acanthocheilonema reconditum* and *Acanthocheilonema dracunculoides* were also detected in 22% of the screened rural dogs from Lampa (Metropolitan Region) [28]. In South America, *Leishmania infantum* (syn. *Leishmania chagasi*) is the most important etiological agent of canine visceral leishmaniosis, while *Leishmania braziliensis* mostly causes cutaneous forms in dogs [29]. There are no previous reports in Chile of *Leishmania* spp. Similarly, *Hepatozoon* spp. and piroplasmid parasites have been poorly investigated in Chile, despite the presence of suitable vectors (e.g. *R. sanguineus*) and the report of *Hepatozoon canis* in the whole South America [30,31] and of *Hepatozoon felis* or *Hepatozoon americanum*, in wild canids in Argentina [32,33]. There are no studies describing the presence of canine *Hepatozoon* spp. in Chile. Canine babesiosis in South America is caused by the intraerythrocytic protozoan parasites *Babesia vogeli* and *Babesia gibsoni* [34,35]. Recently, *B. vogeli* has been reported in Chile [36].

Dog population in Chile was estimated at 4.059.200 individuals [37], and owned free-roaming dogs (i.e. characterized by the lack of continuous direct supervision and irresponsible ownership) are common in Chile [38]. Owned free-ranging dogs are considered the intermediate stage between well-managed pet with movement restriction and feral dogs without human control and management [39]. In Chile, prophylactic measures such as antiparasitic treatments are infrequently applied to rural dogs by their owners [13]. This

is why these animals are useful sentinels for vector and pathogen environmental pressure in a given area [40]. Free-ranging dog lifestyle is indeed considered an important risk factor in the epidemiology of zoonosis [41]. In fact, outdoor and/ or hunting lifestyle has been associated with higher exposure to some CVBP when comparing with indoor and pet lifestyle [42,43].

It is likely that the actual burden of CVBP in the Chilean dog population has been underestimated. The aim of this study is to determine the presence and co-occurrence of nine groups of the most relevant CVBP in free-ranging, owned, rural dogs of central Chile, and to evaluate infection risk factors and potential "hidden" hematological alterations associated to pathogen co-infection by two or more pathogens.

Methods

Study area and dog sampling

The study was conducted in the Metropolitan Region of Chile (33°26'16"S, 70°39'01"W), which holds the highest human population density of the country (461.8 habitants/km²) [44]. This region has a typical Mediterranean climate, with dry, hot summers and rainy winters. Mean annual temperature is around 14.7°C (minimum and maximum absolute annual temperature= -1.1 and 37.3 °C) and annual precipitation is around 243.3 mm [45]. Typical vegetation is sclerophyllous scrub and forest [45].

From 2016 to 2018, 111 free-roaming rural dogs were sampled and examined. All sampled animals were free-ranging (without permanent confinement). Age (based on tooth eruption) and sex of dog were recorded, and general clinical sign examination carried out. Dogs were inspected for ticks for 5 minutes, and the arthropods collected were preserved in 95% ethanol. Blood obtained from the cephalic vein was collected in two separated EDTA tubes and a further tube with serum separator. The serum was removed after centrifugation and frozen at -20°C until biochemistry analysis. Hematological analyses were performed on whole blood and the remaining sample frozen at -20° until molecular analysis.

Laboratory analysis

DNA extraction from 100 µl of blood was performed using DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. DNA was eluted in 200 µl of elution buffer. An internal control PCR targeting the RPS19 gene for canine genomic DNA was carried out in all samples examined [46]. Primers and protocols for pathogen DNA detection are presented in Supplementary Table 1. Briefly, DNA of hemotropic *Mycoplasma* spp., *Bartonella* spp., *Rickettsia* spp., Anaplasmataceae (*Anaplasma* and *Ehrlichia*), Piroplasmida (*Babesia* and *Theileria*) and *Hepatozoon* spp. was screened by conventional PCR (cPCR) with the primers and run protocols described in Millán et al. [33]. Samples scored positive for *Mycoplasma* were examined with specific primers for *M. haemocanis* (Mhc) and for *Candidatus M. haematoparvum* (CMhp) to detect coinfections [47,48]. *Trypanosoma cruzi* was detected and quantified by real-time PCR following the protocols described by Yefi-Quintero et al. [49]. *Leishmania* spp. DNA was screened by qPCR using the protocol described by Francino et al. [50] and positive samples were further analyzed by cPCR using primers and run protocol previously described Cortes et al. [51] for

sequencing purposes. Filaroids were screened by cPCR as described by Casiraghi et al. [52]. To avoid cross-contamination, DNA extraction, mixing of DNA-free PCR reagents and the addition of the template DNA were carried out in separated areas with separate equipment and solutions. PCR products were visualized on a 2% agarose electrophoresis gel, and later purified and sequenced by Sanger technique. Obtained sequences were then compared with those available in GenBank by BLAST analyses (<http://www.ncbi.nlm.nih.gov/blast>). Collected ticks were identified using morphological criteria [53].

Hematology and serum chemistry

The following hematological parameters were analyzed through manual and automatic cell counter (HumaCount 80TS[®], Human, Germany): hematocrit (HCT), red blood cell (RBC), platelet (PLT) and total leukocyte count (WBC), hemoglobin concentration (HGB), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC). Relative leukocyte differentiation was performed by microscopic observation. The following serum biochemistry parameters were evaluated using Analyzer BA400[®] (BioSystems, Spain): total proteins, albumin, calcium, phosphorus, cholesterol, glucose, creatinine, urea, blood urea nitrogen (BUN), aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and Gamma-glutamyl transferase (GGT).

Data analysis

Confidence intervals for prevalence were calculated using the “EpiR” package [54] of R software [55]. Parasitological terms follow Bush et al. [56]. Differences in the occurrence of pathogens, the existence of coinfection and the number of pathogens per host depending on the dog’s sex (male/female) and age (young/adult) were evaluated. For *A. platys*, Mhc and CMhp, the prevalence and abundance of *R. sanguineus* were also analyzed as independent variables. Generalized linear mixed models (GLMMs) were used to study the binary variables (i.e., pathogen occurrence= absence/presence; pathogen coinfection= not coinfecting/ coinfecting) and fixed and random effects. GLMMs handle non-normal data by using link functions and exponential family distributions and incorporate random effects [57]. Study zone (Andean hillside, central valley and coastal hillside) was included as random effect. GLMMs were analyzed using the “lme4” package [58] of R software [55] with binomial error (logit-link function). The best model was selected using the “dredge” function from the “MuMIn” package, which generates, given a full model, a subset of models and selects the best model that best fit the data, based on Akaike information criterion corrected to sample size (AICc). The overall fit of best model was assessed by residuals analysis and comparison with the null model (with an intercept and random effects only), using the likelihood ratio test. Individuals with information on all factors were included in the models. In the case that a category of the independent variables had not any positive animal, the evaluation of that variable was carried out by Fisher's exact test. In that case, that variable was removed from the full model of GLMM analysis. Differences in hematological and biochemistry values were tested using Student’s *t* or Mann Whitney U depending of data distribution. Initially, differences between adult and young dogs were evaluated. In case of not finding significant differences between ages, these were pooled to assess the association between parameter and co-infection status, and otherwise were analyzed separately. All statistical analyses were carried out using R software [55].

Results

Pathogen occurrence and co-infection patterns

At least one CVBP was detected in 75% of the dogs (Table 1). Anaplasmatataceae DNA was found in 40 dogs (36%) (Table 1, Figure 1) and all the obtained sequences corresponded to *A. platys*. The overall prevalence of hemoplasmas was 40.5% (95%CI= 31.4 - 49.7), with CMhp and Mhc DNA being confirmed, respectively, in 34 and in 31 dogs. DNA of *T. cruzi* was detected in 19 dogs (17%) with a parasite load of one *T. cruzi* parasite equivalent/ml. *Leishmania* sp. DNA was detected in five dogs (4.5%; 95%CI= 0.6-8.4). No readable sequence was obtained by cPCR products. One dog (0.9%; 95%CI= 0.0-2.6) was positive to *A. reconditum*, and the obtained sequence showed 99.4% identity with that available in GenBank (JF461456.1). All dogs were negative for *Rickettsia* spp., *Bartonella* spp., Piroplasmida and *Hepatozoon* spp. Thirty-eight dogs (34.2%; 95%CI= 25.4-41.1) were infected with more than one pathogen (Figure 1). Among them, 30 animals were infected by two pathogens, seven by three pathogens, and one by four pathogens. The most common co-infection pattern was CMhp – Mhc (14 samples). CMhp was involved in 71.0% of the co-infections (n=27), Mhc in 57.8% (n=22) and *A. platys* in 50% of them (n=19).

Risk factor analysis

The probability of being infected by *A. platys* was four times higher (OR=4.13, 95%CI= 1.60-10.66; z-value=2.93; p =0.003) for a young than for an adult dog (Table 1, Figure 2). Adult age was associated with a higher prevalence for CMhp (Fisher's p=0.0001) and Mhc (OR=5.49, 95%CI= 1.2-25.01, z-value=2.51, p= 0.01). A total of 507 ticks were retrieved, chiefly *R. sanguineus* (505 specimens, for an infestation prevalence of 45.9%; 95%CI= 36.8-55.4). Dogs infected by *A. platys* showed higher abundance of *R. sanguineus* than those non-infected (z-value=1.947, p=0.05; Table 1, Figure 3). The prevalence and abundance of *R. sanguineus* were not related to the presence of any other agent. Adult dogs had five times higher probability of being co-infected than young ones (OR=4.9, 95%CI= 1.4-17.8, z-value=2.44, p=0.01) (Figure 2).

Clinical, haematological and biochemical findings

Most of the animals were considered apparently healthy in the clinical exploration. Only eight of the dogs (7.2%) presented pale mucous membranes, without differences between co-infected and non-co-infected animals (Fisher's p=1). Co-infected animals showed higher white blood cell count (WBC) (t= 2.01, p<0.05) and segmented neutrophil count (t=2.46, p<0.05) and GGT levels (U= 583.5, p<0.03) (Figure 4; Supplementary file 1).

Discussion

The present study is the most extensive study ever in Chile, including the most relevant CVBP in dogs. We found a widespread occurrence of CVBP in rural, free-ranging dogs of central Chile, with three quarters of the dogs positive to at least one pathogen. Previous studies in other parts of the world showed that rural dogs are frequently exposed to or infected by different vector-borne pathogens [59,60]. The outdoor activity

of these dogs exposes them to a range of vectors, which is, together with the absence of antiparasitic prophylactic management, the most likely reasons for our findings. In this sense, higher rates of exposure or infection were found in rural dogs when compared with their urban counterparts [61–63]. In the Metropolitan Region of Chile, the prevalence of *R. sanguineus* and *Ctenocephalides canis* was indeed higher in rural than in urban dogs [64].

Anaplasma platys was the only Anaplasmataceae confirmed in this study. This picture is similar to that reported in other geographical areas where *R. sanguineus* is the only tick species infecting dogs (e.g. [65,66]). The absence of *E. canis*, a bacterium that was reported in northern Chile [11], is not surprising considering that only the tropical lineage of *R. sanguineus* is able to transmit *E. canis* [67], and that in central Chile (where our study was conducted), only the temperate lineage has been previously reported [12]. Accordingly, the seroprevalence of 69% using an *A. phagocytophilum*-based IFI commercial kit registered in dogs from Santiago [15] probably corresponded to *A. platys*, due to the serologic cross-reaction among species within the family Anaplasmataceae [68]. The observed prevalence in our study can be considered high when compared with other studies in rural dogs. It is close to the prevalences reported by Brown et al. [69] in free-roaming dogs associated with remote Aboriginal communities in Australia, but higher than other studies carried out in Brazil [62], Ivory Coast [70], Kenya [70] and Uganda [59]. The higher probability of *A. platys* infections in young dogs in our study was already been recorded in a previous study in Africa [70], most likely due to a primary exposure of juvenile individuals to the pathogen [71,72] and might be related to the lower levels CD8 T lymphocytes found in young dogs [73], which has a role in clearance of rickettsial infections [74]. In agreement with our results, other studies found that dogs infested with *R. sanguineus* were more likely to be infected with or exposed to *Anaplasma* spp. than uninfested dogs [62,75,76]. Overall, our results suggest that the risk of infection with *A. platys* is more associated with the tick abundance than just the presence of the tick.

The overall prevalence of hemoplasmas in our study was similar to the prevalence observed in rural dogs in southern Chile [21], and in rural or free-ranging dogs worldwide, such as Australia [77] and Brazil [78]. Rural environments were suggested to be a risk factor for hemoplasma infections when compared with urban environments [21,79], and, accordingly, in free-ranging compared with domestic pet dogs [80]. We found similar prevalence for both hemoplasma species, coinciding with the findings by Soto et al. [21], although these authors did not detect any co-infection. The observed higher infection percentage in older dogs may be explained by an increased probability of exposure throughout life and/or by the characteristic long-term bacteremia of hemoplasma infection [81,82]. Lack of hemoplasma clearance was reported in infection follow-up studies [83,84].

To the best of our knowledge, this survey represents the first molecular detection of *T. cruzi* in dogs in the Metropolitan Region of Chile, although the presence of parasitized dogs in this region was known [23,85]. The only previous study in the country was conducted in two regions in the North of Chile [86]. It is known that dogs are competent hosts of importance in the cycle of *T. cruzi* in endemic areas [87,88]. Despite of this, there have been few molecular studies in rural dogs in the Americas [89–91]. Rural dogs have been suggested as a bridge between the domestic and sylvatic transmission cycles [89], and this can be the case in our study area, where all of the studied dogs live outdoor and some of them accompany muleteers in

areas where triatomines abound [25]. Further studies should aim to characterize the genetic diversity of *T. cruzi* in the region.

The presence of *Leishmania* spp. is herein reported for the first time in Chile, though it is endemic in some neighboring areas of Argentina [29,92,93]. Phlebotomine sandflies are present in Chile (i.e. *Lutzomyia isoposi* and *Lutzomyia* sp.) [94], but none of these species has known vectorial capacity. If confirmed, our findings would markedly extend the distribution of *Leishmania* spp., though the species identity has not been ascertained. Due to the importance of this parasite for veterinary medicine and public health, the proper characterization of the cases should be further investigated.

A higher prevalence of microfilariae of *A. reconditum* was detected at Knott's test in previous studies conducted in Chile [27,28] and this could be due to the number of animals tested or to the lower occurrence of specific vectors (i.e., fleas and lice) in the sampled animals. *Ctenocephalides felis*, in particular, is considered a well established intermediated host [95–97]. The globally distributed *C. felis* is also a common parasite of dogs in the Metropolitan Region of Chile [13,64] although they were found at low prevalence on the dogs included in this study and lice were not found at all (data not shown).

Absence or a very low number of *Rickettsia* and *Bartonella* PCR-positive dogs reported here has been previously observed elsewhere [98,99], though a high prevalence of the pathogen has been detected in ectoparasites vectors [17,100]. In absence of acute infections, low blood bacterial levels could be observed for these pathogens, being detect only by cPCR [101,102].

Piroplasmid and *Hepatozoon* spp. DNA was not detected during this survey. *Babesia vogeli* was recently found in Chile [36], is surprising we had not found it despite been present the competent vector *R. sanguineus*. Absence of *Hepatozoon* spp. and *B. gibsoni* could be due to the geographical isolation of Chile from the rest of the continent. Nevertheless, the arrival of imported cases of dogs infected with *H. canis*, and/or *B. gibsoni* must be considered a risk for the Chilean canine population, also considering the widespread presence of *R. sanguineus* in the country and the circulation of dogs among countries.

A third of the studied dogs were co-infected with two or more pathogens. Co-infection is considered frequent in CVBD-endemic areas, especially in dogs living environments with high vector density and without antiparasitic treatment [8]. Interestingly, although *A. platys* was the most prevalent agent in our study, was not the pathogen most commonly associated with co-infections in dogs, in contrast with previous studies in areas where *R. sanguineus* is prevalent [71] in areas. In our case, hemoplasma species were common in cases of coinfection, and multiple infections has indeed been considered as risk factor for hemoplasma infection [80,103].

Higher WBC and segmented neutrophil level were found in co-infected animals. No consistent leukogram abnormalities have been associated with canine hemoplasmosis or anaplasmosis [21,81,104]. However, increased leukogram values have been associated with *T. cruzi* infections [105]. On the other hand, higher GGT values were found in co-infected animals. Anyway, almost all the GGT values were into the range of the reference values [106]. It is possible that our findings may be explained by absence of acute stages of infection. Chronically infected dogs usually present low bacteremia or parasitemia [8]. Thus, dogs with

chronic or “hidden” infections used to be apparently healthy with absent or minor hematological abnormalities [8,72] . For example, most of the cases of canine haemoplasmosis used to be chronic subclinical infections and infected dogs seemed unable to clear the infection [82]. Therefore, as suggested before, co-infection complicates the diagnosis based on clinical examination and hematological and biochemistry abnormalities alone [8].

Conclusion

Rural free-ranging dogs of central Chile are infected or parasitized by a range of agents of veterinary and zoonotic interest, being a remarkable number of dogs infected by more than one pathogen. It is important to remark that those clinically healthy but infected dogs could be acting as subclinical carriers of different CVBP, possibly contributing to the spreading of some of these pathogens to potential vectors and among their owners, other dogs, or protected wild carnivores.

Declarations

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Ethics declarations

Ethics approval and consent to participate

This study was approved by the authorities in bioethics from Universidad Andres Bello under authorization 08/2016. Dog owners signed an informed consent form before samples were taken.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The data supporting the conclusions of this article are included within the article. Raw data used or analyzed during the present study are available from the corresponding author upon reasonable request.

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Author Contributions

AC and JM conceived and designed the study. AC, SDC and JM performed field work, and sample and data collection. AC, SDC, CMSM, CH and MSL carried out laboratory analysis, AC, JM, SDC and MSL analyzed the data. AC, JM, PC and DO wrote and contributed in the manuscript. All authors read and approved the final manuscript.

Abbreviations

ALP: alkaline phosphatase

ALT: alanine transaminase

AST: aspartate aminotransferase

BUN: blood urea nitrogen

CBVP: Canine vector-borne pathogens

CMhp: *Candidatus* Mycoplasma haematoparvum

cPCR: polymerase chain reaction

CVBD: Canine vector-borne diseases

DNA: deoxyribonucleic acid

EDTA: Ethylenediaminetetraacetic acid

GGT: gamma-glutamyl transferase

GLMM: generalized linear mixed models

HCT: hematocrit

HGB: hemoglobin concentration

MCHC: mean corpuscular hemoglobin concentration

MCV: mean corpuscular volume

Mhc: *Mycoplasma haemocanis*

OR: Odds Ratio

PCR: polymerase chain reaction

PLT: platelet

qPCR: quantitative polymerase chain reaction

RBC: red blood cell

WBC: total leukocyte count

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Table

Table 1. Occurrence of selected canine vector-borne pathogens in rural dogs in Chile and the occurrence of co-infections depending on host sex and age.

Factors	<i>Anaplasma platys</i>	<i>Candidatus Mycoplasma haematoparvum</i>	<i>Mycoplasma haemocanis</i>	<i>Trypanosoma cruzi</i>	Co-infection
	Oc% (95%CI)	Oc% (95%CI)	Oc% (95%CI)	Oc% (95%CI)	Oc% (95%CI)
Overall prevalence	36.0 (27.1-44.9)	30.6 (22.0-39.2)	27.9 (19.6-36.3)	17.1 (10.1-24.1)	
Sex					
Female	30.9 (16.9-44.9)	23.8 (10.9-36.6)	21.4 (9.0-33.8)	28.6 (14.9-42.2)	38.1 (23.4-52.7)
Male	39.1 (27.6-50.6)	34.8 (23.5-46.0)	31.9 (20.8-42.8)	10.1 (3.0-17.2)	33.3 (22.2-44.4)
Age					
Adult	28.7 (19.2-38.2)*	39.1 (28.8-49.3)*	33.3 (23.4-43.2)*	17.2 (9.3-25.1)	41.4 (31.0-51.7)*
Juvenile	62.5 (43.1-81.9)*	0*	8.3 (0.0-19.4)*	16.7 (1.7-31.5)	12.5 (0-25.7)*
R.s abundance					
	MA±SE	MA±SE	MA±SE		
Infected	7.2 ±2.6*	5.3±1.4	7.0±2.9		
No-infected	3.0±0.6*	4.1±1.3	3.5±0.8		

Oc: Occurrence; CI: 95% Confidence Interval; * significant differences between groups; R.s; *R. sanguineus*; MA, mean abundance; SE, standard error

Figures

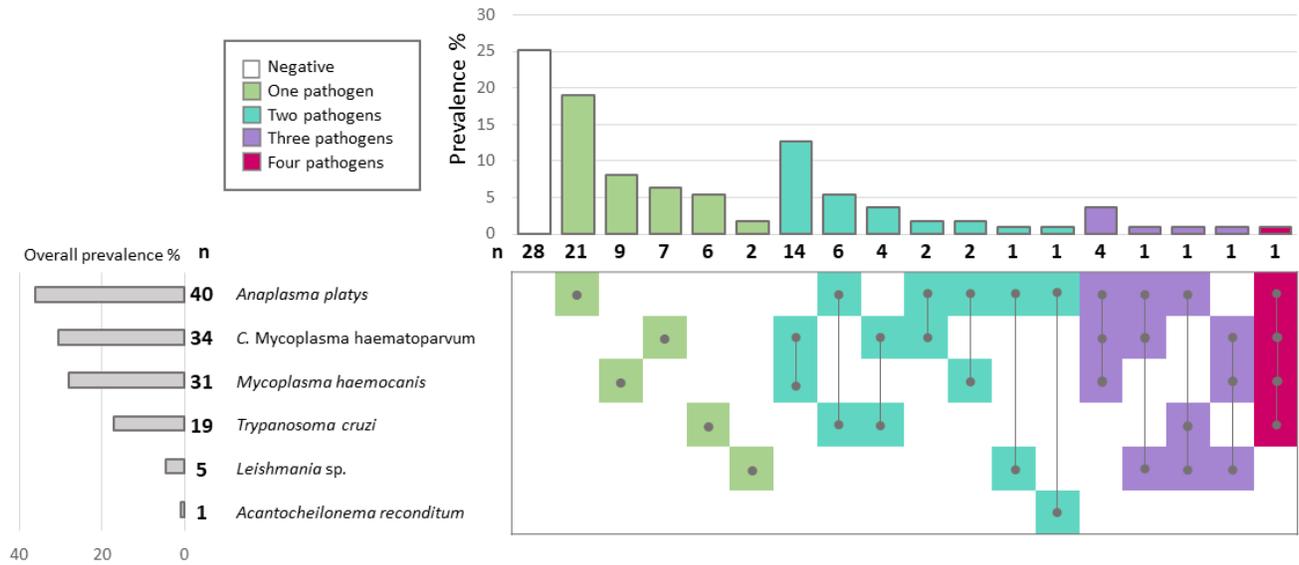


Figure 1

Number of positive animals and observed prevalence for each pathogen and each co-infection pattern in rural dogs sampled in central Chile.

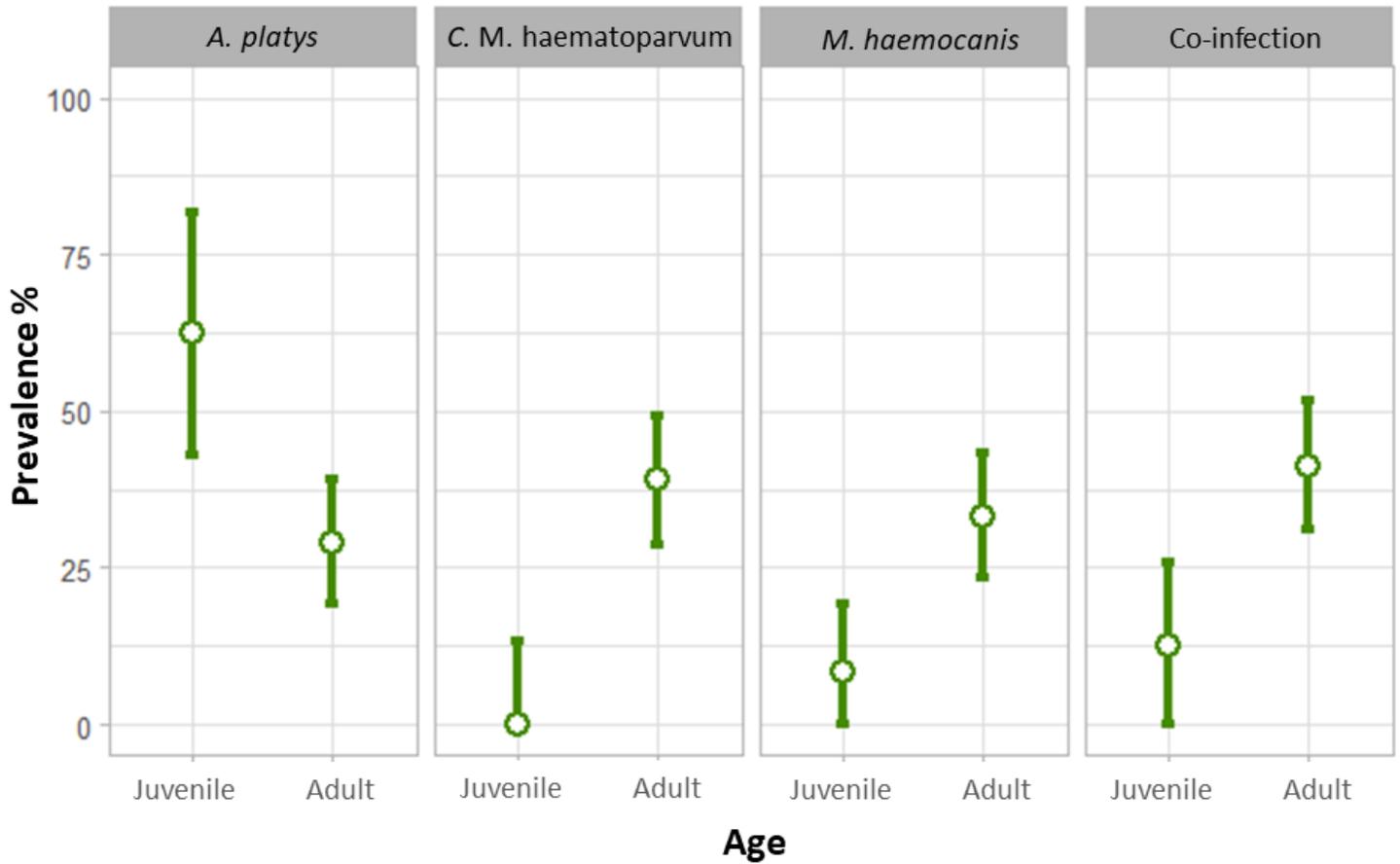


Figure 2

Significant differences in prevalence of *Anaplasma platys*, *Candidatus Mycoplasma haematoparvum*, *Mycoplasma haemocanis*, and co-infection with these pathogens plus *Trypanosoma cruzi*, *Leishmania* spp. and *Acanthocheilonema reconditum* depending on the age class of rural dogs sampled in central Chile.

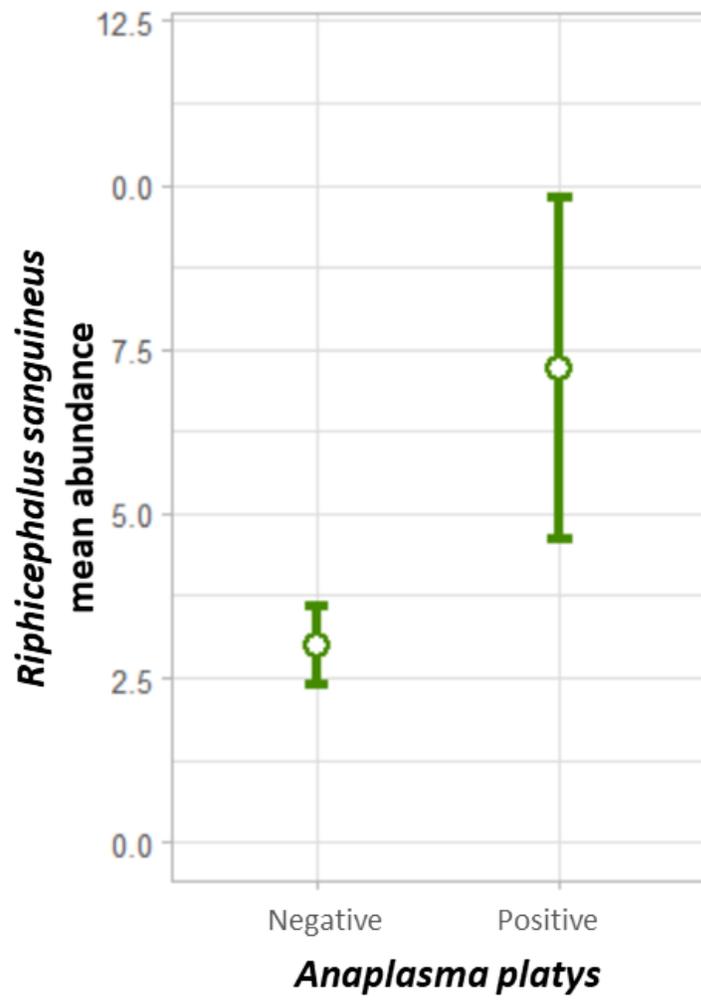


Figure 3

Abundance of *Rhipicephalus sanguineus* depending on the *Anaplasma platys* infection status.

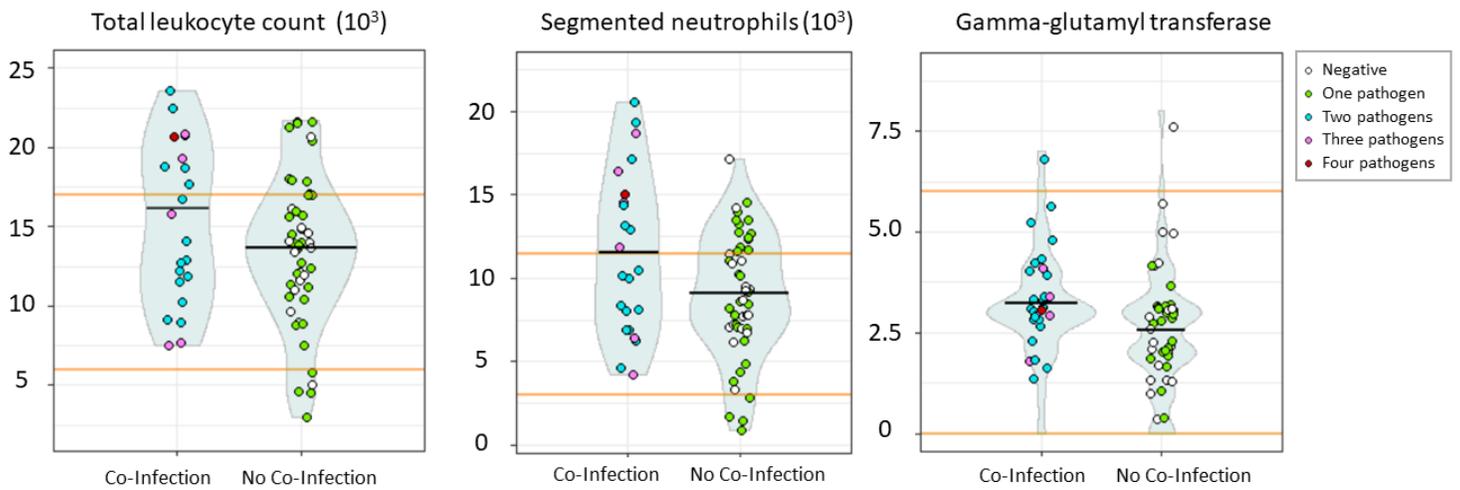


Figure 4

Significant differences in total leukocyte count, segmented neutrophil count and gamma-glutamyl transferase (GGT) depending in the co-infection status. Black lines indicate de mean and orange lines the maximum and minimum reference values based on Thrall et al. [106].

Supplementary Files

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