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Sección Especial

LOS MAMÍFEROS COMO HOSPEDADORES DE PARÁSITOS

Artículo

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DOMESTIC DOGS AS HOST OF ECTOPARASITES CARRYING *RICKETTSIA*, *BARTONELLA* AND *MYCOPLASMA* IN URBAN, PERI-URBAN AND RURAL AREAS FROM CENTER ARGENTINA

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ABSTRACT. The domestic dog (*Canis lupus familiaris*) plays a vital role in transmitting zoonotic ectoparasites and vector-borne pathogens, often being an important source of pathogens in spillover and spillback processes between domestic and wild animals. The aim of this study was to analyze the diversity and prevalence of ectoparasites and their associated bacteria, *Rickettsia*, *Bartonella*, and *Mycoplasma*, in dogs from urban, peri-urban, and rural environments in central Argentina. A total of 180 dogs were examined, and 308 ectoparasites were collected. Diversity and prevalence (P) for the environment were: Urban (P_{total} = 78%) [*Ctenocephalides felis felis* (P = 78.1%); *Rickettsia felis* (P = 25%); *Bartonella* sp. (P = 8.3%); *Mycoplasma suis* (P = 8.3%)]; Peri-urban (P_{total} = 83%) [*C. felis felis* (P = 80%); *Rhipicephalus sanguineus* s.l. (P = 20%); *R. felis* (P = 19.2%); *Bartonella* sp. (P = 25%); *M. suis* (P = 3.8%)]; Rural (P_{total} = 50%) [*Pulex irritans* (P = 45.4%); *R. sanguineus* s.l. (P = 15.1%); *R. felis* (P = 7.4%); *M. suis* (P = 8.7%)]. These results present new insights into bacteria distribution across environments, emphasizing the role of dogs in their circulation. *Pulex irritans*, a flea with the highest prevalence in foxes, exclusively found in the rural environment, supports the hypothesis that wild and domestic sympatric carnivores share ectoparasite species. The detection of *M. suis* for the first time in all analyzed ectoparasite species and environments raises questions regarding the potential reservoir role of dogs for this pathogen. Furthermore, it suggests that dogs infested with ectoparasites may be exposed to an unknown range of potentially invasive vector-borne pathogens. These findings reinforce the importance of parasitological and epidemiological studies in domestic mammals under the "One Health" paradigm.

RESUMEN. PERROS DOMÉSTICOS COMO HOSPEDADORES DE ECTOPARÁSITOS PORTADORES DE BACTERIAS *RICKETTSIA*, *BARTONELLA* Y *MYCOPLASMA* EN ÁREAS URBANAS, PERI-URBANAS Y RURALES DEL CENTRO DE ARGENTINA. El perro doméstico (*Canis lupus familiaris*) desempeña un papel fundamental en la transmisión de ectoparásitos y patógenos transmitidos por vectores, y muchas veces es una fuente importante en el proceso de traspaso de patógenos entre animales silvestres y domésticos. El objetivo de este trabajo fue analizar la diversidad y prevalencia de ectoparásitos y bacterias de los géneros *Rickettsia*, *Bartonella* y *Mycoplasma* en perros provenientes de ambientes urbanos, peri-urbanos y

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rurales del centro de Argentina. Se examinaron 180 perros, y se recolectaron 308 ectoparásitos. La diversidad y prevalencia (P) por ambiente fue: urbano (P_{total} = 78%) [*Ctenocephalides felis felis* (P = 78,1%); *Rickettsia felis* (P = 25%); *Bartonella* sp. (P = 8,3%); *Mycoplasma suis* (P = 8,3%)]; periurbano (P_{total} = 83%) [*C. felis felis* (P = 80%); *Rhipicephalus sanguineus* s. l. (P = 20%); *R. felis* (P = 19,2%); *Bartonella* sp. (P = 25%); *M. suis* (P = 3,8%)], y rural (P_{total} = 50%) [*Pulex irritans* (P = 45,4%); *R. sanguineus* s.l. (P = 15,1%); *R. felis* (P = 17,4%); *M. suis* (P = 8,7%)]. Aportamos nueva información sobre la distribución de las bacterias en distintos ambientes, y se destaca el papel del perro para su circulación. La presencia exclusiva de *Pulex irritans*, pulga con mayor prevalencia en zorros, en el ambiente rural, refuerza la hipótesis de que carnívoros silvestres y domésticos simpátricos comparten especies de ectoparásitos. La detección de *M. suis* por primera vez, en todos los ambientes y especies ectoparásitas, plantea interrogantes sobre el papel potencial del perro como reservorio de este patógeno. Además, sugiere que los perros infestados con ectoparásitos pueden estar expuestos a una variedad desconocida de patógenos transmitidos por vectores. Estos resultados refuerzan la importancia de los estudios parasitológicos y epidemiológicos en mamíferos domésticos bajo el paradigma de "Una Salud".

Palabras clave: *Canis lupus familiaris*, garrapatas, patógenos transmitidos por ectoparásitos, pulgas, Una Salud.
Key words: *Canis lupus familiaris*, ectoparasite-borne pathogens, fleas, One Health, ticks.

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INTRODUCTION

The domestic dog (*Canis lupus familiaris* Linnaeus 1758) was the first domesticated species and the only animal known to have entered into a domestic relationship with people during the Pleistocene (Freedman et al. 2014; Perri et al. 2021). Since that period, dogs have co-evolved alongside humans, and today dogs fill many roles in society, e.g., as pets, watchdogs, hunting dogs, herders, trackers, or guides (Perri et al. 2021). This close relationship and intense contact, however, comes at a cost: dogs are widely recognized as playing a role in the transmission of zoonotic parasites and pathogens (Wells et al. 2012; Chomel 2014; Durden & Hinkle 2019; Mendoza Roldan & Otranto 2023). In addition, dogs have been attributed to the origin of diverse epidemics affecting wild carnivores (Cevidanes et al. 2021). For instance, when considering wild carnivores that exhibit solitary behavior and infrequent intraspecific contacts, which hinders the transmission of parasites and pathogens, it is commonly presumed that the epidemiology of disease agents in these species is influenced by the presence of reservoirs, such as the domestic dog (Otranto et al. 2015; Millán et al. 2019).

The most common ectoparasite species in domestic dog populations worldwide are the fleas (Siphonaptera) *Ctenocephalides canis* (Curtis 1826), *Ctenocephalides felis* (Linnaeus 1758) and *Pulex irritans* (Bouché 1835) and the ticks (Ixodida) *Rhipicephalus sanguineus sensu lato* (Latreille 1806) and *Ixodes ricinus* (Linnaeus 1758) (e.g., González et al. 2004; Wells et al. 2012; Troyo et al. 2012; Abarca et al. 2016). These cosmopolitan and generalist ectoparasite species act as vectors of the etiological agents of diseases of public and animal health importance, such as various rickettsioses, bartonellosis, hemoplasmosis, and bubonic plague (Bitam et al. 2010; Durden & Hinkle 2019), infections whose reservoirs are wild, domestic, and synanthropic mammals and for which there is scarce information on their presence in Argentina (Nava et al. 2008, 2018; Romer et al. 2011; Cicuttin et al. 2014; Mascarelli et al. 2016; Armitano et al. 2019; Borrás et al. 2019; Ruiz et al. 2021).

Although the geographical distribution of some ectoparasites is strictly dependent on host movement, the increased mobility and worldwide distribution of pets have resulted in a rapid expansion of some arthropod vectors and the pathogens they transmit (Shaw et al. 2001). In addition, the presence and abundance of ectoparasites could be affected by the type of domestic dog management. In urban areas, dogs may reach high densities, and frequent contact of potentially disease-spreading dogs with others can be of serious health concern (Traub et al. 2005; Wang et al. 2006). Dog densities in more rural areas are usually lower, and individuals are perhaps less likely to frequently encounter parasites from conspecifics. However, in rural areas, dogs may be more likely to come into contact with sympatric

wild mammals, which may foster the exchange of zoonotic parasites between wildlife, livestock, and humans (Salb et al. 2008; Alexander & McNutt 2010). In this sense, domestic dogs can share several ectoparasite species with wild carnivores living in the same area, and, moreover, untreated domestic carnivores can be useful sentinels for ectoparasite infestation pressure in outside environments (Dantas-Torres et al. 2012; Otranto et al. 2015).

Some research suggests that climate change plays a significant role in influencing the occurrence and transmission of vector-borne diseases within an ecosystem since it is a main factor in altering the development parameters and distribution ranges of arthropod species that act as vectors (Crkvencic & Šlapeta 2019). On the other hand, it has been demonstrated that the detrimental effects of climate change are intensified by anthropogenic disturbances, such as agricultural and livestock activities, as they often lead to environmental alterations (Altizer et al. 2013; Hassell et al. 2016). In this sense, these changes can potentially create new pathways for the transmission of numerous unidentified populations of pathogens carried by wild ectoparasites (Bitam et al. 2010).

The Pampas region, characterized by natural and cultivated pastures, serves as the primary agricultural-livestock area in Argentina. However, it is important to note that this form of production significantly contributes to environmental transformations (Solbrig & Viglizzo 2000). Surprisingly, there is only one study available that focuses on the presence of ectoparasite-borne agents in dogs from this specific Argentine region, and it is limited to ticks (Borrás et al. 2019). Furthermore, information on the diversity and prevalence of ectoparasites and arthropod-borne agents, some of which have zoonotic potential, in dogs living in different environments in Argentina is still limited. Therefore, the aim of this study was to evaluate the diversity and prevalence of fleas and ticks and their associated bacteria of genus *Rickettsia*, *Bartonella*, and *Mycoplasma*, in dogs from different environments (urban, peri-urban, and rural) in central Argentina.

MATERIALS AND METHODS

Study area and sample collection

The study area was focused on two localities in the northwest of the province of Buenos Aires: Pergamino (33°53'22" S; 60°34'11" W) and Junín (34°34'10" S; 60°57'35" W). The area is situated in the Pampa ecoregion, which is characterized by a prairie ecosystem and a temperate sub-humid climate. The region experiences average annual temperatures ranging from 14 °C to 16 °C, and an annual rainfall between 700 and 1200 mm. Specifically, the study area falls within the "agrarian triangle of Argentina" renowned as the most fertile region in the country. Approximately 90% of the land surface in this region is dedicated to agricultural activities (Ministerio de Agricultura, Ganadería y Pesca 2017).

The study area was divided into three environments: urban, peri-urban, and rural, according to the territorial cadaster of the province of Buenos Aires instituted by Provincial Law N° 10.707, based on characteristics of the constructions, level of urbanization, and following the land divisions implemented by the districts (Instituto Nacional de Estadísticas y Censos 2022) and confirmed through the use of digital cartography analyzed with the Geographic Information System (QGIS Development Team 2021). The sampling sites were categorized based on the type of environment: urban areas included veterinary clinics and residential homes; peri-urban areas encompassed municipal kennels and residential homes, and rural areas consisted of private fields. The sampling was conducted during the warm months, as this period is associated with a higher prevalence and abundance of fleas and ticks (González et al. 2004; Dantas-Torres 2010; Maggi et al. 2013).

A stratified random study was conducted, where each stratum corresponded to an environment (urban, peri-urban, and rural). A total of 180 domestic dogs were considered in this study. The sample size for each of the three strata was estimated based on a previous study in the region on ticks and *Erlichia* in dogs (Borrás 2018), following the methodology of the Programa de Muestreo Estadístico en Sanidad Ambiental (ProMESA) (León & Duffy 2010), which indicates that 50% of the dog population is distributed in the urban region; 25% in the peri-urban area, and 25% in the rural zone. Domestic dogs were inspected with the consent of their owners. Individuals were thoroughly examined in various areas, including the head, ears, neck, chest, abdomen, armpit, back, perineal region, and fore and hind limbs. Fleas and ticks were removed using combs, tweezers, or by hand. All ectoparasites collected from each infested dog were preserved in Eppendorf tubes with 96% ethanol for subsequent identification and molecular studies at the Centro de Bioinvestigaciones (Pergamino, Argentina).

Morphological identification of ectoparasites

Identification of the ectoparasites was carried out through a morphological study following conventional techniques specific to each taxonomic group. Fleas were cleared in 10% KOH, dehydrated in an increasing series of ethanol dilutions (80° to 100°), diaphanized in eugenol, and mounted in Canada balsam for subsequent identification with an optic microscope, following Johnson (1957) and Smit (1987). On the other hand, ticks did not require any prior preparation and were identified following Nava et al. (2018).

Genomic DNA extraction

Fleas and ticks were washed and cut between the third and fourth abdominal tergites using a sterile scalpel. The material used to handle the ectoparasites was sterilized between each sample. Genomic DNA extraction was carried out from each individual ectoparasite per host using the Chelex®-100 method (Bio-Rad Laboratories, CA, USA)

adapting the procedure described by Miura et al. (2017) as follows: 5% Chelex®-100 resin in sterile distilled water was prepared, 100 μL of the homogenized mixture was placed in a tube together with the individual ectoparasite sample and incubated in a dry bath at 95 °C for 10 minutes. Then the material was centrifuged for 5 minutes at 14,000 rpm and 50 μL was taken from the supernatant. Following the DNA extraction, the fleas' and ticks' exoskeletons were recovered and stored in 96% ethanol; they were subsequently mounted for species identification.

In the cases in which the extraction was unsuccessful, we proceeded to the CTAB protocol (Doyle & Doyle 1987), where each ectoparasite was disintegrated with a mortar. Subsequently, depending on the amount of pellet observed, DNA from each specimen was eluted in 30-50 μL of Tris-EDTA buffer solution and stored at -20 °C under sterile conditions.

PCR amplification of *Rickettsia* spp., *Bartonella* spp., and *Mycoplasma* spp.

The presence of *Rickettsia* spp. was screened using the citrate synthase (*gltA*) and outer membrane protein B (*ompB*) genes, *Bartonella* spp. through the RNA polymerase beta-subunit (*rpoB*) gene, and *Mycoplasma* spp. with the 16S ribosomal RNA (*16S*) gene.

For the amplification, the polymerase chain reaction (PCR) program started with an initial denaturation for 5 minutes at 95 °C, followed by 40 cycles (95 °C for 30 s, gene-specific annealing temperature for 30 s, and 74 °C for 40 s), and a final extension step at 74 °C for 5 minutes (Table S1). The PCR reaction was set to a final volume of 20 μL, containing: 25-100 ng of template DNA, 1.5 mM $MgCl_2$, 0.2 μM of each primer, 0.2 mM of each dNTP, 1X reaction buffer, 0.5 U of Pegasus DNA polymerase, and ultrapure sterile water to come to final volume. In conventional PCR, 2 μL of genomic DNA was used, while as nested PCR was performed, 2 μL of genomic DNA was used in the first round of amplification, and 1 μL of DNA from the first reaction was used in the second round. All amplifications were performed in conjunction with negative (distilled water) and positive (DNA of *Rickettsia parkeri* provided by INEVH "Dr. Julio I. Maiztegui", ANLIS Malbrán, DNA of *Bartonella henselae* provided by Departamento de Bacteriología, INEI-ANLIS Malbrán, and DNA of *Mycoplasma suis* provided by Servicio Central de Laboratorio del Hospital de Clínicas, IGEVET, Universidad Nacional de La Plata) controls. DNA fragment amplification was confirmed by electrophoresis on a 1% m/v agarose gel stained with ethidium bromide (10 mg/μL) and visualized under UV light.

In those samples in which the PCR was positive, we proceeded to purify using 10U of Exonuclease I and 1U of FastAP thermosensitive alkaline phosphatase, incubating at 37 °C for 15 minutes and then at 85 °C for another 15 minutes to stop the reaction, and finally sequenced by Macrogen® Company.

Molecular data analysis

The obtained sequences for the genes were analyzed and manually edited using the BioEdit software (Hall 2004).

Using the nBLAST algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi), a homology comparison was performed against the GenBank nucleotide database to assign an identity to each sequence along with its statistical significance.

The complete set of gene sequences was employed for a multiple alignment performed with the ClustalW algorithm and the MEGA v.11 software (Tamura et al. 2021) together with sequences taken from the GenBank data base. The resulting alignment was checked and manually corrected. Moreover, phylogenetic trees were built using the Maximum Likelihood (ML) clustering methods, both for individual genes and for concatenated sequences, as in the case of the genus *Rickettsia*. In this latter case, the Farris test (Farris et al. 1994) was initially conducted using PAUP* methods based on parsimony inference (Swofford & Sullivan 2009) to assess the suitability of these genes. Subsequently, the Mesquite program (Maddison & Maddison 2021) was employed to concatenate these sequences. The evolutionary history was inferred using the ML method based on the Tamura 3-parameter (I + G) model with 10,000 replicates of random-addition taxa and tree bisection and reconnection branch swapping. All positions were weighted equally.

Prevalence calculation

The prevalence of each ectoparasite species and bacteria genus was calculated for each type of environment (urban, peri-urban, and rural), according to (Bush et al. 1997). The prevalences were tested through chi-square (X^2) using the Quantitative Parasitology (QPweb) software version 1.0.14 (Reiczigel et al. 2019).

RESULTS

A total of 308 ectoparasites were collected from 180 domestic dogs (92 urban, 50 peri-urban, and 38 rural), identified as Siphonaptera, Pulicidae: *Ctenocephalides felis felis* (N = 210), and *Pulex irritans* (N = 44), and Ixodida, Ixodidae: *Rhipicephalus sanguineus* s. l. (N = 54) (**Table** 1). All dogs were parasitized by at least one ectoparasite. The values of prevalence, total, and for each ectoparasite species for each environment are presented in **Table** 1. The highest total ectoparasite prevalence was obtained for peri-urban environments (83%). *Ctenocephalides felis* was the most prevalent species, both in urban (78%) and peri-urban (80%) environments. No significant differences in prevalence were found among the three environments. The genomic DNA extraction was successful in 87 ectoparasite samples.

Rickettsia spp. was detected by PCR through *gltA* and *ompB* genes in 19.5% (17/87) of the ectoparasites analyzed (**Table** 1). For *gltA*, nBLAST analysis indicated 100% (query cover 100%; e-value < 0.0) identity with *Rickettsia* spp. in all cases. Because this gene is highly conserved among rickettsiae, we only assigned the genus level to it. To confirm this information, the *ompB* gene was used, which gives an identity of 100% (query cover 100%; e-value < 0.0) for the species *Rickettsia felis*. When the two genes were concatenated, it gave an identity of 100% with *R. felis* (query cover 100%; e-value < 0.0). Phylogenetic

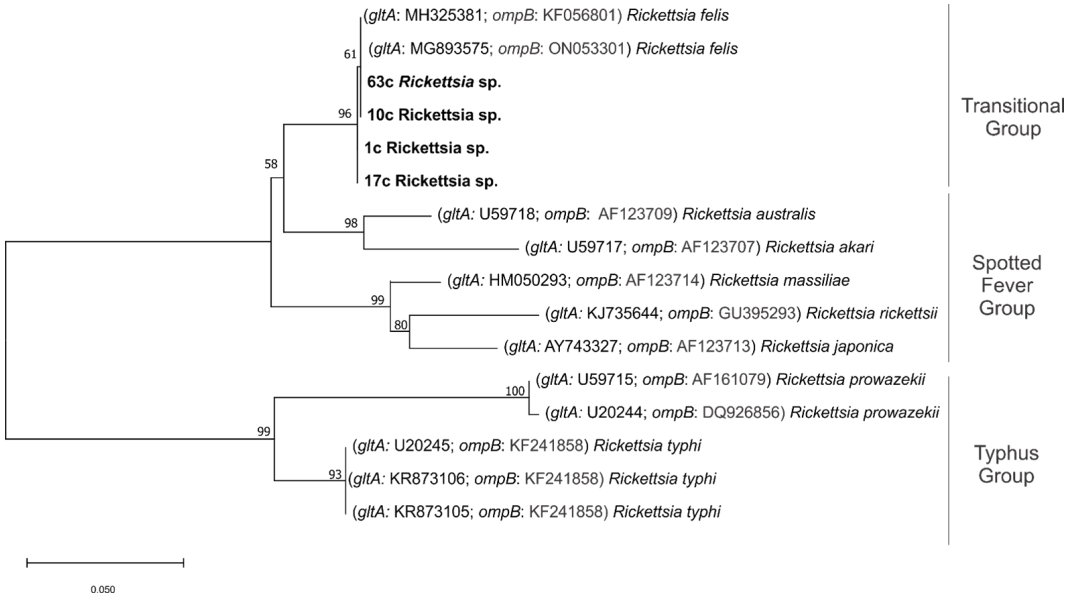

Fig. 1. Concatenated phylogenetic tree obtained with ML methodology for 1016 bp fragment of the *gltA* and *ompB* genes of the bacterial genus *Rickettsia*. Sequences obtained in this study can be seen in bold with their internal ID, and letters indicate the ectoparasitic species (c: *C. felis felis*; r: *R. sanguineus* s.l.; p: *P. irritans*). In the nodes, bootstrap values > 50% are shown.

analyses through ML inference were inferred from the *ompB* analyzed separately (Fig. S1), as well as by concatenating *gltA* and *ompB* genes, resulting in a total length of 1016 bp (**Fig**. 1). In the analyses, it can be observed that the sequence obtained in this study is grouped with *R. felis*.

Bartonella spp. was detected by PCR for the *rpoB* gene in 12.6% (11/87) of the ectoparasites analyzed (**Table** 1). The nBLAST analysis indicated 99% (query cover 100%; e-value = $7e^{-27}$) identity with *Bartonella* spp. Because this gene is highly conserved in the *Bartonella* genus, we can only confirm that taxonomic level.

Mycoplasma spp. was detected by PCR for the *16S* gene in 5.74% (5/87) of the ectoparasites analyzed (**Table** 1). The nBLAST analysis indicated 100% (query cover 100%; e-value = $2e^{-137}$) identity with *Mycoplasma suis*. Phylogenetic analyses through ML using the *16S* gene show that sequences obtained in this study are grouped with *M. suis*, confirming what was obtained (**Fig**. 2).

Coinfection of bacteria of different genera (*Rickettsia* and *Mycoplasma* and/or *Rickettsia* and *Bartonella*) was observed in some samples of ectoparasites (**Table** 1).

The prevalence of each bacterial genus in each ectoparasite species is shown in **Table** 1. The highest prevalence was obtained for *R. felis* (19.5%), which was detected in all ectoparasite species, with higher values in fleas (21% and 25%) than ticks (11%).

DISCUSSION

This study contributes to the knowledge of domestic dogs as hosts for ectoparasites carrying pathogenic bacteria in urban, peri-urban, and rural areas in central Argentina. Furthermore, our findings contribute to expanding the known geographic distribution of the bacteria *Rickettsia*, *Bartonella*, and *Mycoplasma*, as well as the range of potential vectors associated with these bacteria.

Parasites found in companions play a significant role in the transmission of zoonotic infectious diseases that affect humans worldwide. Furthermore, the global mobility and widespread distribution of pets have contributed to the rapid spread of certain arthropod vectors and the pathogens they carry (Shaw et al. 2001). For instance, domestic dogs can serve as hosts for ticks and fleas, which act as vectors or transmitters of a wide variety of zoonotic pathogens and are often an important source of pathogens in spillover and spillback processes between domestic and wild animals (Klimpel et al. 2010; Wells et al. 2012; Otranto et al. 2015; Millán et al. 2019). From an epidemiological perspective, understanding the prevalence of ectoparasites in their respective hosts and environments provides

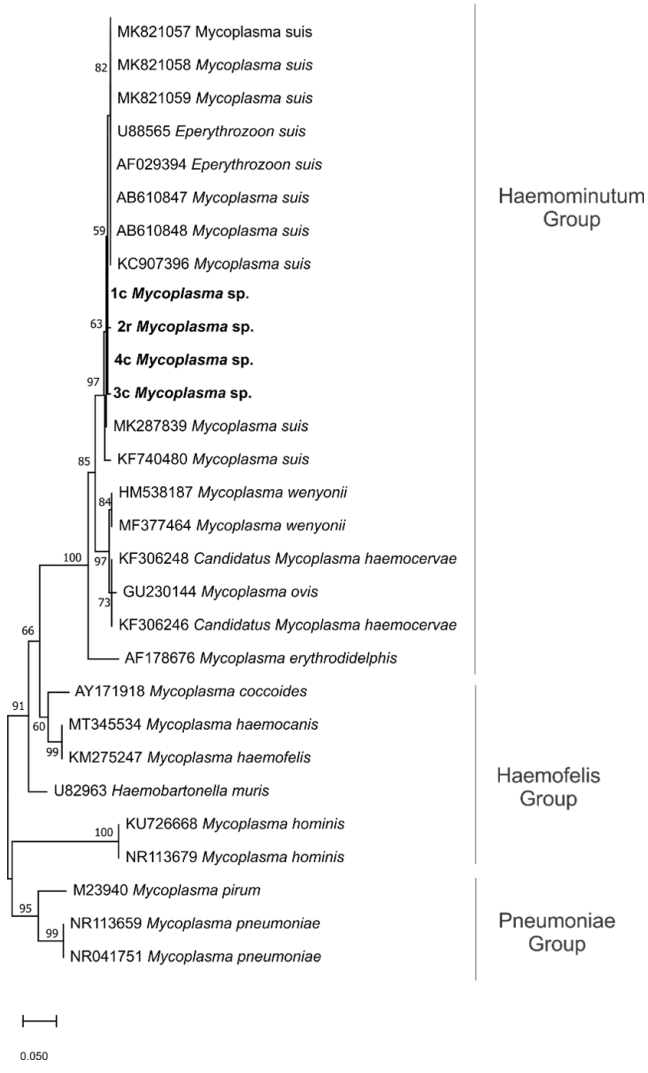

Fig. 2. Phylogenetic tree obtained with ML methodology for a 366 bp fragment of the *16S* gene of the bacteria genus *Mycoplasma*. Sequences obtained in this study can be seen in bold with their internal ID, and letters indicate the ectoparasitic species (c: *C. felis felis*; r: *R. sanguineus* s.l.; p: *P. irritans*). In the nodes, bootstrap values > 50% are shown.

valuable insights into their adaptation to different ecological parameters, which can in turn impact their effectiveness as disease vectors within an ecosystem (Bitam et al. 2010).

In accordance with our results, bibliographic records indicate that the most frequent species in dog ectoparasite assemblages are fleas *Ctenocephalides* spp. and *P. irritans* and ticks *R. sanguineus* s.l. (González et al. 2004; Xhaxhiu et al. 2009; Wells et al. 2012; Troyo et al. 2012; Abarca et al. 2016). These ectoparasite species, according to their geographic distribution and habitats, could be considered synanthropic and cosmopolitan. Although it is known that the host affinity of fleas and ticks varies from specific species *sensu stricto* to generalists (Nava & Guglielmone 2013; Sanchez et al. 2023), the breadth of hosts that ectoparasite species can utilize varies geographically or temporally, indicating that this specificity condition is strongly influenced by local environmental conditions (Krasnov et al. 2008). This could explain our results, wherein certain environments showed the absence of specific

Table 1

Diversity and prevalence of ectoparasites and bacteria in dogs from urban, peri-urban, and rural environments in central Argentina. N: total number of ectoparasite; P: prevalence.

Environment	Ectoparasite (N; P)	*Rickettsia felis* (P)	*Bartonella* sp. (P)	*Mycoplasma suis* (P)
Urban	*Ctenocephalides felis felis* (72; 78%) Total prevalence 78%	25%	8.3%	8.3%
Peri-urban	*Ctenocephalides felis felis* (138; 80%) *Rhipicephalus sanguineus* s.l. (34; 20%) Total prevalence 83%	25% -	25% -	5% -
Rural	*Pulex irritans* (44; 45.4%) *Rhipicephalus sanguineus* s.l. (20; 15.1%) Total prevalence 50%	21.4% 11.1%	- -	7.1% 11.1%
TOTAL	**308; 91.6%**	**19.5%**	**12.6%**	**5.7%**

ectoparasite species. For example, the unique tick species collected on the surveyed dogs, *Rhipichepalus sanguineus* s.l., although it uses dogs as its main feeding source, can act as hosts in other animals, including humans (Dantas-Torres 2010). This tick can be found in indoor homes, and the off-host hiding preferences also include cracks or between rocks in peri-domestic grounds (Dantas-Torres 2010). However, in this study, *R. sanguineus* s.l. was not found in dogs from urban areas, and its prevalence in both the peri-urban and rural environments was low, less than 20%. These results are in agreement with previous studies in the region (Pergamino) where prevalence values of less than 10% were reported for this species of tick (Borrás 2018).

About flea-host associations, the higher prevalence of *C. felis felis* obtained in our study is consistent with bibliographic records in dogs (Troyo et al. 2012; Krishna Murthy et al. 2017; Kumsa et al. 2019). This flea also infects multiple species of domestic and wild animals and can adapt to various environmental conditions (Durden & Hinkle 2019). In this study, *C. felis felis* was collected with a high prevalence (≈80%) in both the urban and peri-urban environments but was not found in the rural environment. To analyze this point, it is important to consider that the study area comprises the highest agronomic production in the country (Ministerio de Agricultura, Ganadería y Pesca 2017). Intensive agricultural practices and the removal of native vegetation are the main causes of biodiversity loss (Medan et al. 2011; Marrero et al. 2014), with potentially drastic consequences for ecosystem services (Chapin et al. 2000; Philpott & Armbrecht 2006; Halstead et al. 2014). Additionally, the increased use of agrochemicals in this rural environment may indirectly affect ectoparasite species diversity (Halstead et al. 2014).

Regarding *P. irritans*, bibliographic records show a greater abundance and prevalence in foxes than in dogs (Millán et al. 2019; Cevidanes et al. 2021). An important point to note is that the presence of foxes (*Lycalopex*) is common in the study area, mainly in environments that correspond to the rural environment of this study (Lucherini & Luengos Vidal 2008; Chemisquy et al. 2019; Luengos Vidal et al. 2019; Scioscia et al. 2022). Additionally, the authors of this study have observed (e.g., *Lycalopex gymnocercus*) through fecal samples, roadkill incidents, and/or direct sightings at the sampling sites (Pers. Obs.). In this sense, the record of *P. irritans* only in the rural environment reinforces the hypothesis that wild and domestic carnivores share ectoparasite species. With respect, genetic analyses of host associations of *R. sanguineus* s.s. and *Ctenocephalides* spp. suggest that dogs are acting as the maintenance host population, and occasional spillovers of those ectoparasites seem to occur from dogs to foxes (Cevidanes et al. 2021). In contrast, foxes appear to be natural hosts for *P. irritans*, while dogs with free-ranging behavior can occasionally become infested by these species (Millán et al. 2019).

On the other hand, it has been proven that domestic dogs can be reservoirs and transmitters of bacteria with zoonotic relevance, such as *Rickettsia*, *Bartonella*, *Mycoplasma*, and *Ehrlichia* (Walker et al. 2008; Breitschwerdt et al. 2010; Levin et al. 2012; Grasperge et al. 2012; Maggi et al. 2013; Iannino et al. 2018; Barbosa et al. 2021).

The genus *Rickettsia* currently includes 32 species, with a significant number of strains that have not yet been characterized (Diop et al. 2020). These species are classified into four phylogenetic groups:

ancestral, typhus, spotted fever, and the transition (Parola et al. 2013; Merhej et al. 2014; Brown & Macaluso 2016). All groups, except the ancestral, are capable of causing disease (Parola et al. 2013). In this study, we detected the presence of *R. felis* in all studied ectoparasite species as well as in the three types of environments. This finding was further supported by phylogenetic inference, where our sequences are observed within the transitional group (**Fig**. 1). *Rickettsia felis* is the etiological agent of the SFG, an emerging pathogen reported worldwide, and *C. felis* is considered its primary vector and reservoir in the environment (Brown & Macaluso 2016). Although fleas are known to maintain this bacterium through transovarial transmission (Wedincamp & Foil 2002), domestic dogs and cats are considered mammalian reservoir hosts for *R. felis* (Hii et al. 2011). In Argentina, *R. felis* has been detected in fleas such as *C. felis* (collected on dogs from Northeast Argentina: Nava et al. 2008; Oscherov et al. 2011); *P. irritans* (collected on domestic and feral pigs from Central Argentina: Ruiz et al. 2021), and *Polygenis (Polygenis) axius axius* (collected on rodents from Central Argentina: Melis et al. 2020), as well as in the louse *Haematopinus suis* (associated with domestic and feral pigs from central Argentina) (Ruiz et al. 2021). Therefore, this study represents the first record of *R. felis* in ectoparasites *C. felis felis*, *P. irritans*, and *R. sanguineus* s.l. collected from domestic dogs in central Argentina.

The genus *Bartonella* consists of more than 30 species, and 17 of them have been associated with human pathologies and domestic animals, highlighting its classification as an emergent pathogen (Breitschwerdt et al. 2010; Buffet et al. 2013; Gutiérrez et al. 2017). Several studies suggest the presence of adaptive coevolution between these bacteria, their mammalian reservoirs, and the vector arthropods that usually parasitize these mammals (Breitschwerdt 2014; Gutiérrez et al. 2017). In this sense, ectoparasites play a crucial role in this dynamic, efficiently transmitting a wide variety of *Bartonella* species (Buffet et al. 2013). In this study, *Bartonella* was only detected in *C. felis felis*, both in urban and peri-urban environments. This flea species has previously been identified as a potential vector of *Bartonella* spp. (Bouhsira et al. 2013). Our findings represent the first record of *Bartonella* spp. in the region since in Argentina it has been previously detected in *C. felis felis* of domestic dogs from Northeast Argentina (Oscherov et al. 2011; Urdapilleta et al. 2020) and, moreover, was detected in other flea species, *P. irritans* and *Neotyphloceras crackensis*, in foxes and sigmodontine rodents, respectively, from Patagonia (Millán et al. 2019; Cicuttin et al. 2019).

The genus *Mycoplasma*, also known as hemotrophic mycoplasmas or hemoplasmas, poses a threat to animal health as these bacteria reside in erythrocytes and cause deformities and damage to these blood cells (Neimark et al. 2001). These species are classified into three phylogenetic groups: Haemominutum, Haemofelis, and Pneumoniae (Zhou et al. 2009). *Mycoplasma haemocanis* infects dogs worldwide (Torkan et al. 2014), including Argentina, being the most prevalent species (Mascarelli et al. 2016). In Northern Argentina, 77.1% of dogs were found to be infected with hemoplasmas, and *M. haemocanis* has also been found in wild canids (Mascarelli et al. 2016). Additionally, in Argentina, *M. haematoparvum* and *M. suis* have been detected less frequently in the blood of dogs (Mascarelli et al. 2016). In this study, *M. suis* was first reported in the three analyzed ectoparasites, *C. felis felis*, *P. irritans*, and *R. sanguineus* s.l. Regarding the phylogenetic relationship between Haemoplasma species, our results agree with previous studies (**Fig**. 2; Zhou et al. 2009). Regarding the transmission routes of *M. suis*, Song et al. (2014) demonstrated an association between infection with this bacterium and the presence of mosquitoes and flies, suggesting their potential contribution to the natural spread of *M. suis*. In addition, there are reports about other *Mycoplasma* species being mechanically transmitted by ectoparasites such as fleas, ticks, and lice (Woods et al. 2005; Song et al. 2014; Acosta et al. 2019). *Mycoplasma suis* is a hemotrophic pathogen primarily affecting swine; however, it has recently been reported in the ectoparasites of domestic and wild pigs from the Pampas region (Acosta et al. 2019). In this sense, the detection of this bacterium in all the species of ectoparasites analyzed and in the three environments studied raises questions about the potential role of dogs as a reservoir of *M. suis*. These findings suggest that dogs infested by ectoparasites may be exposed to an unknown range of potentially invasive pathogens vector borne.

Canine vector-borne diseases are among the most complex of all infectious diseases to diagnose, mitigate, control, and prevent. This study provides valuable new information about previously unreported or underreported infections caused by canine vector-borne pathogens (CVBP) in the ectoparasites of dogs in Argentina. Considering that anthropogenic disturbances are one of the main factors influencing the circulation of CVBPs, this study is particu-

larly relevant as it was conducted in the primary agricultural-livestock area of Argentina. The identification of pathogen-ectoparasite associations across all analyzed environments (urban, peri-urban, and rural) suggests that dogs may serve as potential epidemiological links between the wild and domestic fauna of the region.

In conclusion, the findings of this study underscore the significance of conducting parasitological and epidemiological investigations in domestic mammals within the framework of the One Health paradigm. These results emphasize the need for significant enhancements in clinical diagnosis, veterinary practices, and vector control and surveillance measures. These improvements are crucial in addressing the challenges posed by canine vector-borne pathogens in the region.

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ONLINE SUPPLEMENTARY MATERIAL

Table S1 - **Figure** S1