



Tick-borne microorganisms in *Amblyomma tigrinum* (Acari: Ixodidae) from the Patagonian region of Argentina

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Abstract

This study presents the results of the molecular detection of tick-borne microorganisms in *Amblyomma tigrinum* Koch collected near the city of Viedma, Río Negro, Argentina. Ticks were collected in their non-parasitic stage, on pet dogs and on *Lycalopex gymnocercus* (Pampa fox). Also, six tick samples from humans were analyzed. All ticks were morphologically identified to species level and genomic DNA was extracted. The DNA samples were examined by end point PCR assays to amplified DNA of *Anaplasma* sp., *Babesia* sp., *Ehrlichia* sp., *Rickettsia* sp. and *Theileria* sp. Although all tested DNA samples from the collected ticks resulted negative to the detection of Piroplasmida and *Rickettsia* spp., 16 samples (16.5%, including all hosts) were positive in the 16S rDNA gene PCR that detects bacteria from the Anaplasmataceae family. Phylogenetic analysis of seven obtained partial sequences resulted in the identification of three bacteria: two *Ehrlichia* spp. (related to *Ehrlichia* sp. strain Iberá and strain Viedma) and *Candidatus* *Anaplasma* *boleense*. The latter finding represents the first detection of this novel *Candidatus* species in *A. tigrinum*. Based on the results of this study, it must be assumed that the diversity of bacteria of the Anaplasmataceae family in Argentina is greater than previously thought, and that these bacteria can infect a wide range of domestic and wild animals.

Keywords Ticks · *Anaplasma* · *Ehrlichia* · Wildlife · South America

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Introduction

In Argentina, *Amblyomma* is the tick genus with the highest species richness; it contains 25 species (Guglielmone et al. 2021). *Amblyomma tigrinum* Koch is a neotropical species exclusive to South America that has environmental plasticity and has been reported in most of the phytogeographical regions of Argentina (Cuervo et al. 2021). The main hosts of adult stages of *A. tigrinum* are domestic and wild carnivores of the family Canidae and Felidae, whereas immature stages primarily fed on small rodents of the families Caviidae and Cricetidae, and on birds (mainly several families of Passeriformes) (Guglielmone et al. 2021; Flores et al. 2023). Also tick parasitism on humans is reported (Guglielmone et al. 2021). There are two wild canids in Argentine Patagonia: *Lycalopex culpaeus* and *Lycalopex gymnocercus* (Luengos Vidal et al. 2019; Pía et al. 2019). *Lycalopex gymnocercus* (Pampa fox) is described as a species with high tolerance to anthropic environments (Luengos Vidal et al. 2019).

The family Anaplasmataceae contains obligate intra-cellular α -proteobacteria from the genera *Aegyptianella*, *Anaplasma*, *Ehrlichia*, *Neorickettsia* and *Wolbachia* (Dumler et al. 2001, 2015). Within this family, the species of the genera *Anaplasma* and *Ehrlichia* are transmitted by hard ticks (Rar et al. 2021). Bacteria of the genus *Rickettsia* are widespread all over the world and various species are able to produce several pathological effects in humans and grave infection can be lethal (Parola et al. 2013). In Argentina, the potential vectors for these obligate intra-cellular bacteria are some species of the genus *Amblyomma* (Nava et al. 2017). *Babesia* and *Theileria* are apicomplexan hemoparasites belonging to the order Piropasmida that are transmitted by different genera of hard ticks to mammals or birds and act as causative agents of several diseases of veterinary and public health importance (Schnittger et al. 2022).

In the last years, various uncultured *Ehrlichia* spp. were detected in *A. tigrinum* from peri-urban areas of central Argentina (Cicuttin et al. 2017) and Esteros del Iberá wetland in Corrientes province, northeastern Argentina (Eberhardt et al. 2020). Further, *A. tigrinum* were found naturally infected with *Rickettsia parkeri* in Cordoba province, central Argentina (Romer et al. 2014) and *Candidatus Rickettsia andeanae* in Tucuman province, northwestern Argentina (Saracho Bottero et al. 2015). However, the knowledge about the presence of tick-borne microorganisms in the Patagonian region of Argentina is scarce. *Borrelia burgdorferi* sensu lato was detected in *Ixodes* sp. cf. *I. neuquenensis* and *I. sigelos* (Sebastian et al. 2016), *Candidatus Rickettsia andeanae* and *Ehrlichia* sp. in *Amblyomma pseudoconcolor* (Sebastian et al. 2022), and *Hepatozoon* sp. in *A. tigrinum* (Millán et al. 2018). Therefore, the aim of this study was to get an overview of the occurrence of tick-borne microorganisms belonging to the genera *Anaplasma*, *Babesia*, *Ehrlichia*, *Rickettsia* and *Theileria* in *A. tigrinum* collected in the Patagonian region of Argentina.

Materials and methods

Study area and sample collection

Viedma, the capital city of Río Negro province, is located in the extreme northeast of Argentine Patagonia (40° 48' S, 62° 59' W), within the phytogeographic province 'Monte' characterized by xerophytic shrubs (Oyarzabal et al. 2018). The climate is temperate and

semi-arid. The mean annual temperature is 14 °C and the mean annual precipitation is 270 mm, with peaks in autumn and spring (Godagnone and Bran 2009). Around 80,000 people live in Viedma according to the estimate for the year 2020 (Dirección de Estadística y Censos de la Provincia de Río Negro, Viedma, Argentina). It is a small town with an evident and important population of pets and stray dogs (Lartigau et al. 2019). In recent years, the increase in livestock and agricultural production as well as the advance of urbanization on wild areas, has increased interface environments. So, in the peri-urban area, the presence of wildlife is frequent (Luengos Vidal et al. 2019; Pía et al. 2019).

Ticks were collected between 2020 and 2022 in a peri-urban area on dogs, humans, *L. gymnocercus*, and also from the environment as free-living stages. The ticks that were parasitizing on *L. gymnocercus* were collected from road-killed specimens a few kilometers from Viedma. Every *L. gymnocercus* was immediately placed in a plastic container to facilitate the collection of ticks. In the laboratory, each animal was checked for tick infestation for 20 min from the snout to the tail. The use of wild animals found dead was approved by Secretariat of Environment and Climate Change of the Province of Río Negro (File No. 08526SAYDS 2015/218/222). All the ticks were preserved in 96% alcohol.

Morphological identification of ticks, molecular detection of tick-associated microorganisms and phylogenetic characterization

Collected ticks were morphologically identified according to a taxonomic key described by Nava et al. (2017). After cleaning the ticks with sodium hypochlorite solution (10%), PBS-buffer and distilled water, whole genomic DNA was extracted from each tick sample individually using a commercial DNA extraction kit (High Pure PCR Template Preparation Kit, Roche, Germany). Afterwards, all DNA samples were tested by three conventional PCR assays: (I) detection of the *gltA* gene, specific for *Rickettsia* spp. (Labruna et al. 2004); (II) detection of the 16S rDNA gene, specific for bacteria of the Anaplasmataceae family (Aguiar et al. 2008); and (III) detection of the 18S rDNA gene, specific for the order Piroplasmida (Soares et al. 2011) (see Table S1). *Anaplasma marginale*, *Babesia bovis* and *Rickettsia rickettsii* were used as positive control, whereas ultrapure PCR-water acted as negative control.

In case of positive results, amplicons were purified (High Pure PCR Product Purification Kit) and sequenced (Sanger sequencing carried out by Macrogen, Seoul, South Korea). Obtained sequences were compared with sequences deposited in GenBank. Further, the sequences were edited and aligned using BioEdit Sequence Alignment Editor (Hall 1999). The phylogenetic analyses were carried out with the maximum-likelihood (ML) method by using the program MEGA X (Kumar et al. 2018). Best fitting substitution model was determined with the Akaike Information Criterion using the ML model test implemented in MEGA X. Support for the topologies was tested by bootstrapping over 1000 replications and gaps were excluded from the comparisons.

Results and discussion

In total 97 *A. tigrinum* ticks (61 females, 36 males) were collected. Besides, one female specimen of *Rhipicephalus sanguineus* was collected on a dog. The distribution of ticks was as follows: 58 *A. tigrinum* (28 females, 30 males) on six specimens of *L. gymnocercus*;

Table 1 *Amblyomma tigrinum* collected on dogs, humans, *Lycalopex gymnocercus*, and also from the environment in Río Negro province, Argentina between 2020 and 2022 with results of the molecular detection of tick-borne microorganisms

Host	Host ID	Coordinates		<i>Amblyomma tigrinum</i>		Positive PCR samples			<i>Rickettsia</i> spp.
		Latitude (S)	Longitude (W)	Female	Male	Anaplasmataceae	Apicomplexa		
<i>Lycalopex gymnocercus</i>	LG 38	41° 01' 39"	62° 49' 44"	7	15	2FF/3MM	0	0	
	LG 40	41° 01' 39"	62° 49' 44"	14	8	3FF/3MM	0	0	
	LG 90	40° 53' 19"	62° 53' 37"	1	0	1F	0	0	
	LG 95	40° 58' 49"	62° 50' 23"	0	1	0	0	0	
	LG 100	41° 01' 20"	62° 49' 07"	2	2	0	0	0	
	LG 101	41° 01' 20"	62° 49' 07"	4	4	1M	0	0	
	CAN 1	40° 49' 57"	62° 58' 59"	7	1	1F	0	0	
	CAN 2	41° 02' 41"	62° 49' 33"	4	2	1M	0	0	
	CAN 3	40° 50' 43"	62° 53' 26"	11	1	0	0	0	
	CAN 4	40° 49' 57"	62° 58' 59"	1	0	0	0	0	
Human	HS 1	41° 02' 49"	62° 49' 45"	3	1	0	0	0	
	HS 2	41° 02' 49"	62° 49' 45"	1	0	0	0	0	
	HS 3	41° 02' 49"	62° 49' 45"	0	1	0	0	0	
Environment (non-parasitic)	AMB	40° 49' 32"	62° 58' 19"	6	0	1F	0	0	
Total				61	36	8FF/8MM	0	0	

F(F) = female(s); M(M) = male(s)

27 *A. tigrinum* (23 females, 4 males) on four dogs; six *A. tigrinum* (4 females, 2 males) on three humans and six *A. tigrinum* (6 females) from the environment (Table 1).

All DNA samples from the collected ticks tested negative for the detection of Piroplasmida and *Rickettsia* spp., whereas 16 samples (16.5%) were positive for the 16S rDNA gene that detects bacteria from the Anaplasmataceae family (Table 1). Positive ticks were collected on four *L. gymnocercus*, two dogs and one as free-living specimen from the environment (Table 1). Unfortunately, the amplification of further *Anaplasma* sp. and/or *Ehrlichia* sp. specific genes such as *dsb*, *groEL*, *groESL* and *gltA* resulted negative.

Seven amplicons of the 16S rDNA of Anaplasmataceae, one from each host ID, were selected, purified and sequenced. Analysis of the obtained sequences resulted in the observation of three groups of sequences: (I) one group including four 16S rDNA gene fragments of 307–360 bp (GenBank acc. nrs. OR885903-OR885906) detected in *A. tigrinum* of *L. gymnocercus* and dogs that are 100% similar to each other and 99.7% similar to sequences of *Candidatus Anaplasma boleense* (GenBank acc. nrs. OQ730408-OQ730411) detected in goats from Argentina (Sebastian et al. 2023), (II) one group including two partial sequences of 359–360 bp (GenBank acc. nrs. OR885908 and OR885909) detected in a free-living *A. tigrinum* tick and from one specimen collected on *L. gymnocercus* which were 100% equal to each other and highly similar (98.9%) to a large number of sequences of uncultured *Ehrlichia* sp. and *Ehrlichia ruminantium* [e.g., GenBank acc. nrs. CP033456 (unpubl. sequence, goat, South Africa), MN120892 (unpubl. sequence, cattle, Cameroon), NR_074513 (type strain *E. ruminantium*; Frutos et al. 2006), OQ552620 (Seo et al. 2023)], and (III) one partial sequence of 363 bp (GenBank acc. nr. OR885907) detected in *A. tigrinum* collected on *L. gymnocercus* that shows high sequence similarity to various uncultured *Ehrlichia* spp. including an *Ehrlichia* sp. strain Iberá detected in *A. tigrinum* from Argentina (GenBank acc. nr. MN256462; Eberhardt et al. 2020).

The obtained sequences were used to construct a maximum-likelihood tree (Fig. 1). One group of sequences (GenBank acc. nrs. OR885903-OR885906) are situated in a clade within a reference sequence of *Ca. A. boleense* detected in a goat from Argentina (GenBank acc. nr. OQ730410). The other sequence group (GenBank acc. nrs. OR885908 and OR885909) forms a clade with a sequence of an uncultured *Ehrlichia* sp. detected in *Amblyomma pseudoconcolor* also from the Patagonian region of Argentina (GenBank acc. nr. OP744461). The partial sequence of the *Ehrlichia* sp. detected in *A. tigrinum* collected on *L. gymnocercus* (GenBank acc. nr. OR885907) forms a clade with *Ehrlichia* sp. strain Iberá detected in *A. tigrinum* from Argentina (GenBank acc. nr. MN256462).

Candidatus A. boleense was firstly detected in *Hyalomma asiaticum* from China (Kang et al. 2014). Later findings of this bacterium in other ticks, mosquitoes, domestic and wild animals from various parts of the world (Guo et al. 2016; Lu et al. 2017; Koh et al. 2018; Fernandes et al. 2019) suggest that *Ca. A. boleense* is widely distributed and associated with a wide range of invertebrate and vertebrate organisms. Also in Argentina, *Ca. A. boleense* was detected in *Rhipicephalus microplus* and goats (Sebastian et al. 2023). However, these findings were made in the North of Argentina in the phytogeographic province ‘Chaqueña’ (Oyarzabal et al. 2018) whereas the finding of the current work was made not only in a different tick species but also in a contrasting ecological area. The results of the present work suggest that *Ca. A. boleense* could be widely distributed in Argentina and circulating in different animal species. Nevertheless, the vector and non-vector transmission routes and the pathogenicity of *Ca. A. boleense* remain unknown.

The 16S rDNA partial sequences of the detected *Ehrlichia* sp. strains in *A. tigrinum* collected on *L. gymnocercus* and from the environment are closely related to an *Ehrlichia* sp. strain recently detected in *A. pseudoconcolor* collected from *Chaetophractus villosus*

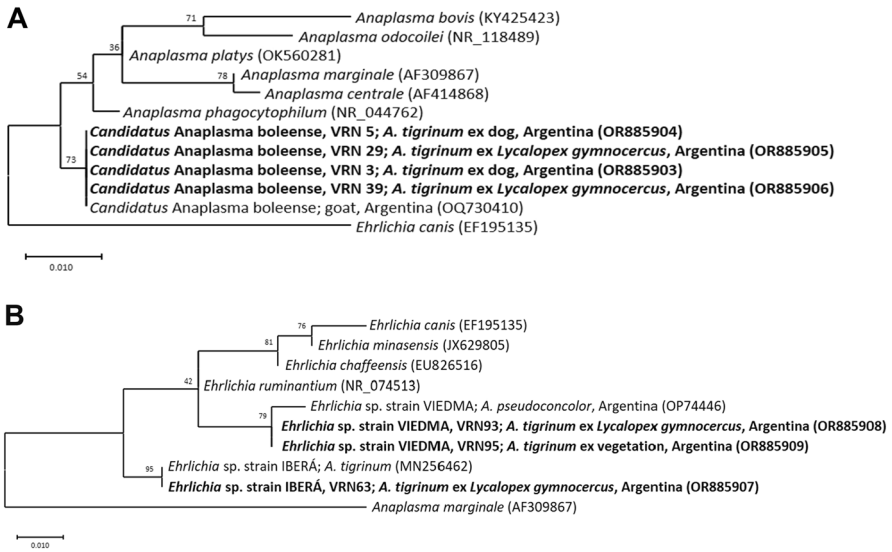


Fig. 1 Maximum-likelihood tree constructed from partial 16S rDNA gene sequences of various *Anaplasma* (A) and *Ehrlichia* (B) species detected in animals and ticks. Substitution models: (A) Hasegawa-Kishino-Yano (HKY) model with gamma distribution; (B) HKY model with gamma distribution and invariant sites. Sequences generated in this study are written in bold letters. Numbers represents bootstrap support generated from 1000 replications. GenBank accession numbers are given in parentheses

at the same sample site of the current study (Sebastian et al. 2022). We propose to name these reports as *Ehrlichia* sp. strain Viedma. Several *Ehrlichia* sp. strains were previously reported in Argentina (e.g., Tomassone et al. 2008; Cicuttin et al. 2017, 2020; Monje et al. 2019; Fagnoli et al. 2020; Tarragona et al. 2022; Vaschalde et al. 2023). Eberhardt et al. (2020) detected *Ehrlichia* sp. strain Iberá in *A. tigrinum* ticks collected on a road-killed *L. gymnocercus* from the Esteros del Iberá wetland in Corrientes province, Northeastern Argentina. The sequence of the strain detected in *A. tigrinum* collected on *L. gymnocercus* in this study was identical to the sequence of *Ehrlichia* sp. strain Iberá reported by Eberhardt et al. (2020).

Two other strains of *Ehrlichia* sp.—Cordoba and San Luis—were previously found infecting *A. tigrinum* ticks associated with dogs in peri-urban areas of central Argentina (Cicuttin et al. 2017).

Although some tick-borne microorganisms, including *B. burgdorferi*, *Ca. R. andeanae*, *Ehrlichia* sp. and *Hepatozoon* sp., were previously reported in ticks from the Patagonian region of Argentina (Sebastian et al. 2016, 2022; Millán et al. 2018), the knowledge about the presence of tick-borne microorganisms in this part of Argentina remains scarce. The absence of *Rickettsia* infection in the analyzed ticks is interesting as *A. tigrinum* is commonly infected by *Ca. R. andeanae* and *R. parkeri* (Romer et al. 2014; Saracho Bottero et al. 2015; Krawczak et al. 2023). Nevertheless, there is no report about *Rickettsia* infection in *A. tigrinum* in the Patagonian region of Argentina.

In this study we present the molecular detection of three bacteria of the Anaplasmataceae family in *A. tigrinum* ticks. *Candidatus A. boleense* was firstly detected in *A. tigrinum* and there is evidence suggesting that it can infect non-phylogenetically related animal species. *Ehrlichia* sp. strain Viedma is a novel strain of this genus and may be present in this geographical region associated with the tick species *A. pseudoconcolor* and *A. tigrinum*.

Also, a second detection of *Ehrlichia* sp. strain Iberá in *A. tigrinum* is reported. Nevertheless, further studies on pathogenicity, geographical distribution and transmission cycles of these bacteria as well as the amplification of other genes for better phylogenetic classification of the detected strains are needed.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10493-023-00874-4>.

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Author contributions Conceptualization: MW and PSS; Data curation: MW and PSS; Funding acquisition: MW, SDA, PSS and SN; Investigation: MW, PSS, ELT and FSS; Methodology: MMW and PSS; Project administration: PSS; Resources: MW; Supervision: PSS; Visualization: PSS; Writing—original draft: MW and PSS; Writing—review & editing: MW, PSS, ELT, FSS, SN.

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Data availability Not applicable.

Declarations

Competing interests The authors declare no competing interests.

Ethical approval The use of wild animals found dead was approved by Secretariat of Environment and Climate Change of the Province of Río Negro (File No. 08526SAYDS 2015/218/222).

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