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Detection of *Rickettsia monacensis* and *Rickettsia amblyommatis* in ticks collected from dogs in Costa Rica and Nicaragua

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ABSTRACT

The neotropical climate of Central America provides ideal conditions for ticks, which may transmit several human pathogens, including spotted-fever group *Rickettsia*. Dogs may act as sentinels or reservoirs for human tick-borne diseases due to shared tick species. Here, ticks were collected from 680 client-owned dogs in Nicaragua and Costa Rica, and a total of 316 tick pools were investigated for *Rickettsia* infection by quantitative real-time PCR (qPCR) targeting the *gltA* gene. Subsequently, up to six further genomic targets (*16S* rDNA, *gltA*, *sca4*, *ompA*, *ompB* and the 23S-5S intergenic spacer) were investigated for *Rickettsia* species determination. The predominant tick species was *Rhipicephalus sanguineus* sensu lato (s.l.) (19.9% of dogs infested in Costa Rica, 48.0% in Nicaragua), followed by *Ixodes boliviensis* (3.1% in Costa Rica / none in Nicaragua) and *Amblyomma ovale* (4.8% in Costa Rica, 0.9% in Nicaragua). In total, 22 of 316 tick pools containing 60 of 1023 individual ticks were *Rickettsia*-positive as determined by qPCR, resulting in a minimum infection rate (MIR) of 2.2%. In detail, MIR in *Rh. sanguineus* s.l. was 0.7% (7/281 pools), in *I. boliviensis* 33.3% (12/13 pools) and in *A. ovale* 9.7% (3/22 pools). For 11 of 12 positive *I. boliviensis* pools and one of six positive *Rh. sanguineus* s.l. pools, the species could be determined as *R. monacensis*. *R. amblyommatis* was identified in one *Rh. sanguineus* s.l. pool from Costa Rica and one *A. ovale* pool from Nicaragua. Nine of 12 *R. monacensis*-positive tick pools were collected in San Rafael de Heredia, Costa Rica, indicating a high local occurrence in this area. This study supports recent evidence that *R. monacensis* is present on the American continent. Its high local occurrence among dog-associated *I. boliviensis*, which may also parasitize humans, in Costa Rica gives cause for concern, as *R. monacensis* is also pathogenic to humans.

1. Introduction

Rickettsioses are caused by worldwide distributed obligate intracellular bacteria of the genus *Rickettsia*, which are usually arthropod-associated. Members of the spotted-fever group (SFG) *Rickettsia*, which are mainly transmitted by ticks, may cause febrile disease in humans with symptoms ranging from mild to life-threatening (Parola et al., 2005). More than 20 species with known pathogenic potential for humans have been identified within this group to date, and multiple candidate species and subspecies have been proposed (Parola et al., 2013).

The neotropical climate of Central America provides ideal conditions for ticks. For example, more than 40 tick species are found in Panama, at least 15 of which parasitize humans (Esser et al., 2016). In contrast, only few tick-borne SFG *Rickettsia* with known pathogenic potential for humans have been detected in continental Central America so far. Until recently, *R. rickettsii*, the causative agent of Rocky Mountain spotted fever, was the only recognized agent of spotted fevers in Central America, responsible for cases in Mexico, Panama, Costa Rica (Hun et al., 2008; Tribaldos et al., 2011; Zavala-Castro et al., 2006) and presumably also Guatemala (Eremeeva et al., 2013). Recently, *Rickettsia* sp. strain Atlantic rainforest, which has caused cases of spotted fever in Brazil (Spolidorio et al., 2010), has been found in ticks in Belize (Lopes et al., 2016). *Rickettsia africae*, the causative agent of African tick-bite

Abbreviations: SFG, spotted-fever group; qPCR, quantitative real-time PCR; MIR, minimum infection rate

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fever, has been detected in ticks in Nicaragua (Vogel et al., 2018). Another pathogenic species, *Rickettsia parkeri*, has been reported to occur in ticks in Belize (Polsomboon et al., 2017). Moreover, *R. monacensis*, which may cause a Mediterranean spotted fever-like illness (Jado et al., 2007; Madeddu et al., 2012), has been identified in an *Ixodes boliviensis* specimen in Costa Rica (Campos-Calderón et al., 2016). Previously, the known distribution of this *Rickettsia* species, which was first isolated from *I. ricinus* in Germany (Simser et al., 2002), was restricted to Europe, northern Africa and Asia (Parola et al., 2013; Shin et al., 2013). However, this pathogen was recently also detected in a dog on the Cape Verde archipelago (Lauzi et al., 2016), and in an *Ixodes* sp. tick carried by a migratory songbird captured in Texas, United States of America (Cohen et al., 2015). These findings indicate a widespread geographic distribution and the capability of range-expansion via transport by migratory birds.

Additionally, several tick-borne *Rickettsia* with unknown or unclear pathogenic potential occur in continental Central America: *Rickettsia amblyommatis* (Karpathy et al., 2016) has been detected in ticks from Costa Rica, Panama, Belize, Nicaragua, Honduras and Mexico (Bermúdez et al., 2011; Hun et al., 2011; Novakova et al., 2015; Polsomboon et al., 2017; Sánchez-Montes et al., 2016; Vogel et al., 2018), and *R. bellii* has been found in ticks in Costa Rica, El Salvador and Panama (Barbieri et al., 2012; Ogrzewalska et al., 2015; Bermúdez Castillero and Troyo Rodríguez, 2018). Furthermore, an uncultivated *Rickettsia* sp. closely related to *R. monacensis*, named strain IbR/CRC (Troyo et al., 2014) as well as another candidate species, *Candidatus* Rickettsia nicoyana (Moreira-Soto et al., 2017) and a *Rickettsia* sp. related to *R. asemboensis* (Troyo et al., 2016) have been detected in Costa Rican ticks. Similarly, *Rickettsia* sp. strain Colombianensi has been described from ticks in Honduras, Colombia and northern Brazil (Luz et al., 2018; Miranda et al., 2012; Novakova et al., 2015) and *Rickettsia* sp. ARAGOI from Nicaragua (Vogel et al., 2018).

Dogs live in intimate contact to humans, and a study has shown that pet ownership is significantly correlated with the risk of tick encounters (Jones et al., 2017). In fact, 10 of 21 tick species reported to parasitize dogs in Panama have also been found to bite humans (Esser et al., 2016). Thus, dogs may serve as sentinels for human tick-borne diseases, and, furthermore, might constitute a reservoir for certain pathogens, including some species of *Rickettsia* (Levin et al., 2011). Indeed, a previous study conducted in Costa Rica has shown that dogs living in areas associated with human spotted-fever outbreaks were more likely to be SFG *Rickettsia* seropositive compared to other dogs (Moreira-Soto et al., 2016). In the present study, ticks collected from client-owned dogs in Nicaragua and Costa Rica were analyzed for *Rickettsia* spp. in order to evaluate the potential risk for humans.

2. Materials and methods

Research and sample export permits were obtained from the Nicaraguan and Costa Rican authorities, respectively (permit no. 28927 and 00101785). From September to December 2013, dogs were presented to the veterinarian for various reasons in seven different cities in western Nicaragua and at 23 different locations in Costa Rica (Fig. 1, Table 1). Almost all dogs were presented to the veterinarian by their owners. Dogs at animal shelters were only included at one location in Nicaragua (Managua) and one location in Costa Rica (San Rafael, Heredia). During clinical examinations of dogs, tick infestation was assessed visually and ticks (adults, nymphs and larvae) were collected into sterile tubes. Ticks were conserved in 70% ethanol and identified based on morphological characteristics according to the guide of Barros-Battesti et al. (2006). Infestation rate for each tick species was compared between Nicaragua and Costa Rica using X^2-tests or Fisher Exact tests when appropriate.

From each tick-infested dog, a maximum of 3 adult tick pools (each containing max. 5 adult ticks of the same species) were selected for further analyses. DNA was extracted from pooled ticks using the Nucleospin® 8 Blood Kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany) as described previously (Tappe and Strube, 2013). Each pool was tested for *Rickettsia* DNA using two different quantitative real-time PCRs (qPCRs). First, a qPCR targeting the citrate synthase (*gltA*) gene based on a primer-probe combination by Stenos et al. (2005) was carried out at the Institute for Parasitology, University of Veterinary Medicine Hannover, as described by Tappe and Strube (2013). Second, positive samples were sent to the Bundeswehr Institute of Microbiology and subjected to another qPCR targeting the *gltA* gene, as described by Wölfel et al. (2008). Only tick pools with a positive result in both assays were regarded as confirmed positives. These were subjected to up to seven additional conventional PCRs and subsequent sequencing in order to achieve a *Rickettsia* species determination. The investigated targets, published in previous protocols, included partial sequences of *ompA*, *ompB* and the 23S-5S intergenic spacer. For three samples, additional targets were investigated for possible sequence variation (*16S* ribosomal DNA, *gltA*, *sca4*; Table 2). Subsequent Sanger sequencing was conducted by an external contractor (GATC Biotech, Konstanz, Germany). Sequences were analyzed using BioEdit Alignment Editor Version 7.1.1 (Hall, 1999) and compared with sequences deposited in the GenBank database of the National Centre for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990). Species determination based on the percentage nucleotide identity at each locus followed the criteria of Fournier et al. (2003).

Minimum infection rates were calculated for each tick species by dividing the number of *Rickettsia*-positive tick pools by the total number of ticks represented in all tested tick pools.

3. Results

In total, 680 dogs were examined (329 in Nicaragua, 351 in Costa Rica), of which 638 were client-owned, whereas 12 dogs from Managua, Nicaragua, and 30 dogs from San Rafael, Heredia, Costa Rica, were sampled at animal shelters. Ticks were found on 37.4% of the examined dogs, with significantly lower infestation prevalences in Costa Rica than in Nicaragua, as detailed below. A total of 1023 adult ticks were collected from 159 dogs in Nicaragua and 96 dogs in Costa Rica. The predominant tick species was *Rhipicephalus sanguineus* s.l. (956 adult specimens, 19.9% of dogs infested in Costa Rica, 48.0% in Nicaragua [X^2-test, $P < 0.001$]), followed by *I. boliviensis* (36 adult specimens, 3.1% of dogs infested in Costa Rica, none in Nicaragua [Fisher Exact test, $P < 0.001$]) and *Amblyomma ovale* (31 adult specimens, 4.8% in Costa Rica, 0.9% in Nicaragua [Fisher Exact test, $P = 0.002$]).

In total, 22/316 tick pools (containing 60 of the total 1023 individual ticks) originating from 20 different dogs were *Rickettsia*-positive as determined by qPCR, resulting in an overall MIR of 2.2%. In detail, 7/281 *Rh. sanguineus* s.l. pools, 12/13 *I. boliviensis* pools and 3/22 *A. ovale* pools were *Rickettsia*-positive. The corresponding MIRs were 0.7%, 33.3% and 9.7%, respectively. The distribution of tick species, numbers of *Rickettsia*-positive pools and MIRs per country are presented in Table 3.

For 11 of 12 positive *I. boliviensis* pools and one of six positive *Rh. sanguineus* s.l. pools, the species could be determined as *R. monacensis*, based on evaluation of up to six genetic loci as shown in Table 4. A comparison to recently described rickettsiae from Central American *Ixodes* ticks (e.g. *Rickettsia* sp. IbR-CRC1, *Rickettsia* sp. Barva1) was only possible for the partial *gltA*-gene due to different genomic regions of the used targets. In particular, the comparison of the partial *gltA*-gene showed identities ranging from 98.3 to 100% to these strains, as well as 100% identity to the *R. monacensis* reference strain IrR/Munich (*Rickettsia* sp. IbR-CRC1: KJ507211–KJ507214: 100%, KJ507215: 99.1%, KJ507216: 98.3%; uncultured *Rickettsia* sp. [KU001172]: 100%; *Rickettsia* sp. Barva1 [KF702332]: 100%; reference strain IrR/Munich [LN794217.1]: 100%). Nine of the *R. monacensis*-positive pools were