



Study of the presence of *Borrelia* spp. in ticks of the genus *Amblyomma* from Argentina

Gabriel L Cicuttin^{1*} ; María N. De Salvo¹ ; Paula Díaz-Pérez¹ ; Daniela Lamattina² 
Evelina L. Tarragona³ ; Viviana M. Orcellet⁴ ; Santiago Nava³ 

¹Instituto de Zoonosis Luis Pasteur, Ministerio de Salud del Gobierno de la Ciudad Autónoma de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina

²Instituto Nacional de Medicina Tropical, Ministerio de Salud de la Nación, Almaguer y Ámbar S/N, Misiones, Argentina

³Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Rafaela. Consejo Nacional de Investigaciones Científicas y Técnicas, Rafaela, Santa Fe, Argentina

⁴Universidad Nacional del Litoral, Facultad de Ciencias Veterinarias, Esperanza, Santa Fe, Argentina.

*Correspondence: gcicuttin@gmail.com

Received: October 2020; Accepted: May 2020; Published: June 2020.

ABSTRACT

Objective. The aim of the present study was to detect *Borrelia* spp. in different species of ticks of the genus *Amblyomma* from Argentina. **Materials and Methods.** *Amblyomma* tick specimens collected from various provinces of Argentina (Entre Ríos, Misiones, Formosa, Salta, Córdoba, San Luis and Buenos Aires) were analyzed by nested PCR for a 306 bp fragment of the gene *fla* of the genus *Borrelia*. **Results.** A total of 422 tick specimens (*Amblyomma aureolatum*, *Amblyomma brasiliense*, *Amblyomma ovale*, *Amblyomma sculptum*, *Amblyomma tigrinum*, *Amblyomma tonelliae* and *Amblyomma triste*) from various regions of Argentina were studied. All samples were negative by PCR for a fragment of the gene *fla* of *Borrelia* spp. **Conclusions.** The tick species of the genus *Amblyomma* analyzed in the present study are important for public health; although infection with bacteria of the genus *Borrelia* was not detected. However, considering the few previous antecedents in the region, future studies on this genus of ticks are necessary.

Keywords: *Borrelia*; *Amblyomma*; Argentina (Source: CAB, TGN)

RESUMEN

Objetivo. El objetivo del presente estudio fue detectar la presencia de *Borrelia* spp. en especies de garrapatas del género *Amblyomma* en Argentina. **Materiales y métodos.** Se analizaron especímenes de garrapatas del género *Amblyomma* colectadas de distintas provincias de Argentina (Entre Ríos, Misiones, Formosa, Salta, Córdoba, San Luis y Buenos Aires) mediante una PCR anidada para un fragmento de 306 pb del gen *fla* del género *Borrelia*. **Resultados.** Se analizaron 422 ejemplares de las especies *Amblyomma aureolatum*, *Amblyomma brasiliense*, *Amblyomma ovale*, *Amblyomma sculptum*, *Amblyomma tigrinum*, *Amblyomma tonelliae* y *Amblyomma triste* de diversas regiones de Argentina. Todas las muestras estudiadas resultaron negativas mediante la PCR. **Conclusiones.** Las especies de garrapatas del género *Amblyomma* analizadas en el presente estudio son de importancia en salud pública; si bien no se detectó infección con bacterias del género *Borrelia*, considerando los escasos antecedentes previos, futuros estudios en esta temática son necesarios en este género de garrapatas.

Palabras clave: *Borrelia*; *Amblyomma*; Argentina (Fuente: CAB, TGN).

How to cite (Vancouver).

Cicuttin GL, De-Salvo MN, Díaz-Pérez P, Lamattina D, Tarragona EL, Orcellet VM, Nava S. Study of the presence of *Borrelia* spp. in ticks of the genus *Amblyomma* from Argentina. Rev MVZ Córdoba. 2021; 26(3):e2199. <https://doi.org/10.21897/rmvz.2199>



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INTRODUCTION

Ticks of the genus *Amblyomma* (Family Ixodidae) comprise approximately 135 species worldwide, being the genus with the highest specific richness in the neotropical region (1). Although the range of hosts for ticks of the genus *Amblyomma* includes all classes of terrestrial vertebrates, most of the species present in the southern cone of America feed on mammals, although birds are also important hosts for the immature stages of many species (1). Different species of *Amblyomma* were reported infesting humans in the southern cone of America, such as *Amblyomma aureolatum* (adult stages), *Amblyomma brasiliense* (all stages), *Amblyomma coelebs* (all stages), *Amblyomma dubitatum* (all stages), *Amblyomma neumanni* (all stages), *Amblyomma ovale* (adults), *Amblyomma parvum* (adults), *Amblyomma sculptum* (all stages), *Amblyomma tigrinum* (adults), *Amblyomma tonelliae* (all stages) and *Amblyomma triste* (adults); all species present in Argentina (1). In addition, some of these species are vectors for various pathogenic microorganisms (e.g. *Rickettsia rickettsii* and *Rickettsia parkeri*) for humans (1).

The genus *Borrelia* (Family Spirochaetaceae) includes more than 30 genospecies transmitted by arthropods with different degrees of pathogenicity (2,3). Bacteria of the genus *Borrelia* are contained in three groups: *Borrelia burgdorferi* sensu lato (BBG), transmitted mainly by ticks of the genus *Ixodes*; Relapsing Fever (RFG), transmitted by ticks and lice; and a third group also transmitted by ticks (REP) (3,4). BBG consists of approximately 18 formally described genospecies, including the main agents of Lyme disease: *Borrelia burgdorferi* sensu stricto, *Borrelia afzelii*, and *Borrelia garinii*, all transmitted by ticks of the *Ixodes ricinus* complex in the northern hemisphere (2,3). Within RFG *Borrelia* transmitted by ticks, there are genospecies transmitted by soft ticks (family Argasidae), e.g., *Borrelia hispánica*, *Borrelia turicatae* and *Borrelia anserina* and genospecies transmitted by hard ticks (family Ixodidae), e.g. *Borrelia miyamotoi* and *Borrelia theileri* (2,3). Finally, the REP includes *Borrelia* spp. transmitted by hard ticks that have been detected in different regions of the world. *Borrelia* REP name is due that was originally found in reptiles or reptile-associated ticks, but has also been detected in non-reptile-related hard ticks, as well as birds (2,3,4,5).

The association of *Borrelia* spp. with ticks of the genus *Amblyomma* has been less studied regarding the association between *Borrelia* and ticks of the genus *Ixodes*. In America, RFG genospecies (*Borrelia lonestari*) were detected in *Amblyomma americanum* from the USA (6,7) and REP genospecies in *Amblyomma maculatum* from the USA (4), *Amblyomma longirostre* from Brazil (5) and French Guiana (8), *Amblyomma geayi* from French Guiana (8), *A. aureolatum* from Argentina (3) and *Amblyomma dissimile* from Mexico (9). Furthermore, recently dos Santos et al (10) reported *Borrelia* sp. from a separate group to REP and RFG in *A. brasiliense*.

Considering the few studies about the potential role of ticks of the genus *Amblyomma* as vectors of *Borrelia* spp. in South America, the objective of this work was to analyze the presence of genus *Borrelia* in different species of this genus of ticks in Argentina.

MATERIALS AND METHODS

Samples. The tick specimens used in this study, collected in the parasitic and free-living phase, correspond to material already published in Cicuttin et al (11,12,13), Lamattina et al (14), Mastropaolo et al (15) and Tarragona et al (16). The data of sampling methods, environmental characteristics of the collection sites and taxonomic determination are detailed in them. The ticks collected in the provinces of Entre Ríos and Formosa were extracted manually on the hosts or by flag method in their free-life phase in vegetation, and identified following the descriptions and keys presented in Nava et al (1).

Extraction of DNA. The ticks were processed individually. The nucleic acids were extracted using AxyPrep Multisource Genomic DNA MiniPrep Kit (Axygen Biosciences, USA), High Pure PCR Template Preparation Kit (Roche, Germany) and using phenol-chloroform-isoamyl alcohol. The details of the methodology used were previously published; endogenous controls were not used (11,12,13,14,15,16).

Molecular detection of *Borrelia* spp. Nested PCR for a 306 bp fragment of the *fla* gene was performed using the primers: Fla-LL 5'-ACATATTCAGATGCAGACAGAGGT-3', Fla-RL 5'-GCAATCATAGCCATTGCAGATTGT-3', Fla-LS 5'-AACAG-CTGAAGAGCTTGAATG-3' and Fla-RS 5'-CTTTGATCACTTTCATTCT-AATAGC-3' (6). *Borrelia burgdorferi* s.s. (positive) and nuclease-free water (negative) were used as controls.

Ethical aspects. This study was carried out following the principles emanating from law 25.326/2000 (Protection of personal data), law 3.301/2009 (Protection of the Rights of Subjects in Health Research) and the Declaration of Helsinki (1964) from the World Medical Association and subsequent updates. It was also in accordance with the "International Guiding Principles Applicable to Biomedical Research with Animals" of the Council for International Organizations of Medical Sciences approved by the Consultative Committee on Medical Research of the World Health Organization (1985), the "International Standards for biomedical research with animals" of the Pan American Health Organization (1990) and Title 7: "Animal Welfare" of the Terrestrial Animal Health Code of the World Organization for Animal Health (2011).

The project had the approval of the Teaching and Research Committee of Instituto de Zoonosis Luis Pasteur.

RESULTS

A total of 422 specimens of the species *A. aureolatum*, *A. brasiliense*, *A. ovale*, *A. sculptum*, *A. tigrinum*, *A. tonelliae* and *A. triste* were processed. Table 1 shows the detail by species, stage, host (or vegetation for ticks free-life phase) and collection location.

All the samples analyzed were negative by PCR for the fragment of the *fla* gene of the genus *Borrelia*.

Table 1. Specie, locality of origin, collection source (hosts / vegetation) and number of analyzed specimens of the ticks studied.

Specie	Province	Location	Host / vegetation	Stage	n	Total
<i>Amblyomma aureolatum</i>	Entre Ríos	Colón (32°13'S, 58°08'O)	Dog	Adult	4	37
		Feliciano (30°23'S, 58°45'O)	Dog	Adult	2	
		La Paz (30°45'S, 59°39'O)	Dog	Adult	8	
			Cat	Adult	1	
		Paraná (31°43'S, 60°31'O)	Dog	Adult	19	
			Cat	Adult	2	
<i>Amblyomma brasiliense</i>	Misiones	Parque Nacional Iguazú (25°41'S, 54°27'O)	Human	Adult	1	92
			Vegetation	Adult	47	
		Parque Provincial Puerto Península (25°43'S, 54°33'O)	Vegetation	Nymph	35	
			Vegetation	Adult	5	
			Vegetation	Nymph	5	
<i>Amblyomma ovale</i>	Formosa	El Bagual (25°59'S, 58°24'O)	<i>Taraba major</i>	Nymph	4	8
				Larva	2	
<i>Amblyomma sculptum</i>	Salta	Parque Nacional El Rey (24°15'S, 64°40'O)	Vegetation	Adult	80	80
		Nono (31°46'S, 64°59'O)	Dog	Adult	4	
<i>Amblyomma tigrinum</i>	Córdoba	Salsipuedes (31°09'S, 64°19'O)	Vegetation	Adult	11	99
			Vegetation	Adult	42	
		San Luis	Cortaderas (32°30'S, 65°00'O)	Cat	Adult	
Merlo (32°20'S, 65°00'O)	Dog		Adult	23		
<i>Amblyomma tonelliae</i>	Salta	El Tunal (25°14'S, 64°25'O)	Vegetation	Adult	52	52
<i>Amblyomma triste</i>	Buenos Aires	INTA E.E.A. Delta del Paraná (34°11'S, 58°50'O)	Vegetation	Adult	35	54
		General Lavalle (36°22'S, 56°21'O)	Vegetation	Adult	19	
Total						422

DISCUSSION

This is the first study to analyze the presence of *Borrelia* spp. in ticks *A. sculptum*, *A. tigrinum* and *A. tonelliae* and the first in Argentina for the species studied (except for of *A. triste* and *A. aureolatum*).

In Argentina, the previous research of *Borrelia* in the genus *Amblyomma* was limited to a single work. Cicuttin et al (3) analyzed 432 *A. aureolatum* and 65 *A. triste* ticks collected in the Reserva Ecológica Costanera Sur (Buenos Aires City), finding 1.7% of *A. aureolatum* positive for *Borrelia* sp. phylogenetically related to the REP group. In that study, only nymphs of *A. aureolatum* were positive (9/52 -17.3% - in nymphs collected on vegetation and 5/11 -45.4% - in those collected on birds), highlighting the possible role of the birds as amplifying hosts of *Borrelia* sp. REP (3). Similarly, the findings of *Borrelia* sp. REP in Brazil were carried out in nymphs of *A. longirostre* collected on birds (5), while in French Guiana it was detected in larvae of *A. longirostre* and *A. geayi* parasitizing birds (8) and in Mexico it was detected in nymphs and adults of *A. dissimile* collected from toads (9). On the other hand, *Borrelia* sp. REP group in USA was obtained from adults of free-living *A. maculatum* (4). The *Borrelia* genospecies of the REP found in USA, Brazil and French Guiana are closely related to each other (possibly the same species), but not to *Borrelia* sp. REP found in *A. aureolatum* from Argentina (8). The *Borrelia* sp. recently found in *A. brasiliense* from Brazil is related to sequences of *Borrelia* spp. reported in Ethiopia and Ivory Coast, conforming a separate group of REP and RFG (10). In that study, *Borrelia* sp. was detected in 1 group of 6 nymphs collected from vegetation (out of 266 studied) (10); in comparison, in our study only 40 nymphs of *A. brasiliense* were analyzed.

Regarding the association between BBG and the genus *Amblyomma*, an extensive review by Stromdahl et al. (17) shows that the few positive findings (0.1% in almost 30,000 specimens studied) in *A. americanum* from USA correspond to studies in ticks collected on hosts (the DNA detected is possibly related to the ingestion of infected blood from the host) or suspected contamination in the laboratory. On the other hand, in a review on experimental studies to determine the vector competence of *A. americanum* in the transmission of *B. burgdorferi* s.l., it is concluded that this species contributes minimally or in no way to the transmission cycle of Lyme borreliosis in USA (18). The presence of antimicrobial peptides (defensins) with lytic activity against *B. burgdorferi* s.l. it has been considered as an important factor (18).

Finally, various studies in *A. americanum* from the USA report 1-3% infection with *Borrelia lonestari*, a species belonging to the RFG (7).

In the present study, no DNA of bacteria of the genus *Borrelia* was detected in different species of ticks of public health importance belonging to the genus *Amblyomma*. Considering the scarce previous antecedents, future studies on this subject are necessary, especially in the case of tick species that frequently parasitize birds, reptiles and amphibians, since these hosts could act as potential reservoirs and amplifiers in the enzootic cycle of borreliae-associated with *Amblyomma* in the region.

Conflicts of interest

None.

Acknowledgements

This work has been partially funded by the Grant for Clinical and Epidemiological Research 2018 (Alberto J. Roemmers Foundation).

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