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Original article

Rickettsial infections in ticks from reptiles, birds and humans in Honduras

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ABSTRACT

Ticks were collected from captive reptiles, wild birds, and incidentally from humans at two locations in Honduras and part of these were tested for the presence of *Rickettsia* using polymerase chain reaction. The following species of ticks were found: *Amblyomma dissimile* on Iguanidae reptiles, *Amblyomma longirostre* and *Amblyomma nodosum* on birds, and *Amblyomma mixtum* (*Amblyomma cajennense* complex) on humans. *A. dissimile* was infected with *Rickettsia* sp. strain Colombianensi. Both *A. longirostre* and *A. mixtum* were infected with *Candidatus* 'Rickettsia amblyommii'. This study provides the first report of rickettsial infections in ticks from reptiles, birds and humans in Honduras. New host – *Amblyomma* tick associations are documented.

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1. Introduction

Information regarding tick fauna and their hosts in Honduras is limited to a few collection records. To our knowledge, only the following 13 species of ticks have been reported in Honduras, all belonging to the Ixodidae family: *Amblyomma dissimile* (Bequaert, 1932), *Amblyomma coelebs*, *Amblyomma sabanerae, Amblyomma scutatum, Amblyomma maculatum* (Guglielmone et al., 2003a; Jones et al., 1972), *Amblyomma auricularium* (Guglielmone et al., 2003b), *Amblyomma rotundatum* (Pietzsch et al., 2006), *Amblyomma mixtum* (*Amblyomma cajennense* complex) (Nava et al., 2014), *Rhipicephalus (Boophilus) microplus, Rhipicephalus sanguineus* sensu lato, *Dermacentor nitens, Ixodes bequaerti*, and *Ixodes boliviensis* (Guglielmone et al., 2003a). Indeed, this list seems to be highly incipient, probably reflected by the low number of tick surveys that have been conducted in the country.

Relapsing fever and Rocky Mountain spotted fever are the only tick-borne diseases suspected in the country, but they have not been confirmed (Chen and Wilson, 2009; McCown and Grzeszak, 2010; Peacock et al., 1971). In other Central American countries, this knowledge is also limited, although some rickettsial infections in ticks and various arthropod hosts have been reported. In El Salvador, spotted fever and typhus group rickettsioses have been diagnosed (Kováčová et al., 1996; WHO, 1993) and *Rickettsia bellii* has been detected in ticks (Barbieri et al., 2012). In Guatemala, *Rickettsia prowazekii* (Peacock et al., 1971), *Rickettsia typhi* (Parola et al., 2007), and *Rickettsia felis* (Troyo et al., 2012) have been detected and human spotted fever group cases also have been reported recently (Eremeeva et al., 2013). In Nicaragua, cases of spotted fever group have been suspected but no vectors identified (Peacock et al., 1971). In Costa Rica, infections in humans and/or ticks caused by *Rickettsia rickettsii* (Fuentes, 1979; Fuentes et al., 1985; Hun et al., 1991, 2008), *Rickettsia akari* (Peacock et al., 1971), *Candidatus* 'Rickettsia

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amblyommii' (Hun et al., 2011; Ogrzewalska et al., 2015), *R. felis* (Hun et al., 2011; Troyo et al., 2012), *R. bellii* (Ogrzewalska et al., 2015), and two novel *Rickettsia* sp. agents (Troyo et al., 2014; Ogrzewalska et al., 2015) have been reported. In Panama, *R. typhi* (Calero, 1948), *R. rickettsii* (de Rodaniche and Rodaniche, 1950; de Rodaniche, 1953; Estripeaut et al., 2007), *Ca.* 'R. amblyommii' (Bermúdez et al., 2009, 2011), and *R. felis* (Bermúdez et al., 2011) have been found.

The objective of the present work was to investigate rickettsial infection among ticks collected on reptiles, wild birds and humans in Honduras. It is the first study of this type from this country.

2. Materials and methods

Ticks were collected at two study locations: 1. Centro de Investigación y Jardín Botánico Lancetilla, Tela, Atlantida, lowland close to the Caribbean coast, 15°44′ N and 87°27′ W, 30 m.a.s.l. Birds were trapped in the Wilson Popenoe Arboretum comprising a large collection of trees, shrubs, and epiphytes surrounded by an area composed from virgin broadleaf forest and secondary forest. 2. Isla de Utila, Islas de la Bahía, a small Caribbean island 33 km far from the mainland of Honduras, 16°06′ N and 86°54′ W, 25 m.a.s.l. Terrestrial ecosystems on the island cover mangrove forest, wetlands, remnants of broadleaf forest and pastures. Birds were caught in the rest of the forest near village of Puerto Este. Ticks from reptiles were collected from captive iguanas at the Iguana Research and Breeding Station in Utila on 1 September 2014.

Wild birds were trapped using ornithological mist nets and examined for the presence of ectoparasites, including ticks, from 10 to 19 August 2014 on the mainland and from 21 to 30 August 2014 on the island. Each bird was identified in accordance with Garrigues and Dean (2007) and Howell and Webb (1995). Bird scientific names follow the checklist of Clements et al. (2014). After examination, all birds were released back into the wild. Additionally, ticks were looked for and collected from people working with birds during the field work at both study locations. The ticks were collected during naked-eye examination using tweezers and preserved in 96% ethyl alcohol.

For taxonomic identification of ticks, adults were identified morphologically according to Onofrio et al. (2006) and nymphs according to Martins et al. (2010). Larvae were identified using molecular tools as described elsewhere by amplifying a ≈460 bp fragment of the tick mitochondrial 16S rDNA gene (Mangold et al., 1998), Sanger dideoxy sequencing, and comparing the obtained sequences with those available in National Center for Biotechnology Information (NCBI) Nucleotide BLAST database. Additionally, three nymphs identified to species level according to Martins et al. (2010) were examined using the same molecular procedure in order to confirm the morphological identification.

Adult and immature ticks were submitted to DNA extraction using the guanidine isothiocyanate phenol technique, as previously described (Sangioni et al., 2005). Detection of rickettsial DNA in tick DNA extracts was performed by polymerase chain reaction (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). Samples that yielded visible amplicons of the expected size by the *gltA*-PCR were further tested by a second PCR assay using primers Rr190.70 and Rr190.701 targeting a 631 bp fragment of the *ompA* gene, which occurs only in *Rickettsia* species of the spotted fever group (Regnery et al., 1991; Roux et al., 1996). PCR products were DNA sequenced and analyzed using BLAST to determine similarities to other *Rickettsia* species.

The obtained sequences of tick and rickettsial DNA were accessed via NCBI Nucleotide database.

3. Results

A. dissimile (12 nymphs, 7 males, 4 females) and *Amblyomma* spp. (18 larvae) were found on captive iguanas (Table 1). Eleven (48%, *n* = 23) and 1 (100%, *n* = 1) *Ctenosaura bakeri* and *Iguana iguana* were infested, respectively. In total, 280 birds were captured and overall 25 (9%) birds were found parasitized by ticks (Table 1). Tick prevalence on birds were 13% (23/177) and 2% (2/103) in Lancetilla and Utila, respectively. Two species of ticks were found on birds: immature stages of *A. longirostre* (7 larvae, 6 nymphs) and *Amblyomma nodosum* (2 nymphs). The remaining 54 larvae belonged to *Amblyomma* spp. All 9 ticks collected on people in Lancetilla were identified as nymphs of *A. mixtum* (*A. cajennense* complex). Ticks identified by molecular methods are shown in Table 2.

A. dissimile (4 nymphs, 4 males, 3 females), 4 *A. mixtum* nymphs, 4 *A. longirostre* nymphs, and 5 *Amblyomma* spp. larvae were deposited into the tick collection at "Coleção Nacional de Carrapatos" (CNC) of the University of São Paulo, Brazil (accession numbers CNC-3037, CNC-3038, CNC-3039, CNC-3040, CNC-3041, CNC-3042, CNC-3043). The remaining 95 ticks, comprising 4 different species (8 nymphs, 3 males and 1 female of *A. dissimile*, 5 nymphs of *A. mixtum*, 7 larvae and 2 nymphs of *A. longirostre*, 2 nymphs of *A. nodosum*), and 67 unidentified larvae of *Amblyomma* sp. collected from birds, iguanas and humans were individually tested for the presence of *Rickettsia*.

Four (6%) out of 67 *Amblyomma* spp. larvae [3 collected from *C. bakeri* and 1 from *Geothlypis formosa* (Passeriformes: Parulidae) from Ísla de Utila] and 3 (38%) out of 8 nymphs of *A. dissimile* (2 collected from *C. bakeri* and 1 from *I. iguana* from Ísla de Utila) yielded positive results by *gltA* PCR, which yielded DNA sequences (350 bp) identical to each other and 100% identical to the corresponding sequences of *Rickettsia* sp. strain Colombianensi (JF905456), and to uncultured *Rickettsia* sp. clone Necocli 190 (JX519583) from Colombia. The *ompA* sequences obtained from these 7 ticks were identical to one another and showed 99.6% similarity (448/450 bp) with the corresponding sequence of *Rickettsia* sp. strain Colombianensi from Colombia (JF905458). This rickettsia was denominated *Rickettsia* sp. strain Colombianensi genotype Utila.

One (14%) out of 7 *A. longirostre* larva and 12 (18%) out of 67 *Amblyomma* spp. larvae [collected from 1 individual of *Xiphorhynchus guttatus* (Passeriformes: Furnariidae) from Jardín Botánico Lancetilla, Tela] yielded positive results for the *gltA*- and *ompA*-PCR. The *gltA* partial sequence was 100% identical (350/350 bp) to the corresponding sequence of *Ca.* 'R. amblyommii' strain RV1 from Costa Rica (KF702331) and the *ompA* partial sequence was 100% identical (433/433 bp) to the corresponding sequence of *Ca.* 'R. amblyommii' strain RV2 from Costa Rica (KF702333). This rickettsia was denominated *Ca.* 'R. amblyommii' genotype Tela2.

Four (80%) out of 5 *A. mixtum* nymphs collected from humans in Jardín Botánico Lancetilla, Tela yielded positive results for the *gltA*- and *ompA*-PCR. The *gltA* partial sequences (350 bp) were identical to each other, to *Ca.* 'R. amblyommii' isolate AMB18 from Panama (KM652483), and to *Ca.* 'R. amblyommii' isolate AcCR from Costa Rica (JF694089). The *ompA* partial sequences obtained from the 4 nymphs (526 bp) were identical to one another, to *Ca.* 'R. amblyommii' strain GAT-30 V (CP003334), to *Ca.* 'R. amblyommii' isolate TX051 (EF689731), and to *Ca.* 'R. amblyommii' strain Texas A&M (EF194096) from Texas. This rickettsia was denominated *Ca.* 'R. amblyommii' genotype Tela1.

GenBank nucleotide sequence accession numbers for the partial sequences of *Rickettsia* spp. generated in the present study are KP835791, KP835792 and KP835793 for the *gltA* gene, and KP835794, KP835795 and KP835796 for the *ompA* gene.

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