

Ticks (Acari: Ixodidae) of northern Misiones Province, Argentina

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Abstract

Information on distribution and hosts of hard ticks of northern Misiones Province in Argentina is presented in this study. Ticks were identified by morphological characters and by sequences of a 410 bp fragment of the mitochondrial 16S rRNA gene. A total of 292 ticks collected from the environment and wild and domestic mammals were identified: *Amblyomma coelebs*, *A. brasiliense*, *A. dubitatum*, *A. incisum*, *A. ovale*, *Haemaphysalis juxtakochi* and *Rhipicephalus microplus*. The presence of *A. coelebs* was confirmed for Argentina and the first record of *H. juxtakochi* in Misiones was presented. Some of the species as *A. incisum*, *A. ovale* and *R. microplus* are ticks of medical and veterinary importance. Therefore, further studies on the vectorial role of these species in Misiones Province are necessary to determine their epidemiological relevance.

Key words: Ticks, Ixodidae, Misiones, Argentina

Introduction

Ticks are among the most important arthropod vectors of pathogens affecting humans and domestic and wild animals. They transmit a greater variety of infectious agents than any other blood-sucking arthropods group. Also, ticks can cause paralysis, toxicoses, irritation and allergy, and are able to produce large economic losses due to deleterious effects on livestock (Sonenshine 1991, Jongejan & Uilenberg 2004).

In Argentina, 37 species of hard ticks were recorded, including 25 species of *Amblyomma* (Guglielmone & Nava 2006, Nava *et al.* 2009, Nava *et al.* 2014 a, b), 1 of *Dermacentor*, 2 of *Haemaphysalis*, 9 of *Ixodes* and 2 of *Rhipicephalus* (Guglielmone & Nava 2005). In the particular case of Misiones Province, information on tick species is scarce. So far the species recorded for Misiones are *Amblyomma aureolatum* (Pallas, 1772), *Amblyomma calcaratum* Neumann, 1899, *Amblyomma incisum* Neumann, 1906, *Amblyomma longirostre* (Koch, 1844), *Amblyomma tigrinum* Koch, 1844, *Ixodes loricatus* Neumann, 1899, *Rhipicephalus microplus* (Canestrini, 1888), *Amblyomma ovale* Koch, 1844, *Amblyomma dubitatum* Neumann, 1899, *Rhipicephalus sanguineus* sensu lato and *Amblyomma brasiliense* Aragão, 1908 (Boero 1957; Boero & Delpietro 1971; Guglielmone & Mangold 1984; Ivancovich & Luciani 1992; Sinkoc *et al.* 1998; Mastropaolo *et al.* 2004; Nava *et al.* 2012; Guglielmone & Nava 2014; Welschen *et al.* 2014).

In Misiones Province, the Paranaense Rainforest covers an area of 26,860 km² and has the largest forest fragments of the Atlantic Forest ecoregion, one of the most biodiverse ecosystems in

the world. The Paranaense Rainforest still contains the original set of large vertebrates, including large predators and large herbivores, and it is known for its high level of endemism (Di Bitteti *et al.* 2003). Considering the limited information on hard ticks for Misiones, the aim of this paper is to present new information on distribution and hosts of tick species in the north of this Argentinean Province.

Materials and methods

The sampled area is located at north of the province of Misiones, where areas that protect old forests are in close contact with farms of small and medium size. Three sites were selected to collect free-living ticks: 1) Iguazú National Park (INP) (25° 33'S, 54° 17'W), with primary rainforest, lagoon and palm trees environments; 2) the private reserve San Sebastián de la Selva (25° 51'S, 53° 58'W), which is part of the biological corridor Urugua-í-Foerster, has 100 ha of grassland environments that combine capuera, primary forest, lagoon and swamp, and houses a large community of mammals, including a large population of *Hydrochoerus hydrochaeris* (Linnaeus, 1766); 3) the private reserve and ecolodge Surucuá (25° 32'S, 54° 08'W), a reservation of 120 km of primary forest which western edge contacts the INP. Questing ticks were collected from vegetation by using cloth flags and preserved in 96% ethanol. Samples on trapped animals were taken in INP (25° 40'S, 54° 25'W), in Puerto Península Provincial Park (PPPP) (25° 43'S, 54° 32'W) and in the touristic section Área Cataratas of the INP (25° 40'S, 54° 26'W). Ticks attached to road-killed animals were also collected at the National Route N°12, which traverses several protected areas. In addition, ticks were collected on cattle from two private agricultural establishments in the northeast of the province, both with silvopastoral livestock production (25° 40'S, 54° 2'W).

Ticks were identified following Guglielmone and Viñabal (1994), Barros-Battesti *et al.* (2006) and Martins *et al.* (2014), and by comparison with reference material deposited in the Tick Collection of INTA Rafaela. To confirm the nymphs determination performed with morphological characters, 10 representative specimens were selected from which DNA was extracted. In addition, two larvae collected on a human in the Surucuá reserve and an adult female collected in the San Sebastián reserve, which morphological diagnosis was difficult, were also included in the molecular analysis.

DNA was extracted by proteolytic digestion with Proteinase K and purified with a solution of phenol-chloroform-isoamyl alcohol and absolute ethanol. A 410 bp fragment of the mitochondrial 16S rRNA gene was amplified by a Polymerase Chain Reaction (PCR) using primers and conditions described in Mangold *et al.* (1998). Each of the sequences was aligned with each other and with the corresponding sequences of the *Amblyomma* species available in GenBank, using the BioEdit Sequence Alignment Editor (Hall 1999) with the CLUSTAL W program (Thompson *et al.* 1994). A pairwise estimate of percent sequence divergence was determined using MEGA version 5.0 (Tamura *et al.* 2011).

Results

A total of 292 ticks (38 larvae, 51 nymphs, 100 females and 103 males) belonging to 7 species were collected: *Amblyomma coelebs* Neumann, 1899, *A. brasiliense*, *A. dubitatum*, *A. incisum*, *A. ovale*, *Haemaphysalis juxtakochi* Cooley, 1946 and *R. microplus*. Details of hosts and localities for each tick species are presented in Table 1.

TABLE 1. Ticks collected in northern Misiones Province. (n): Number of specimens examined.

Site	Host/environment	Number of ticks collected				
		Species	Larvae	Nymphs	Females	Males
National Route N°12	<i>Cerdocyon thous</i> (3)	<i>A. ovale</i>	—	—	5	2
		<i>Amblyomma</i> cf. <i>A. incisum</i> - <i>A. brasiliense</i>	—	1		
		<i>A. coelebs</i>	—	1		
	<i>Puma yagouaroundi</i> (2)	<i>A. coelebs</i>	—	1		
		<i>A. ovale</i>	—			1
	<i>Procyon cancrivorus</i> (1)	<i>A. ovale</i>	—			5
	<i>Galictis cuja</i> (1)	<i>A. ovale</i>	—		3	11
<i>Nasua nasua</i> (1)	<i>A. ovale</i>	—		11	8	
Apepú (25°33'S, 54°17'W)	Human (4)	<i>Amblyomma</i> sp.	15	1		
		<i>A. coelebs</i>		20		
	Primary forest	<i>Amblyomma</i> sp.	11			
Área Cataratas (25°40'S, 54°26'W)	<i>Nasua nasua</i> (17)	<i>Amblyomma</i> cf. <i>A. incisum</i> - <i>A. brasiliense</i>		6		
		<i>A. brasiliense</i>		1		
		<i>A. ovale</i>			43	31
		<i>Amblyomma</i> sp.				1
		<i>A. coelebs</i>		9		
Northeastern Misiones (25°40'S, 54°2'W)	Cattle (10)	<i>R. microplus</i>			8	11
IPN (25°40'S, 54°25'W)	<i>Puma concolor</i> (1)	<i>A. incisum</i>		1		
		<i>Amblyomma</i> cf. <i>A. incisum</i> - <i>A. brasiliense</i>		1		
		<i>A. ovale</i>			15	21
		<i>H. juxtakochi</i>			1	
San Sebastián (25°51'S, 53°58'W)	Pasture	<i>Amblyomma</i> sp.	10			
		<i>A. dubitatum</i>			1	
Surucúa (25°32'S, 54°08'W)	Human (1)	<i>A. brasiliense</i>	1			
		<i>A. coelebs</i>	1			
Puerto Península Provincial Park (25°43'S, 54°32'W)	<i>Panthera onca</i> (1)	<i>Amblyomma</i> cf. <i>A. incisum</i> - <i>A. brasiliense</i>		1		
		<i>A. ovale</i>			13	12
		<i>A. coelebs</i>		3		

The comparison of the 16S rDNA sequences confirmed the presence of one larva of *A. coelebs* (Genbank accession number: KM519936), one nymph (Genbank accession number: KM519940) and one larva (KM519941) of *A. brasiliense*, one nymph of *A. incisum* (KM519939), eight nymphs of *A. coelebs* (two haplotypes: seven sequences with haplotype I (Genbank accession number: KM519936) and one sequence with haplotype II (Genbank accession number: KM519937)), and one female of *A. dubitatum* (KM519938). For all four species, the intraspecific genetic divergence among sequences from Misiones and those deposited in Genbank never exceeded 2%.

Representative specimens of the tick species collected during this study were deposited in the National Institute of Tropical Medicine (INMeT) of Puerto Iguazú, Argentina.

Discussion

Through this study the presence of *A. coelebs* in Argentina was confirmed. Recently it was shown that previous records of *A. coelebs* in Argentina (Salta and Jujuy) correspond in fact to *Amblyomma hadanii* Nava, Mastropaolo, Mangold, Martins & Guglielmone, 2014 (Nava *et al.* 2014b). Thus, the presence of *A. coelebs* in Argentina is only confirmed for the province of Misiones. The findings of *A. coelebs* nymphs parasitizing humans constitute the first record of this association for Argentina. This tick is a widely distributed species in the Neotropics, from Mexico to northern Argentina (Guglielmone *et al.* 2004), but the public health importance of this tick is not yet determined. There are previous studies where specimens of *A. coelebs* were found infected with *Rickettsia amblyommii*, an organism of unknown pathogenicity (Labruna *et al.* 2004). This finding and the parasitism in humans reported in Misiones highlight the need for further studies to determine the relevance of *A. coelebs* as a vector of potentially pathogenic rickettsiae. Finally, nymphs of this species were found parasitizing *Cercopithecus thous* (Linnaeus, 1766), *Nasua nasua* (Linnaeus, 1766), *Puma yagouaroundi* (Geoffroy, 1803), *Panthera onca* (Linnaeus, 1758) and humans, indicating that the immature stages of *A. coelebs* are catholic feeders.

In this study, *H. juxtakochi* is reported for the first time in Misiones Province, and to our knowledge, the association of an adult of *H. juxtakochi* parasitizing *Puma concolor* (Linnaeus, 1771) is also recorded for the first time. Specimens of *A. incisum*, *A. brasiliense*, *A. ovale* and *A. dubitatum* were also collected, confirming the presence of these species in northern Misiones. Specimens of these species are frequently found parasitizing humans in South America (Guglielmone *et al.* 2006, Szabó *et al.* 2006), and two of them, *A. ovale* and *A. dubitatum*, were found infected with the human pathogens *Rickettsia* sp. strain Rainforest and *Rickettsia parkeri*, respectively (Szabó *et al.* 2013; Lado *et al.* 2014). Therefore, it is inferred that these species may act as vectors of rickettsiae in northern Misiones.

Only one specimen of *A. dubitatum* (female) was found in a site with a high population density of *H. hydrochaeris*, which is not unexpected because this rodent is the main host for this tick (Nava *et al.* 2010). Also, the only tick collected on cattle was *R. microplus*. Cattle were examined in areas of close contact of cattle with native wildlife species, but no *Amblyomma* ticks were found on cattle. The parasitism of ticks belonging to *Amblyomma* species is usual in other areas of Centre and Northern Argentina (Guglielmone & Nava 2013). Therefore, further studies are needed to determine if the *Amblyomma* species of Misiones can be parasites of cattle.

Specific determination of several larvae was not possible because the poor condition of several of them, and due to the lack of adequate descriptions to perform comparisons. Also, some nymphs which morphology corresponded to *A. brasiliense* and *A. incisum* could not be determined to species level due to the high morphological similarity among the nymphs of these two tick species (see Martins *et al.* 2014). However, the analysis of the 16S gene sequences confirmed the presence of both species in northern Misiones. Complementary studies should be performed in order to determine which of these two species is more prevalent in the north of Misiones Province.

Acknowledgments

We acknowledge the contribution of the Proyecto Yaguareté Misiones, the Administration of National Parks NEA Delegation, and Dr. D. Salomon, to the Asociación Cooperadora INTA Rafaela and INMeT for their financial assistances.

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Accepted by Zhi-Qiang Zhang: 17 Sept. 2014; published: 1 Dec. 2014