

Polyparasitism and zoonotic parasites in dogs from a rural area of the Argentine Chaco

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ABSTRACT

Dogs play an important role as reservoirs and hosts of multiple pathogens shared with humans and wildlife, which contribute significantly to the global burden of disease. Here, we assessed the occurrence of a broad range of zoonotic and non-zoonotic parasites in dogs from a rural area in the humid Chaco; determined the occurrence of polyparasitism; and explored its association with selected risk factors. In total, 212 dogs were examined serologically to determine *Trypanosoma cruzi* infection and 152 of them also were examined for *Ehrlichia canis*, *Borrelia burgdorferi*, *Anaplasma phagocitophilum*, *Dirofilaria immitis* and *Toxoplasma gondii*. Fecal samples from 85 dogs were examined for intestinal parasites. Seventeen parasite species were seen, 77% of which are zoonotic. The most prevalent parasites were *Ancylostoma caninum* (68.2%), *T. gondii* (55.3%, first report for dogs in Argentina), *Giardia* sp. (25.9%), *Cryptosporidium* sp. (20.0%), *T. cruzi* (16.5%), trematodes (15.3%) and *Toxocara canis* (14.1%). Polyparasitism was found in 96% of the dogs, with up to six parasite species in a single dog, and was significantly associated with age of dog but not with host body condition or sex. The most frequent pair of parasites found together were *T. gondii*-*A. caninum* (46%), *A. caninum*-*T. cruzi* (34%) and *T. gondii*-*T. cruzi* (27%). The prevalence of anemia and leukocytosis was significantly higher in dogs showing the worst body condition. Our findings likely reflect structural poverty, poor sanitation and lack of a safe water supply. Importantly, many of the prevalent parasites seen are threats to human health.

243 words.

1. Introduction

Increased relevance has been given to polyparasitism due to the finding of interspecific interactions that may modify the susceptibility to other parasites, the risk of transmission and even increase morbidity in humans, wild and domestic animals (Vaumourin et al., 2015; Madinga et al., 2017). This has prompted a shift towards a multi-host, multi-parasite paradigm in the investigation and control of infectious diseases (Gibson et al., 2011).

Zoonotic diseases contribute significantly to global disease burden, mostly due to toxoplasmosis, food-borne trematodiasis, cysticercosis,

echinococcosis, leishmaniasis and schistosomiasis, according to DALY (Disability Adjusted Life Year) metrics (Torgerson and Macpherson, 2011). Diarrheal diseases are among the three main causes of DALY (GBD 2016 DALYs and HALE Collaborators, 2017), with zoonotic protozoa, mainly *Cryptosporidium* sp., *Giardia* sp. and *Blastocystis* sp., causing a significant proportion of the global burden (Torgerson and Macpherson, 2011).

Dogs play a complex role in human societies. They play an important role for physical, psychological and social human well-being, but they are also sources of several zoonotic diseases (Chomel and Sun, 2011; Otranto et al., 2017). Polyparasitism has frequently been found in

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dogs worldwide; however, few works include both intestinal and vector borne parasites in their studies. The prevalence of polyparasitism varied between 17 and 51% for intestinal parasites based on previous work (Fontanarrosa et al., 2006; Mateus et al., 2014; Curi et al., 2017) and reached 54% for intestinal and vector-borne parasite (Traversa et al., 2017). In many rural communities of Latin America, dogs are under a regime of little or no restraint, fulfilling the categorization of “free-ranging dogs with owner”, allowing enhanced parasite transmission between them, humans and wildlife (Curi et al., 2017).

Assessing the occurrence of a broad range of zoonotic and non-zoonotic parasites and of polyparasitism in the dog population is the first step before implementing integrated disease control measures to improve human and animal health. The aims of this study were to: identify zoonotic and non-zoonotic dog parasites of relevance to human and animal health in a well-defined rural area of the Argentine humid Chaco; determine the extent of polyparasitism; and explore the association between polyparasitism and body condition, age and sex. Based on previous reports and the fact that the Gran Chaco is a hotspot for NTDs (Hotez, 2014), we hypothesized that there would be a high diversity and prevalence of zoonotic and non-zoonotic dog parasites, a high prevalence of polyparasitism, and the total number of parasite species per dog would be inversely associated with dog body condition and positively with age.

2. Materials and methods

2.1. Study area

This study is part of a broader research project on the transmission and control of Chagas disease in the municipality of Pampa del Indio (26° 2' 0" S, 59° 55' 0" W), Chaco Province, Argentina. Fieldwork was conducted in eight villages (Tacuruzal, Campo Nuevo, Lote Cuatro, Ex Parque, El Gramillar, Santa Carmen, Pampa Bandera and El Palmar) in April and October–November 2013.

Structural poverty affects the local human populations (Gurevitz et al., 2013; Gaspe et al., 2015; Provecho et al., 2017). Approximately half of the population in Pampa del Indio comprise an indigenous ethnic group (Qom) and the rest are creoles (Gaspe et al., 2015), whereas other adjacent villages only have creole residents. Ninety-six percent of all dwellings included in this study belonged to creole residents.

2.2. Study design

In a previous substudy we evaluated the association between infectiousness (as determined by xenodiagnosis), bloodstream parasite load (as assessed by qPCR) and co-infections in the dogs found naturally infected with *T. cruzi* (Enriquez et al., 2016). Herein we report the findings for the population from which these dogs were sampled. This work encompasses the same study population but evaluates a wider and more diverse number of parasites species. To maximize the probability of finding *T. cruzi*-seropositive dogs, sampling targeted dogs from Pampa del Indio that resided in households infested with *T. cruzi*-infected *T. infestans* in 2012, or whose owners reported the presence of *T. infestans* to the surveillance system, or which had mud walls (i.e., a higher probability of harboring triatomines) as described elsewhere (Gurevitz et al., 2012). In total, 69 inhabited dwellings were included. All dogs were examined at their household; owners were interviewed with a standard demographic questionnaire for each dog including age, sex, origin, administration of antihelminthic and type of food.

The body condition of each dog (good, regular or poor) was established by one study researcher (GF Enriquez) based on the degree of development of muscles, external evidence of bone structure, state of fur coat, the existence of fat deposits, and facial expression (Petersen et al. 2001). Handling and examination of dogs were conducted according to the protocol approved by the Ethical Committee for Clinical

Research from Buenos Aires (Protocol No. TW-01-004).

2.3. Serodiagnosis

Serum samples were tested for antibodies to *T. cruzi* by an indirect hemagglutination assay (IHA) and by an in-house enzyme-linked immunosorbent assay (ELISA) as described in Enriquez et al. (2016). An individual was considered seropositive when it was reactive to both assays.

To detect antibodies to *T. gondii*, sera samples were examined by indirect immunofluorescence assay test (IFAT) at the Laboratory of Immunoparasitology, Department of Epizootiology and Public Health, Faculty of Veterinary Science, National University of La Plata, Argentina. The cut-off titer was 100. The same set of sera samples were examined by an immunochromatographic test (Canine Snap 4Dx, IDEXX laboratories, Westbrook, ME, USA) to detect antibodies to *Ehrlichia canis*, *Anaplasma phagocytophilum*, and *Borrelia burgdorferi*, and antigens of *Dirofilaria immitis*, according to the manufacturer's instructions.

2.4. Stool sampling

Stool sampling was performed at households harboring at least one *T. cruzi*-seropositive dog and at households where all dogs were seronegative. We asked the owners to tie their dogs overnight in a sheltered place until our arrival on the next morning, when dogs were released one at a time and followed to where they defecated. Households where dogs could not be tied overnight were excluded from this sampling. Additional stool collections were performed opportunistically (i.e., if a dog defecated while visiting its house a sample was collected and properly labeled). An aliquot of each individual stool was collected in a vial containing sodium acetate-acetic acid-formaldehyde (SAF) (1:3) and immediately homogenized to ensure proper fixation and preservation.

2.5. Coprological examination and intestinal parasites identification

A flotation test using a saturated sodium chloride solution (specific gravity 1.20 g/ml) and a modified sedimentation test were employed for which a saline solution was used and the lipid extraction step was avoided (Garbossa et al., 2013). Helminth identification was performed morphologically by comparison with published records (Bowman et al., 2004; Ash and Orhriel, 2010). Protozoa identification was achieved by examination of a sedimentation sample with a Lugol's iodine stain or with a modified Ziehl-Neelsen technique (Duré et al., 2013). A dog was considered infected with a helminth species when an egg or larvae was detected in at least one methods of enrichment (i.e. flotation or sedimentation), and/or infected with a protozoan when cysts or coccidian oocysts were observed in at least one slide out of the two examined from the sedimentation test (Enriquez et al., 2016).

2.6. Trematode infection

We collected new samples of feces from trematode-positive dogs in December 2014 and January 2015 (nearly 18 months after the cross-sectional study) in an attempt to discard spurious parasites as the sources of the observed eggs in dogs feces. In order to rule out an oral ingestion of trematodes, dog owners were provided with dry commercial food to feed them on a controlled diet for 7 days. Three stool samples were collected from each individual in non-consecutive days during the 7-day diet. Samples were preserved and analyzed as described elsewhere (Enriquez et al., 2016).

2.7. Molecular identification of *Neobalantidium coli*

We sought to confirm the infection of *N. coli* by molecular biology in

samples in which morphologically compatible cysts were observed by microscopic examination. For DNA extraction, the stool sample was placed in a mortar with liquid nitrogen, crushed with a pestle three times, and DNA was extracted with a commercial kit (QIAmp™ DNA Stool Mini Kit, Qiagen) as described in López Arias et al. (2017). In order to confirm the identity of the cysts, PCR amplification and sequence analysis of the 18S rRNA gene were performed as described in López Arias et al. (2017).

2.8. Hematological profile

Hematological profiles were evaluated as described elsewhere (Enriquez et al., 2016). A dog was considered anemic when hemoglobin concentration, red blood cell count (RBC) and/or hematocrit showed values lower than those in the reference range. Leukocytosis, neutrophilia, eosinophilia and lymphocytosis were established when the white blood cell count and/or the percentage of neutrophils, eosinophils or lymphocytes were higher than the corresponding reference range.

2.9. Data analysis

Wilson binomial 95% confidence intervals (CI) were used for proportions (Brown et al., 2001). The Kruskal-Wallis test was used to test differences among villages in the average number of dogs per household and median age (in months). Dogs with a poor or regular body condition were grouped in the same category because there were too few dogs in a poor condition. The association between host body conditions and anemia, leukocytosis, neutrophilia, eosinophilia and lymphocytosis was examined with χ^2 tests. We evaluated the association between infection with (or exposure to) an identified parasite species and selected predictors by means of maximum likelihood multiple logistic regressions with random effects as implemented in STATA (Stata 15.1, Stata Corp, College Station, Texas). The village of residence was included as a random effect in the regression for *T. cruzi* infection because triatomine control actions were usually implemented at village level. For the remaining parasites, control actions were usually undertaken by dog-owners at household level; therefore, the household was included as a random factor. The response variables analyzed was dog infection status (or exposure to) to each parasite species with at least a 5% prevalence in the study population. Selected independent variables were: age of the dog (a continuous variable, in months), sex (a binary variable, 0 = female, 1 = male), administration of anthelmintic (a binary variable only considered for intestinal helminths, 0 no administration, 1 at least one round) and body condition (a binary variable, 0 = good body condition, 1 = regular or poor body condition). Interaction terms were added stepwise and excluded from the final model if they were not significant at the 5% level.

To evaluate whether polyparasitism was aggregated, we estimated the value of the parameter “k” from the negative binomial distribution, expected to be < 5 in case of aggregation (Wilson et al., 2001). In addition, we evaluated the association between polyparasitism and selected predictors by means of maximum likelihood Poisson regression with random effects implemented in STATA (Stata 15.1, Stata Corp, College Station, Texas). The response variable analyzed was number of parasite species per dog. Selected independent variables were: age of the dog, sex and body condition, and the household was included as a random factor. The Wald test was used to examine the hypothesis that all regression coefficients were equal to 0. For both regression analysis, we considered only dogs examined by the full set of diagnostic tests described (n = 77).

We performed a generalized Chi-Square test implemented in R software (version 3.0.1) to detect parasite associations according to Vaumourin et al. (2014). This test was reported to have high power and sensitivity and to perform similarly well in cross-sectional or longitudinal studies of a rodent population exhibiting polyparasitism

(Vaumourin et al., 2014). A threshold prevalence of 15% was used for selecting parasites for this test.

3. Results

In total, 212 out of 226 registered dogs from eight villages were included in this study. Seven dogs were excluded because they were younger than four months of age and maternal antibodies could interfere in serodiagnosis; another seven dogs could not be bled. The mean number of dogs per household was 3.3 (Standard Error, SE = 0.2) and the median age was 24 months (first-third quartiles [Q1–Q3] = 12–60). No significant difference in the mean number of dogs per household and age of dogs was found among villages (Kruskal-Wallis test; df = 5; p = .9 and p = .2, respectively). The sex ratio was significantly skewed to males (male: female = 5.1; $\chi^2 = 102.2$, df = 1, p < .0001).

Dogs were frequently (85%) fed raw meat and viscera from cows, goats and pigs. Additional sources of food were wild animals (armadillos, birds and rodents) hunted by the dogs. Over half (51%) of dog-owning households reported to have administered broad-spectrum antiparasitics to them at least once with over half (52%) of those that treated their dogs administering treatment only once when the animal was < 1 year of age.

3.1. Intestinal parasites

Overall, 75 out of 85 (88%) dogs were infected with at least one intestinal parasite (Table 1). Helminth infections were found in 79% of dogs: *Ancylostoma caninum* in 68.5% (58/85), trematodes in 15.3% (13/85), *Toxocara canis* in 14.1% (13/85), *Taenia* sp./*Echinococcus* sp. in 5.9% (5/85), *Spirocerca lupi* in 5.9% (5/85), *Trichuris vulpis* in 3.5% (3/85), *Dipylidium caninum* in 2.4% (2/85) and *Capillaria* sp. in 1.2% (1/85). Zoonotic rodent parasite eggs also were observed in dog feces as spurious parasites: *Hymenolepis diminuta* in 3 (3.5%) and *Hymenolepis nana* in 2 (2.4%) dogs. *Ancylostoma caninum* were found in all study villages and *T. canis*-infected dogs and trematode-infected dogs were found in six and seven villages, respectively. Trematode eggs were found in fecal samples from three of the five dogs re-examined for trematode infection 18 months later. In all cases, these eggs were yellow-brown in color and their mean length and width were 117 and 63 μ m, respectively (Standard Error, SE = 1.6 for both). Most probably, these eggs belong to *Alaria* sp. However, given that *Fasciola hepatica* is endemic in the province, we cannot rule out this species.

Nearly half of the dogs (49%) were infected with one or more intestinal protozoa. Dogs were found infected with *Giardia* sp. in 25.9% (22/85), *Cryptosporidium* sp. 20.0% (17/85), *Entamoeba* sp. 11.8% (10/85) and *Neobalantidium coli* 2.4% (2/85) (Table 1). *Giardia* sp. were prevalent in all study villages and *Cryptosporidium* sp. in six villages. Only in one from two dogs was DNA extraction successful and *N. coli* was confirmed. The newly-reported sequence is available at GenBank under accession number MH171492.

3.2. Vector-borne parasites and *Toxoplasma gondii* infection

Serum samples were obtained from 212 (97%) dogs. *Trypanosoma cruzi* seroprevalence was 16.5% (35/212) (Table 1). Antibodies against *Ehrlichia canis* were detected in 7.9% (12/152) of the dogs; antibodies anti-*Anaplasma phagocytophilum* in 2.0% (3/152); and antigens of *Dirofilaria immitis* were detected in 0.7% (1/152) of the dogs. No antibodies against *Borrelia burgdorferi* were detected. *Trypanosoma cruzi* and *E. canis*, the most prevalent vector-borne parasite infections in the study area, were found in dogs from six and five villages, respectively. Antibodies against *T. gondii* were detected in 84 of 152 (55.3%) dogs and occurred in all study villages (Table 1).

Table 1
Frequency and prevalence of intestinal and vector-borne parasites found in dogs from Pampa del Indio and adjacent villages, Chaco, 2013.

	Pampa del Indio (San Martín Department)	Quitilipi Department	25 de Mayo Department	General Güemes Department	Total prevalence % (95% CI)
No. of households	13	11	17	28	–
Vector-borne (No. positive samples/No. tested samples)					
<i>Trypanosoma cruzi</i>	9/48	7/21	6/43	13/100	16.5 (12.1–22.1)
<i>Ehrlichia canis</i>	4/32	2/20	4/41	2/59	7.9 (4.5–13.4)
<i>Anaplasma phagocytophilum</i>	0/32	0/20	1/41	2/59	2.0 (0.4–5.9)
<i>Dirofilaria immitis</i>	0/32	1/20	0/41	0/59	0.7 (0.0–4.0)
Other (No. positive samples/No. tested samples)					
<i>Toxoplasma gondii</i>	20/32	10/20	23/41	31/59	55.3 (47.3–62.9)
Intestinal parasites (No. positive samples/No. tested samples)					
<i>Ancylostoma caninum</i>	20/27	7/12	14/20	17/26	68.2 (57.7–77.2)
<i>Toxocara canis</i>	3/27	1/12	3/20	5/26	14.1 (8.1–23.2)
<i>Spirocerca lupi</i>	3/27	1/12	0/20	1/26	5.9 (2.2–13.4)
<i>Capillaria</i> sp.	0/27	0/12	1/20	0/26	1.2 (0.0–0.7)
<i>Trichuris vulpis</i>	1/27	1/12	1/20	0/26	3.5 (0.8–10.3)
Trematodes	6/27	2/12	1/20	4/26	15.3 (9.0–24.6)
<i>Taenia</i> sp./ <i>Echinococcus</i> sp.	0/27	2/12	2/20	1/26	5.9 (2.2–13.4)
<i>Dipylidium caninum</i>	2/27	0/12	0/20	0/26	2.4 (0.1–8.7)
<i>Giardia</i> sp.	9/27	2/12	6/20	5/26	25.9 (17.7–36.1)
<i>Cryptosporidium</i> sp.	6/27	1/12	5/20	5/26	20.0 (12.8–29.8)
<i>Entamoeba</i> sp.	2/27	1/12	1/20	6/26	11.8 (6.3–20.5)
<i>Neobalantidium coli</i>	0/27	0/12	0/20	2/26	2.4 (0.1–8.7)

3.3. Risk factors for host infection

Among intestinal parasites, we found a significant association between age of dog and the infection with trematodes (OR = 1.02; 95% CI = 1.00–1.04; $p = .02$) (Wald $\chi^2 = 7.68$; $p = .02$). There was no association between intestinal parasite infection and sex, body condition or antiparasiticide administration for any species (Table 2).

Trypanosoma cruzi infection was significantly associated with age (OR = 1.04; 95% CI = 1.01–1.06; $p < .01$) (Wald $\chi^2 = 8.26$; $p < .01$), but not with sex (OR = 1.80; 95% CI = 0.47–6.79; $p = .39$) (Table 2). *Ehrlichia canis* infection was significantly associated with age (OR = 1.03; 95% CI = 1.00–1.05; $p = .01$) (Wald $\chi^2 = 6.71$; $p = .01$) (Table 2), and all infected dogs were male.

3.4. Polyparasitism, body condition and hematological profile

At least 17 parasite species were found in the study dogs and most of them were zoonotic (77%). Moreover, of the 77 dogs examined with the full battery of diagnostic tests, none were free from infection and 96% (95% CI = 89–99%) were poly-infected, ranging up to six parasite species in a single dog (Online Resource 1), and ranged from 1 to 5 parasites per dog if only zoonotic species were considered. We found single infections with *T. gondii*, *A. caninum* and *Giardia* sp. The frequency distribution of co-infections showed no aggregation ($k = 20$); 69 dogs (90%) were infected with at least two species (Fig. 1). The number of parasite species per dog was significantly associated with age of dog (IRR = 1.004; 95% CI = 1.001–1.006; $p = .02$) and not with sex (IRR = 0.995; 95% CI = 0.747–1.325; $p = .97$) or body condition (IRR = 1.031; 95% CI = 0.772–1.376; $p = .84$) (Wald $\chi^2 = 5.57$; $p < .02$). The most frequent pair of parasites found together were *T.*

Table 2
Risk factors in dogs infected with vector-borne and intestinal parasites. Pampa del Indio and adjacent villages, Chaco, 2013.

Parasite species	Number of examined dogs	Age	Sex	Use of antiparasitic drug ^c	Body condition
		OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Vector-borne parasites					
<i>Trypanosoma cruzi</i>	212	1.02 (1.01–1.03)**	1.40 (0.41–4.66)	NA	1.66 (0.64–4.32)
<i>Ehrlichia canis</i>	152	1.03 (1.00–1.05)*	– ^a	NA	3.13 (0.66–14.92)
Other					
<i>Toxoplasma gondii</i>	152	1.01 (1.00–1.02)	1.60 (0.58–4.39)	NA	1.04 (0.40–2.71)
Intestinal parasites					
<i>Ancylostoma caninum</i>	85	1.02 (0.98–1.05)	0.21 (0.01–3.73)	0.34 (0.05–2.20)	0.29 (0.03–2.84)
<i>Toxocara canis</i>	85	1.01 (0.99–1.03)	0.47 (0.11–2.07)	0.77 (0.20–3.07)	0.75 (0.13–4.30)
Trematodes	85	1.02 (1.00–1.04)*	0.25 (0.06–1.08)	0.36 (0.08–1.63)	0.37 (0.05–2.56)
<i>Spirocerca lupi</i>	85	1.03 (0.99–1.06)	0.47 (0.03–7.24)	0.73 (0.08–6.30)	1.07 (0.05–25.50)
<i>Taenia</i> sp./ <i>Echinococcus</i> sp.	85	1.00 (0.97–1.03)	– ^b	– ^b	5.24 (0.81–33.97)
<i>Giardia</i> sp.	85	0.99 (0.97–1.01)	2.20 (0.44–10.92)	NA	0.62 (0.18–2.14)
<i>Cryptosporidium</i> sp.	85	0.99 (0.97–1.01)	3.59 (0.43–29.81)	NA	0.97 (0.27–3.43)
<i>Entamoeba</i> sp.	85	0.99 (0.96–1.01)	1.75 (0.20–15.17)	NA	0.74 (0.12–4.53)

* $p < .05$.

** $p < .01$.

^a All *E. canis*-infected were male.

^b Excluded from analysis because of the low frequency of infected dogs.

^c Nine dogs were excluded from analysis because antiparasitic use had not been registered. NA, not applicable. This parasite is not a target of broad-spectrum antiparasitic drugs.

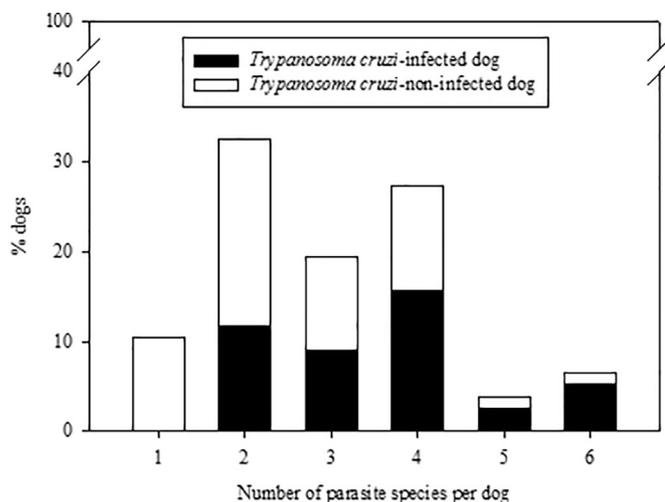


Fig. 1. Number of parasite species per dog. Pampa del Indio and adjacent villages, Chaco, 2013. Includes 77 dogs examined by all serological and coprological tests. Inside each bar, the black area shows the proportion of *T. cruzi*-infected dogs belonging to each category.

gondii-*A. caninum* (in 35 out of 77 dogs, 46%), *A. caninum*-*T. cruzi* in 26 dogs (34%) and *T. gondii*-*T. cruzi* in 21 dogs (27%) (Table 3). *Ancylostoma caninum*, trematodes, *T. cruzi*, *Giardia* sp., *Cryptosporidium* sp. and *T. gondii* were included to perform the generalized Chi-Square test. However, no specific association was found among parasite species ($\chi^2 = 2.37$, $df = 63$, $p = .50$).

Nearly half (46%) of the dogs were anemic, 19% had leukocytosis, 30% eosinophilia, 23% lymphocytosis and 10% neutrophilia. These abnormal hematological values were more frequent in dogs with a regular or poor body condition than in dogs with a good body condition (Table 4). However, significant associations with host body condition were only found for anemia ($\chi^2 = 6.06$, $df = 1$, $p = .01$) and leukocytosis ($\chi^2 = 4.07$, $df = 1$, $p = .03$).

4. Discussion

Our results show that the study dog population was infected with or exposed to multiple parasite species where poly-infected dogs were the rule. Remarkably, most parasites were zoonotic, revealing a potential high risk of disease transmission such as toxoplasmosis, Chagas disease, cutaneous and visceral larva migrans, and giardiasis to the local human population. Dogs may also represent a threat for local wildlife as a source of multiple parasites, as described for some canine viruses in Pampa del Indio (Orozco et al., 2014).

The study of parasite communities has the potential to reveal ecological principles with implications to biodiversity, response of ecosystems to habitat degradation and fragmentation, and control of pest species (Roberts et al., 2001). Poly-parasitism may result from common risk factors and/or direct or indirect-interactions among parasite species (Vaumourin et al., 2015). Therefore, identifying parasite associations, common risk factors and eventual interspecific interactions may

enable combined diagnosis of multiple parasites of public health importance as suggested for Strongyloidiasis and Chagas disease (Salvador et al., 2017; Puerta-Alcalde et al., 2018). In our study, we recorded a rich community of parasites of different species in which each dog could be considered a habitat patch. No dog was free from infection or exposure to the study pathogens and 96% of them were co-infected with at least two parasite species. Zoonotic parasites were found in all dogs and only in 4% as a single infection. However, no significant association between pairs of parasite species was detected among the six species considered, unlike other cross-sectional studies (Mateus et al., 2014; Symeonidou et al., 2017). However, cross-sectional surveys are not the most appropriate design for evaluating interspecific interactions between parasites (Fenton et al., 2014; Vaumourin et al., 2014), thus constituting a limitation of our study, as well as the limited number of dogs examined by all techniques. In addition, interspecific interactions may modify other infection parameters such as the intensity of parasitemia or parasite burden (Telfer et al., 2008), as reported for *T. cruzi* parasitemia and co-infection with *A. caninum* and trematodes in this dog population (Enriquez et al., 2016). Given the relatively high frequency of *T. cruzi*, *A. caninum* and trematode co-infections, future studies may evaluate the existence of common risk factors and elucidate the existence of parasite interactions and the underlying mechanisms.

Zoonotic and non-zoonotic dog parasites exhibit a heterogeneous distribution among different geographical regions. Many biotic and abiotic factors can contribute to this heterogeneity, as well as the diverse preventive strategies adopted by dog owners (Otranto et al., 2017). The overall prevalence of intestinal parasite infection and of each parasite species are among the highest reported in Argentina and elsewhere. Reported intestinal parasite prevalence ranges between 9 and 93% worldwide, and, as in this study, *T. canis*, *A. caninum*, *Giardia* sp. and *Cryptosporidium* sp. are the most frequent species found (Fontanarrosa et al., 2006; Soriano et al., 2010; Cociancic et al., 2018; Rivero et al., 2017; Mateus et al., 2014; Adolph et al., 2017; Traversa et al., 2017).

One limitation of our study is that egg identification mainly relied on morphology; thus, *A. caninum* eggs cannot be differentiated from *Ancylostoma braziliense*, which has been reported from the United States, Surinam and Cuba extending to Brazil and Uruguay (Malgor et al., 1996; Traub et al., 2007; Bowman et al., 2010). Our diagnosis as *A. caninum* eggs was based on the fact that *A. braziliense* has not been described in Argentina to date (Fontanarrosa et al., 2006; Soriano et al., 2010).

We also found the occurrence of *H. nana* and *H. diminuta* eggs in dog feces. Pseudoparasitism may explain these findings (Bowman et al., 2004). Regarding the finding of trematode eggs, no conclusive genus identification was obtained after several attempts (Enriquez et al., 2016). These eggs may belong to *Alaria* sp. since it has a worldwide distribution and was found in carnivorous coprolites in Argentina (Zajac and Conboy, 2012; Fugassa et al., 2018). However, we cannot rule out an infection by *F. hepatica* or spurious findings as was described elsewhere (Marcos et al., 2006), because eggs are morphologically similar and *F. hepatica* is endemic in the Chaco province (Mera y Sierra et al., 2011). Preservation of fecal samples in formalin solution precluded further molecular diagnostic attempts. Although we cannot rule

Table 3

Frequency matrix of the co-occurrence of two parasitic species per dog. Pampa del Indio and adjacent villages, Chaco, 2013.

Parasite species ^a	<i>T. gondii</i>	<i>A. caninum</i>	Trematode	<i>Giardia</i> sp.	<i>Cryptosporidium</i> sp.	<i>T. cruzi</i>
<i>T. gondii</i>	–					
<i>A. caninum</i>	35	–				
Trematode	13	11	–			
<i>Giardia</i> sp.	13	15	3	–		
<i>Cryptosporidium</i> sp.	10	11	2	2	–	
<i>T. cruzi</i>	21	26	7	5	5	–

^a Only dogs examined by the full set of diagnostic tests used.

Table 4
Body condition and hematological profile of dogs. Pampa del Indio and adjacent villages, Chaco, 2013.

Body condition	Number of examined dogs ^a	Mean age, in months (SD)	Anemia % (95% CI) [*]	Leukocytosis % (95% CI) ^{**}	Lymphocytosis % (95% CI)	Neutrophilia % (95% CI)	Eosinophilia % (95% CI)
Good	78	42 (35)	39 (28–50)	14 (8–24)	22 (14–32)	9 (24–17)	27 (18–38)
Regular/Bad	31	31 (37)	65 (47–79)	32 (19–50)	26 (14–43)	13 (5–29)	39 (24–56)
Total	109	41 (34)	46 (37–55)	19 (13–28)	23 (16–32)	10 (6–17)	30 (22–40)

^a Four dogs were excluded because they were not classified for body condition; seven dogs were not evaluated for hemoglobin concentration.

^{*} p = .01.

^{**} p = .03.

out the hypothesis of pseudoparasitism, we found strong evidence for a true trematode infection: i) high overall prevalence (15.3%) in the study dogs; ii) widespread presence in seven distant study villages, and iii) the same trematode-positive dogs had trematode eggs when they were re-examined nearly one year later after being under a controlled diet. Identification of this trematode remains for future investigation.

The prevalence of *T. cruzi*-infected dogs found in this study (16.5%) is within the observed range (10–30%) reported for endemic areas with no recent triatomine control actions (Gürtler and Cardinal, 2015). However, the study villages included mixed levels of vector control status; while villages from Pampa del Indio municipality had been under a supervised community-based vector surveillance program that virtually interrupted domestic transmission after a few years (Gurevitz et al., 2013; Gaspe et al., 2015; Provecho et al., 2017), neighboring municipalities were under less stringent surveillance measures. Given the frequent house reinfestation with *T. infestans* in the absence of effective surveillance, *T. cruzi*-infected dogs are a risk factor for human infection (Gürtler et al., 2007; Gürtler and Cardinal, 2015).

Regarding other vector-borne parasites (*D. immitis*, *Ehrlichia* spp., *B. bugderfori* and *Anaplasma* spp.), infection/exposure prevalence spanned between 1 and 51% (Oscherov et al., 2011; Cuervo et al., 2013; Miró et al., 2013; Dantas-Torres and Otranto, 2016; Movilla et al., 2016; Montenegro et al., 2017). In this work, the observed prevalence was within the range of global estimates and similar or lower than in other Argentine regions (Vezzani et al., 2011; Eiras et al., 2013; Orozco et al., 2013; Cicuttin et al., 2016). Given that *Anaplasma platys* (not *A. phagocytophilum*) was previously found in Argentina and that the serological test used may cross-react between these species (according to the manufacturer's manual), it is highly likely that dogs were exposed to *A. platys*. Our findings extend the geographical distribution of these pathogens and may guide both veterinarians and physicians in diagnosis and treatment (Dantas-Torres and Otranto, 2016; Traversa et al., 2017).

Toxoplasma gondii is a zoonosis in which dogs, despite not being definitive hosts, may play an important role as mechanical shedder of *T. gondii* oocysts (Rengifo-Herrera et al., 2017) and as sentinels of environmental contamination (Benitez et al., 2018). The seroprevalence of *T. gondii* fell within the range reported in dogs from Central and South America (Dubey et al., 2007; Bronson et al., 2008; Alvarado-Esquivel et al., 2014; Acosta et al., 2016; Rengifo-Herrera et al., 2017). We were not able to find any report of *T. gondii* seroprevalence in dogs for Argentina, and thus our study may contribute the first findings. Our finding suggests environmental contamination with *Toxoplasma* oocysts may occur in the study area, to which humans and other vertebrates would also be exposed. Consumption of small mammals and birds are additional sources of infection for dogs (which humans would not share), thus increasing the exposure in them (Alvarado-Esquivel et al., 2014).

Host age was significantly associated with *T. cruzi*, *E. canis*, trematode infections and with the number of parasite species found per dog probably reflecting the effect of cumulative exposure time or age-related changes in exposure risk due to behavioral shifts or seasonality (Wilson et al., 2001). No significant association was found between parasite infection and polyparasitism with host sex, body condition or

reported antiparasitic drug use for any species. The strong bias towards male dogs and lack of accurate records on drug administration schedules may mask any existing association. Regarding body condition and polyparasitism, in Panamá dogs in the worst body condition had higher parasite richness than dogs in better body condition (Fung et al., 2014). Alternatively, if heavier parasite burdens generate a greater damage to host health status, parasite burden and not the number of co-infecting parasite species would be more closely associated with host body condition. This aspect remains for future research because we did not assess parasitic burden.

Because our study design sought to increase the probability of finding *T. cruzi*-infected dogs, its prevalence may be overestimated and may likely affect other prevalence estimates. However, 81% of the study dwellings were selected on the basis of having mud walls, which is the dominant local feature (Gurevitz et al., 2012). One of the strengths of this study is that all samples were collected at the same time or within one day; all samples were individually identified, and stool samples were fresh. This allowed us to perform all diagnostic tests which is essential to evaluate polyparasitism for parasite species with different transmission routes.

The prevalence of anemia was significantly associated with host body condition. Moreover, 90% of anemic dogs exhibited a regenerative anemia, in which the loss of erythrocytes could be due to hemorrhage or hemolysis caused by helminths or hemoparasites, respectively. Nearly 70% of dogs with regenerative anemia were infected with *A. caninum*, well-known for causing anemia and dramatic changes in hematologic parameters (Dracz et al., 2014). Additionally, the hemoparasites *E. canis*, *A. phagocytophilum* and *A. platys* have been associated with anemia in dogs (Gaunt et al., 2010); in our study 7 from the 50 anemic dogs had been exposed at least to one of these parasites. Leukocytosis was significantly higher in dogs with a poor or regular body condition, whereas eosinophilia, lymphocytosis and neutrophilia were also higher in dogs displaying a poor body condition, though not significantly so. The bone marrow produces increased numbers of leukocytes in response to multiple parasitic infections. For example, *A. caninum* infections lead to an increase in leukocytes, eosinophils, and lymphocytes (Loukas et al., 2005; Dracz et al., 2014). However, alterations in the leucogram may also derive from the stress response to animal handling procedures, venipuncture or unregistered inflammatory processes (Dracz et al., 2014; Fung et al., 2014). The associations between parasitic infection and red or white blood cells alterations are hard to reveal using observational studies; additionally, polyparasitism may produce changes in blood cells that differ from changes caused by single infections (Dracz et al., 2014).

5. Conclusions

This study took place in a rural area of the Argentine Chaco where structural poverty, undernourishment and parasitic diseases affect the human population. In consequence, the health status of companion animals is likely to mirror this situation. The high frequency of parasite species and polyparasitism, including dogs with a poor body condition and anemia (potentially related to parasitic infections), are in agreement with the socio-economic and epidemiological context. This

pattern is probably frequent elsewhere in resource-limited areas of the humid Chaco region. The elevated number of zoonotic parasite species and their potential interspecific interactions, as the one that lead to the occurrence of super-infectious *T. cruzi*-infected dogs (Enriquez et al., 2016), merit the design and implementation of integrated disease management based on a One Health framework.

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Ethical statement

Handling and examination of dogs were conducted according to the protocol approved by the Ethical Committee for Clinical Research from Buenos Aires (Protocol No. TW-01-004).

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