



Original article

Rickettsial infections in ticks from wild birds in Paraguay

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ARTICLE INFO

Article history:

Received 18 June 2013

Received in revised form 3 August 2013

Accepted 18 August 2013

Available online 11 November 2013

Keywords:

Birds

Ticks

*Amblyomma**Rickettsia*

Paraguay

ABSTRACT

Ticks were collected from wild birds at 3 locations in Paraguay during the South American winter in August and September 2012. In total, 480 birds belonging to 106 species were examined. Overall, 31 (6.5%) birds representing 21 species were found parasitized by ticks which were identified as *Amblyomma calcaratum* Neumann (2 larvae, 20 nymphs), *Amblyomma longirostre* (Koch) (17 larvae, 3 nymphs), *Amblyomma parvum* Aragão (7 nymphs), *Amblyomma aureolatum* (Pallas) (1 nymph), *Amblyomma ovale* Koch (1 nymph), *Amblyomma tigrinum* Koch (1 larva), and *Amblyomma* spp. (4 larvae). Ticks collected accidentally on humans at the study locations during field work included 1 nymph of *Amblyomma coelebs* Neumann and 54 nymphs of *Amblyomma cajennense* (Fabricius). Most ticks were individually tested for the presence of *Rickettsia* species by polymerase chain reaction targeting rickettsial genes *gltA* and *ompA* and by amplicon sequencing. Two (12%) out of 17 *A. longirostre* larvae were found infected with *Candidatus* 'Rickettsia amblyommii', and 2 (33%) out of 6 *A. parvum* nymphs were infected with *Candidatus* 'Rickettsia andeanae'. This study provides the first report of rickettsial infections in Paraguayan ticks.

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Introduction

Knowledge of Paraguayan tick fauna and tick-borne pathogens is limited. While at least 30 tick species have been confirmed as endemic or established in Paraguay, it is likely that at least 10 additional species are also present in the country (Nava et al., 2007). Regarding the Ixodidae tick family, most Paraguayan records refer to the adult stage and tend to be limited to just a very few collection records. Data about immature stages are even more scarce (Nava et al., 2007).

Bacteria of the genus *Rickettsia* are obligate intracellular organisms that infect invertebrate hosts worldwide. Some of them cause diseases in mammals, including humans, to which they are transmitted by haematophagous vectors such as ticks (Sexton and Walker, 1999). According to a recent review on the distribution of rickettsiae in Latin America (Labruna et al., 2011a), there have been no published reports on the presence of *Rickettsia* in Paraguay, which is in contrast to all neighbouring countries.

The present study reports tick infestation on wild birds in 3 areas of Paraguay as well as the rickettsial infection in these ticks.

Also recorded were data regarding ticks found parasitizing humans during the fieldwork.

Materials and methods

Ornithological mist nets were used to trap wild birds in Paraguay during the 2012 South American winter. Three study locations were as follows: San Rafael National Park, Kanguery Biological Station, 26°30'S, 55°47'W, 183 m a.s.l., a location in the region of the Paraguayan Atlantic Forest (from 17 to 24 August); Teniente Agripino Enciso National Park, Headquarters Area, 21°12'S, 61°39'W, 253 m a.s.l., in the Paraguayan Chaco, a semi-arid region with a very low human population density (from 30 August to 4 September); and Los Tres Gigantes Biological Station, 20°04'S, 50°09'W, 82 m a.s.l., in the Paraguayan Pantanal (from 6 to 10 September).

Trapped birds were examined for the presence of ectoparasites, including ticks. Each individual bird was identified using Narosky and Yzurieta (2006, 2010) and Gwynne et al. (2010). The ticks were collected during naked-eye examination using tweezers and preserved in 96% ethyl alcohol. After examination, all birds were released back into the wild. Additionally, ticks were also collected from people working with birds during field work.

For taxonomic identification of ticks, *Amblyomma* nymphs were identified according to Martins et al. (2010). To identify larvae, we

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used molecular tools as described elsewhere (Ogrzewalska et al., 2011). Briefly, larvae were individually subjected to DNA extraction and then to polymerase chain reaction (PCR) using primers targeting a ~460-bp fragment of the tick mitochondrial 16S rDNA gene (Mangold et al., 1998). Amplified products were purified and DNA-sequenced as previously described (Labruna et al., 2004a) and then compared with NCBI Nucleotide BLAST searches (Altschul et al., 1990). Additionally, 5 nymphs identified to species level according to Martins et al. (2010) were submitted to the same molecular procedure in order to confirm the morphological identification.

Most of ticks were individually tested for the presence of *Rickettsia* by PCR using primers CS-78 and CS-323 targeting a 398-bp fragment of the *gltA* gene that occurs in all *Rickettsia* species (Labruna et al., 2004a), as well as primers Rr190.70 and Rr190.602 targeting a 532-bp fragment of the *ompA* gene present in the majority of those *Rickettsia* species belonging to the spotted fever group (Regnery et al., 1991). PCR products were DNA-sequenced and submitted to BLAST analysis to determine similarities to other *Rickettsia* species (Altschul et al., 1990).

Results

In total, 480 birds were captured, representing 106 species from 27 families and 8 orders (Table 1). Overall, 31 (6.5%) bird specimens representing 21 species from 11 Passeriformes families were found parasitized by ticks (Table 2). Tick prevalence per study location were 0% (0 positive/155 examined) at Los Tres Gigantes Biological Station, 4% (3/69) in Teniente Agripino Enciso National Park, and 11% (28/256) in San Rafael National Park. All collected ticks were *Amblyomma* immature stages and were identified as *Amblyomma calcaratum* Neumann (2 larvae, 20 nymphs), *Amblyomma longirostre* (Koch) (17 larvae, 3 nymphs), *Amblyomma parvum* Aragão (7 nymphs), *Amblyomma aureolatum* (Pallas) (1 nymph), *Amblyomma ovale* Koch (1 nymph), *Amblyomma tigrinum* Koch (1 larva), and *Amblyomma* spp. (4 larvae) (Table 2).

Ticks collected on humans included one nymph of *Amblyomma coelebs* Neumann in San Rafael National Park and 54 nymphs of *Amblyomma cajennense* (Fabricius), one of the latter at Los Tres Gigantes Biological Station and 53 in Teniente Agripino Enciso National Park. Some of these ticks were identified by molecular methods, as reported in Table 3.

A total of 43 ticks collected from birds (2 larvae and 13 nymphs of *A. calcaratum*, 17 larvae and 2 nymphs of *A. longirostre*, 6 nymphs of *A. parvum*, 1 nymph of *A. aureolatum*, 1 nymph of *A. ovale*, and 1 larva of *A. tigrinum*) and 55 ticks collected from humans (1 nymph of *A. coelebs* and 54 nymphs of *A. cajennense*) were tested individually for the presence of *Rickettsia*. Two (12%) out of 17 *A. longirostre* larvae [(1 collected from *Xiphorhynchus fuscus* (Vieillot) (Passeriformes: Dendrocolaptidae), and 1 from *Philydor rufus* (Vieillot) (Passeriformes: Furnariidae) from San Rafael National Park)] yielded positive results by the *gltA* PCR and, after DNA sequencing, showed 100% identity (350/350 bp) with the corresponding sequence of *Candidatus* 'R. amblyommii' strain AL from Brazil (EU274654). The *ompA* fragment amplified and sequenced from these 2 larvae was 99.4% (490/493 bp) similar to *Ca.* 'R. amblyommii' (JQ690638) from the USA and 100% (395/395 bp) to *R. amblyommii* strain AL from Brazil (EU274656). This *Rickettsia* was denominated *Ca.* 'R. amblyommii' strain San Rafael.

Two (33%) out of 6 *A. parvum* nymphs collected from *Cyanocopsa brissonii* Lichtenstein (Passeriformes: Fringillidae) from Teniente Agripino Enciso National Park yielded positive results by the *gltA*- and *ompA* PCR. The *gltA* partial sequences (350 bp) were identical to each other, to *Candidatus* 'Rickettsia andeanae' from Chile (JN180849) and Peru (GU169051), and to *Rickettsia* sp. strain Argentina (EU826510) from Argentina. The

ompA partial sequences (587 bp) from these ticks were 100% identical to the corresponding sequences of *Rickettsia* sp. endosymbiont of *A. maculatum* from the USA (EF524203, EF372578, JQ695859) and of *Rickettsia* sp. strain Argentina (EU826513). Both *Candidatus* 'Rickettsia andeanae', *Rickettsia* sp. strain Argentina, and *Rickettsia* sp. endosymbiont of *A. maculatum* refer to the same agent, which by priority has been named *Ca.* 'Rickettsia andeanae' (Paddock et al., 2010). This *Rickettsia* found in the present study was denominated *Ca.* 'Rickettsia andeanae' strain Agripino Enciso. The remaining tested ticks were found to be negative by the *gltA* PCR; therefore, considered negative for the presence of rickettsiae.

One *A. longirostre* nymph, 1 *A. parvum* nymph, 7 *A. calcaratum* nymphs, and 4 *Amblyomma* spp. larvae were deposited at the tick collection "Coleção Nacional de Carrapatos" (CNC) of the University of Sao Paulo, Brazil (accession numbers CNC2277–CNC2281). GenBank nucleotide sequence accession numbers for the partial mitochondrial 16S rDNA sequences obtained in the present study are KF179340–KF179342 (*A. longirostre* larvae), KF179343 (*A. aureolatum* larva), KF179344 (*A. tigrinum* larva), KF179345 (*A. parvum* larva), KF179346 (*A. calcaratum* consensus of 2 sequences of larvae and 2 nymphs), KF179347 (*A. ovale* nymph), and KF179348–KF179349 (*A. cajennense* nymphs). GenBank nucleotide sequence accession numbers for the partial sequences of the rickettsial *ompA* gene are KF179350 (*Ca.* 'R. amblyommii' strain San Rafael) and KF179352 (*Ca.* 'Rickettsia andeanae' strain Agripino Enciso). GenBank nucleotide sequence accession numbers for the partial sequences of the rickettsial *gltA* gene are KF179351 (*Ca.* 'R. amblyommii' strain San Rafael) and KF179353 (*Ca.* 'Rickettsia andeanae' strain Agripino Enciso).

Discussion

During the present study, we found immature stages of 8 *Amblyomma* species: 6 on birds (*A. longirostre*, *A. calcaratum*, *A. parvum*, *A. aureolatum*, *A. ovale*, and *A. tigrinum*) and 2 (*A. cajennense* and *A. coelebs*) on humans. All these species had been previously reported as established in Paraguay (Nava et al., 2007). To our knowledge, however, the only previous reports of *Amblyomma* ticks on birds in Paraguay were those of Massi Pallarés and Benítez Usher (1982), who reported *A. tigrinum* larvae and nymphs on "charata" [possibly *Ortalis canicollis* (Galliformes: Cracidae)] and *A. cajennense* larvae and nymphs on "charatas" and "cigüeñas" (storks). Therefore, the present study is the first to report *Amblyomma* ticks on passerine birds in Paraguay.

The predominant tick species infesting birds in the present study were *A. calcaratum* and *A. longirostre*. *Amblyomma calcaratum* has been reported in various countries of Central and South America (Guglielmone et al., 2003a). The adult stage parasitizes chiefly anteaters, *Tamandua* spp. and *Myrmecophaga tridactyla*, while subadults feed mostly on wild passerine birds (Labruna et al., 2007c; Ogrzewalska et al., 2009, 2010, 2011). In the present study, we found for the first time 5 additional passerine species being parasitized by this tick: *Dendrocolaptes platyrostris* Spix (Dendrocolaptidae), *Automolus leucophthalmus* (Wied) and *Syndactyla rufosuperciliata* (Lafresnaye) (both Furnariidae), *Turdus rufigiventris* Vieillot (Turdidae), and *Pyrrhocoma ruficeps* (Strickland) (Thraupidae).

Amblyomma longirostre is widely distributed in the Neotropical region (Guglielmone et al., 2003a), where the adult stage feeds primarily on porcupines (*Coendou* spp.) while subadult stages feed primarily on birds, mostly passerines (Labruna et al., 2007c; Ogrzewalska et al., 2008, 2009, 2010, 2012; Nava et al., 2010; Tolesano-Pascoli et al., 2010; Luz et al., 2012). Herein, we report for the first time 2 bird species infested by *A. longirostre*:

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