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Detection of rickettsiae in fleas and ticks from areas of Costa Rica with history of spotted fever group rickettsioses

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ABSTRACT

Outbreaks of spotted fevers have been reported in Costa Rica since the 1950s, although vectors responsible for transmission to humans have not been directly identified. In this study, species of *Rickettsia* were detected in ectoparasites from Costa Rica, mostly from five study sites where cases of spotted fevers have been reported. Ticks and fleas were collected using drag cloths or directly from domestic and wild animals and pooled according to species, host, and location. Pools were analyzed initially by PCR to detect a fragment of *Rickettsia* spp. specific *gltA* gene, and those positive were confirmed by detection of *htrA* and/or *ompA* gene fragments. Partial sequences of the *gltA* gene were obtained, as well as at least one *ompA* and/or *ompB* partial sequence of each species. *Rickettsia* spp. were confirmed in 119 of 497 (23.9%) pools of ticks and fleas analyzed. *Rickettsia rickettsii* was identified in one nymph of *Amblyomma mixtum* and one nymph of *Amblyomma varium*. Other rickettsiae present were '*Candidatus* Rickettsia amblyommii' in *A. mixtum*, *Amblyomma ovale*, *Dermacentor nitens*, and *Rhipicephalus sanguineus* s. l.; *Rickettsia bellii* in *Amblyomma sabanerae*; *Rickettsia felis* in *Ctenocephalides felis*; and *Rickettsia* sp. similar to '*Candidatus* R. asemboensis' in *C. felis, Pulex simulans, A. ovale*, and *Rhipicephalus microplus*. Results show the presence of rickettsiae in vectors that may be responsible for transmission to humans in Costa Rica, and evidence suggests exposure to rickettsial organisms in the human environment may be common. This is the first study to report *R. rickettsii* in *A. varium* and in *A. mixtum* in Costa Rica.

1. Introduction

In Latin America, the only rickettsial diseases recognized during the 20th century were Rocky Mountain spotted fever (RMSF), flea-borne typhus, and epidemic typhus caused by *Rickettsia rickettsii*, *Rickettsia typhi*, and *Rickettsia prowazekii*, respectively (Labruna et al., 2011). The description of new species of these intracellular ectoparasite-borne bacteria has increased in the past 25 years, and emerging human pathogens have been documented worldwide (Parola et al., 2005, 2013). There are now at least 10 more species of *Rickettsia* reported in Latin America, including known human pathogens (*Rickettsia akari*, *Rickettsia felis*, *Rickettsia parkeri*, *Rickettsia massiliae, Rickettsia africae*) and previously undescribed species and genotypes (Pacheco et al., 2007, 2011; Labruna et al., 2011; Miranda et al., 2012; Spolidorio et al., 2012; Troyo et al., 2014). Although infection by some of the rickettsiae present in the region may be severe or have a high fatality rate if untreated, others such as *Rickettsia bellii*, *Rickettsia rhipicephali*, and '*Candidatus* Rickettsia amblyommii' are considered nonpathogenic or have not been associated conclusively with disease in humans (Mediannikov et al., 2007; Labruna et al., 2011; Parola et al., 2013).

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In Central America, the first human cases of spotted fever were documented in Panama and Costa Rica during the 1950s (de Rodaniche and Rodaniche, 1950; Calero et al., 1952; Fuentes, 1979; Hun-Opfer, 2008). In other countries of Central America, reports of spotted fever group (SFG) rickettsiosis are more recent and include diagnosis in a traveler returning from Honduras (Chen and Wilson, 2009), and a possible outbreak that was identified in Guatemala in 2007 with at least one confirmed case (Eremeeva et al., 2012). In El Salvador and Nicaragua, cases of spotted fevers have not been officially confirmed, but there is serological evidence of infection in both countries (Peacock et al., 1971; WHO, 1993). In the case of Belize, there are no reports of human infection with rickettsiae, although the pathogenic *Rickettsia* sp. strain Atlantic rainforest was recently reported infecting ticks in that country (Lopes et al., 2016).

The ecology of rickettsial diseases has been poorly investigated in most countries of Central America (Labruna et al., 2011), despite the evidence of possible transmission in most of the region and recurrent case reports and fatalities in Costa Rica and Panama (Estripeaut et al., 2007; Tribaldos et al., 2011; Arguello et al., 2012; De Lucas et al., 2013; Hun, 2013). In Costa Rica, *R. rickettsii* was identified and confirmed as the species responsible for human cases (Hun et al., 2008), although the most complete ecological studies of RMSF date from the 1980s (Fuentes et al., 1985; Fuentes, 1986). In these investigations, *R. rickettsii* was detected and isolated only on one occasion from an invertebrate host, the rabbit tick *Haemaphysalis leporispalustris* (Fuentes et al., 1985; Fuentes, 1986).

Considering that information concerning vectors and reservoirs of rickettsiae in Costa Rica is limited, a project was initiated in 2008 to detect and identify species of rickettsia in ticks and fleas that may present a risk of contact and infection for the human population. Preliminary results of this study documented the presence and isolation of '*Candidatus* R. amblyommii' and *R. felis* for the first time in Costa Rica (Hun et al., 2011; Troyo et al., 2012a). This paper adds substantial evidence to complete the detection and identification of rickettsiae in different ectoparasite species, collected predominantly from domestic and peridomestic animals in areas of Costa Rica where cases of spotted fevers have historically been diagnosed or are suspected.

2. Materials and methods

2.1. Study sites

The main collection sites have been described previously (Troyo et al., 2012b). Sites are located to the North and East (Caribbean) regions of Costa Rica and have reported cases of spotted fevers in the past (Campbell et al., 1978; Fuentes, 1979; Hun et al., 1991; Hun-Opfer, 2008). Specifically, 5 sites were selected in 7 districts: (1) Turrialba (9°54′N, 83°41′ W; elevation: 650 m.a.s.l.), (2) La Virgen (10°23′N, 84°08′ W; elevation: 190 m.a.s.l.), (3) Limón (9°59′N, 83°02′ W; elevation: 5 m.a.s.l.), (4) Cahuita (9°44′N, 82°50′ W; elevation: 5 m.a.s.l.), 5) Guápiles (10°13′N, 83°47′ W; elevation: 260 m.a.s.l.), Jiménez (10°12′N, 83°44′ W; elevation: 230 m.a.s.l.), and Guácimo (10°12′N, 83°41′ W; elevation: 110 m.a.s.l.). The urban centers of Guápiles, Jiménez, and Guácimo (GP/J/GC) are less than 20 km from each other and were therefore considered to be the same study area (one site). These regions are characterized by continuous wet and warm conditions without distinct seasonality: annual rainfall is 2500–3500 mm, and mean minimum and maximum temperatures are approximately 20 °C and 30 °C, respectively (CRRH, 2008).

The Vector Research Laboratory (Laboratorio de Investigación en Vectores, LIVE) of the University of Costa Rica (UCR), provides the general public with a service of counseling and identification of medically important arthropods. Taking advantage of this service, additional tick and flea samples that were received by the laboratory from other areas of the country were included in the analyses.

2.2. Collection and identification of ectoparasites

Ectoparasites analyzed were collected or received at the LIVE, UCR, between July 2008 and March 2013. At study sites, a non-probabilistic approach was used to target households, farms, and other private properties for collection of specimens. These locations were set preferably in rural environments and in proximity to forested areas. Domestic animals were identified, and 20–25 live animal traps (homemade wooden box, Tomahawk® and/or Havaheart® traps) were placed one day and collected the next day (after 20–24 h) to capture opossums, rodents, and other small mammals in the surrounding areas. Traps were placed, in most cases, for 2–3 consecutive days and at least once every year at each site during 2008–2012. Small animals trapped were measured, photographed, and liberated after inspection and collection of ticks and fleas. Identification of animals was made using general descriptions (Reid, 1997), which were confirmed by an expert mammologist. All methods for trapping and manipulating animals, as well as collecting ectoparasites in this study followed the "Regulations about access to the biodiversity in teaching, social action, and research activities of the University of Costa Rica" (projects A8-127 and B1-041), the "Law of Biodiversity 7788" of Costa Rica, and were approved by University of Costa Rica's Institutional Committee for the Use and Care of Laboratory Animals (CICUA-35-10).

Fleas, ticks, and other ectoparasites were collected from their animal hosts for a period no longer than 1 man-hour per animal using combs and forceps (for example, collection would not take more than 30 min if two people were collecting from an animal, or no more than 15 min if there were 4 people). Ticks and fleas from other wild animals (reptiles, toads, sloths, raccoons) were collected during independent rescue and/or veterinary activities and were brought to the LIVE by third parties for identification and analysis. Specimens from different host species, collection sites, and specific locations (household, farm, property) were placed separately in dry glass vials (ticks), or vials with water or 70% ethanol (fleas and other ectoparasites). Specimens were kept live and at 4 °C (<7 days) or frozen at −20 °C until processing for identification.

When required for species identification or confirmation, some of the specimens were cleared in lactophenol and mounted in Hoyer's medium. Ticks (Ixodida), and fleas (Siphonaptera) were selected and identified by observing morphological characters and using identification keys (Hopkins and Rothschild, 1953; Smit, 1958; Barros-Battesti et al., 2006; Vargas, 2006). Ticks belonging to the *Amblyomma cajennense* species group were considered to be *Amblyomma mixtum*, which is considered to be the only representative species of this group in Central America (Nava et al., 2014). In addition, those identified as *Rhipicephalus sanguineus* were designated as "sensu lato" (s. l.), since the presence of 2 species of this group has been established in Latin America (the tropical lineage is the one present in Central America) (Nava et al., 2015).

Depending on the size and total number collected, specimens were grouped into pools containing usually 1–5 adult ticks (up to 10), 1–20 tick nymphs and/or larvae (up to 50), and 1–10 fleas from the same ectoparasite species, vertebrate host species, and location. Once prepared, all pools were conserved at −20 °C previously to PCR analyses.

In two pools that were of special interest, molecular confirmation of the tick species was performed by amplifying and sequencing a fragment of the mitochondrial 16S ribosomal RNA gene using primers 16S + 1 and 16S-1 (460 bp product) (Black and Piesman, 1994; Mangold et al., 1998). DNA extraction, sequenc-