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Short communication

Infection of *Amblyomma ovale* by *Rickettsia* sp. strain Atlantic rainforest, Colombia

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ABSTRACT

Our goal was to understand rickettsial spotted fevers' circulation in areas of previous outbreaks reported from 2006 to 2008 in Colombia. We herein present molecular identification and isolation of *Rickettsia* sp. Atlantic rainforest strain from *Amblyomma ovale* ticks, a strain shown to be pathogenic to humans. Infected ticks were found on dogs and a rodent in Antioquia and Córdoba Provinces. This is the first report of this rickettsia outside Brazil, which expands its known range considerably.

Introduction

Tobia fever was the first reported outbreak of a rickettsial disease in Colombia. Commonly known in North America as Rocky Mountain spotted fever (RMSF), the illness caused by *Rickettsia rickettsii* affected 20% of the population from the town of Tobia, province of Cundinamarca, Colombia, as described by Patino et al. (1937). After this historical event, RMSF remained epidemiologically silent until 2003 and 2004 when Hidalgo et al. (2007a) confirmed *R. rickettsii* as the cause of death of 2 patients. More recently, 3 additional outbreaks were documented in the provinces of Antioquia (2006 and 2008) and Córdoba (2007) (Acosta et al., 2006; Hidalgo et al., 2007b; Pacheco et al., 2008).

To better understand rickettsial spotted fevers in Colombia, we searched for rickettsiae in ticks from locations where the 2006–2008 outbreaks took place. We collected ticks from domestic and small wild mammals (rodents and opossums) and in the environment in the municipalities of Turbo, Necoclí (Antioquia), and Los Córdobas (Córdoba) during 12 months in 2010 and 2011. We herein report rickettsiae in *Amblyomma ovale* ticks found in a high numbers on dogs.

Materials and methods

The study was conducted in 3 neighboring municipalities (Turbo and Necoclí from Antioquia province and Los Córdobas from Córdoba province), all of them located on the Colombian Atlantic coast. Turbo is at 8°8.272′ N, 76°33.009′ W, and 400 m above sea level (asl), Necoclí is at 8°32.892′ N, 76°34.429′ W, and 182 m asl, and Los Córdobas is at 8°50.195′ N, 76°20.252′ W, and 8 m asl. The climate is hot and humid in all the above municipalities, with an annual average temperature of 28 °C and relative humidity of 85%. The economy of these regions is mainly based on agriculture, with bananas as their main product, followed by plantain, cassava, corn, and rice. Other commercial activities are fisheries, livestock breeding, and beef production and tourism. Finally, the municipalities of Antioquia are rich on forest reserves and secondary woods, normally characterized as humid tropical forest.

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Table 1
Amblyomma ovale ticks processed by PCR with rickettsia-screening primers (*gltA* gene CS 78-323).

Municipality	Positive ticks/total per host species																		
	Dog			Donkey			*P. semispinosus*			*T. talamancae*			*D. marsupialis*			Environment			Total
	M	F	N	M	F	N	M	F	N	M	F	N	M	F	N	M	F	N	
Necoclí	3/22	5/13	1/1	1/1	–	–	–	–	1/13	–	–	–	–	–	–	–	–	–	11/50
Turbo	1/11	2/11	–	–	–	–	–	–	0	–	–	0/3	–	–	0/3	–	–	–	3/28
Los Córdobas	0/14	1/18	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1/1	0/4	2/37

M, male tick; F, female tick; N, nymph.

Ticks collected in this study were identified following an accepted morphological key (Barros-Battesti et al., 2006; Martins et al., 2010).

Ninety-two adult *A. ovale* ticks were collected from domestic animals (91 from 42 dogs, and one from a donkey) and one from the vegetation. Fifty-three of them arrived alive at the laboratory (32 females and 21 males) and were frozen at −80 °C. Those that were already dead (28 males and 12 females) were preserved in 100% isopropanol. Additionally, we collected 24 *A. ovale* nymphs, 16 from rodents (13 from *Proechimys semispinosus* and 3 from *Transandinomys talamancae*), 3 from opossums (*Didelphis marsupialis*), one from a dog, and 4 from vegetation (Table 1).

DNA from ticks was extracted with the DNeasy Blood & Tissue kit (Qiagen®), and samples were processed by PCR assay with primers CS-78 (forward) and CS323 (reverse), which amplify a 401-bp fragment of the citrate synthase gene (*gltA*), previously reported as appropriate for the screening of *Rickettsia* spp. (Guedes et al., 2005). To check for PCR inhibitors, tick DNA was amplified with the primers 16S+ and 16S– as described by Mangold et al. (1998). DNAs found to be positive with the screening primer set were used as templates in additional PCR reactions with primers CS239 (forward) and CS1069 (reverse), which amplify 830 bp of the *gltA* gene, as well as with primers previously described for the *ompA* and *ompB* genes (Guedes et al., 2005). All DNA products were purified and sequenced in a commercial facility. Sequences were assembled with the DNAstar program, and phylogenetic analyses were conducted using MrBayes 3.2.0.

We used frozen samples from PCR-positive ticks for isolation assays. Briefly, we homogenized ticks with a Polytron system in 1 ml of SPG buffer (218 mM sucrose; 3.8 mM KH_2PO_4; 7.2 mM K_2HPO_4; 3.9 mM glutamate monosodium, pH 7.0, filter-sterilized). The homogenate was passed through a 27G needle and incubated onto Vero cell monolayers for 1 h at 34 °C. Cells were maintained in medium with an antibiotic mixture for 3 days. After that, the cells were maintained with antibiotic-free media for 5 weeks with weekly changes of medium. Cells were checked daily for cytopathic effects and weekly for rickettsial antigen by immunofluorescence. Antigen-positive cell cultures were confirmed by PCR.

All the procedures in the current study were approved by the local ethics committee on animal experimentation (file # 53 CEEA from Spanish Comité de Ética para la Experimentación con Animales, Universidad de Antioquia).

Results

All the findings in this report correspond to ticks from the species *A. ovale*, identified according to a previously published morphological key (Barros-Battesti et al., 2006; Martins et al., 2010). Table 1 shows the distribution of ticks found positive for rickettsia.

Of 93 adults ticks gathered in the study, 91 were collected from dogs (one male and one female were stored in isopropanol as a reference for the species). Two more were found on a donkey from Necoclí and in the vegetation of Los Córdobas.

Twelve ticks collected on 8 dogs were positive by PCR using the screening primer set, 3 of them were strongly positive with all primer pairs, one amplified with screening and *ompB* primers (Turbo_19_12, Supplementary Fig. 1), and 8 amplified only with screening primers and produced weak bands. Neither of these weak bands provided good sequencing products for the phylogenetic analysis, therefore only the former 3 ticks (that turned out positive with all the primers) were subsequently used in Fig. 1 (Necoclí_10_11, Necoclí_350, and Necoclí_361). Two out of the 24 *A. ovale* nymphs were also positive with all primers, the individual collected from a dog from Necoclí and a pool of 5 nymphs collected on a Tome's spiny rat male (*Proechimys semispinosus*) from Necoclí (Table 1). These 2 nymph samples were also depicted in the phylogenetic analysis (Fig. 1, Necoclí_364 and Necoclí_367, respectively).

Supplementary Fig. 1 related to this article can be found, in the online version, at doi:10.1016/j.ttbdis.2014.04.018.

One out of 10 samples tested in culture produced a live rickettsial isolate. The *gltA*, *ompA*, and *ompB* sequences of this isolate (Necocli_10_11, Fig. 1, GenBank accession nos. KJ158742, KJ158741, KJ158744) were identical to strain Atlantic rainforest, a *Rickettsia parkeri*-like spotted fever group rickettsia previously reported in *A. ovale* and causing spotted fever in Brazil (Spolidorio et al., 2010). An unprecedented additional sequence downstream of the above-mentioned *ompB* (3392–3749) was also obtained from the same isolate and published in GenBank (accession no. KJ158745) (not shown).

Eight amplicons of the *gltA* gene were sequenced and phylogenetically analyzed. The resulting tree (Supplementary Fig. 1) shows 2 different *Rickettsia* species circulating in *A. ovale* ticks from the study area. One of them corresponds to a single sequence (Necocli_11_2, GenBank accession no. KJ158743) obtained from the tick parasitizing a donkey. This sample was very close to *Rickettsia bellii*, a nonpathogenic *Rickettsia* from the ancestral group. Our sequence has 4 nonsynonymous changes in the amino acid positions 105, 128, 339, and 340 of the *gltA* gene, when compared to the closest *R. bellii* sequence (GenBank accession no. PC000087.1). The remaining 7 sequences were 100% identical to the Atlantic rainforest strain (Spolidorio et al., 2010), one of them obtained from the tick gathered from the environment (Los Córdobas_76d), another one from the Tome's spiny rat (Necoclí_367), and the other 5 taken from dogs (4 from Necoclí of Fig. 1 and Turbo_19_12).

Discussion

Rickettsia sp. strain Atlantic rainforest (also known as strain Bahia) was recently described as the etiological agent of a spotted fever rickettsiosis in 2 Brazilian patients that presented with fever, myalgia, rash, and eschar at the tick bite site. One of the patients had also lymphadenopathy and ulcers on the oral mucosa (Silva et al., 2011; Spolidorio et al., 2010). This novel rickettsial agent is closely related to *Rickettsia parkeri*, *Rickettsia africae*, and *Rickettsia sibirica*, which are human pathogens in different parts of the world (Parola et al., 2005). In Brazil, the strain Atlantic rainforest has been found to be primarily associated with *A. ovale*, which is incriminated as

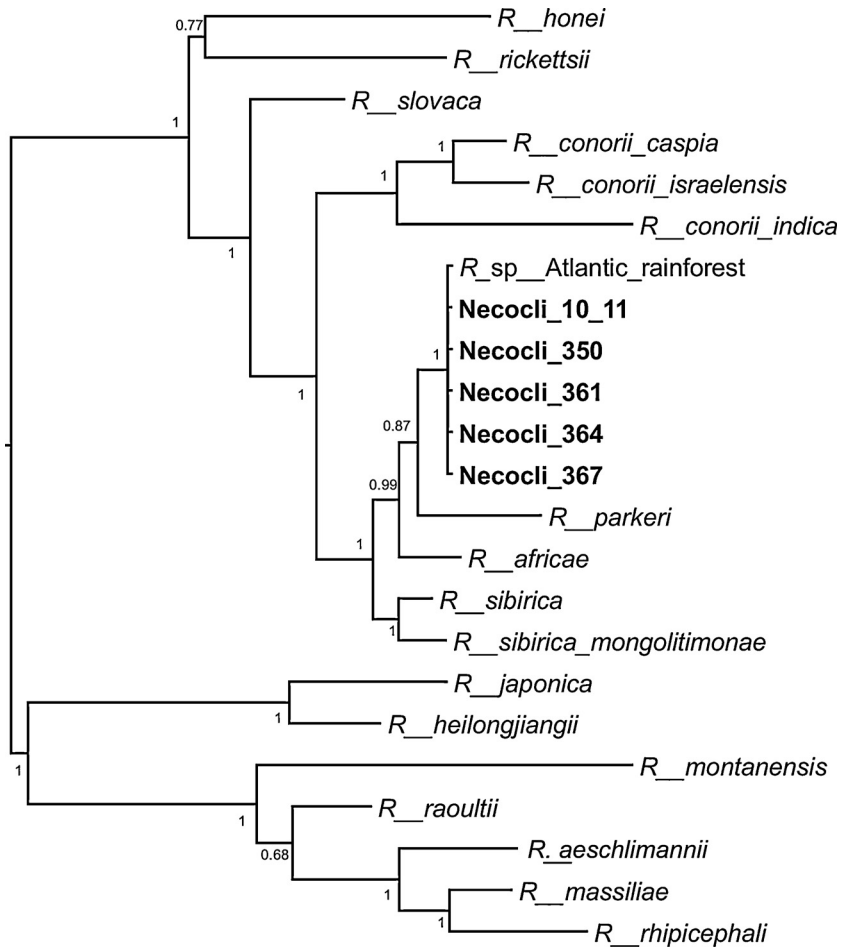

Fig. 1. Bayesian phylogenetic tree of rickettsial *gltA*, *ompA*, and *ompB* genes. Samples from this study are in bold. Two parallel searches were run by 1,000,000 generations sampling every 1000 states. Average standard deviations of split frequencies were <0.01 at the end of the runs. Substitution model used was the General Time Reversible with a discrete Gamma distribution of the variation of the evolutionary rate (GTR+G), previously found as the best model by the Bayesian information criterion. The analysis was performed in MrBayes 3.2.0. There were a total of 2250 positions in the final dataset. The tree was drawn in FigTree 1.4, and it was rooted in the midpoint.

the main vector of the disease (Medeiros et al., 2011; Sabatini et al., 2010; Szabó et al., 2013). Similarly, herein we report *Rickettsia* sp. strain Atlantic rainforest infecting *A. ovale* ticks in Colombia, suggesting that the disease caused by this emerging rickettsial strain could also be occurring in Colombia. This is further supported by the fact that *A. ovale* is known to be an important human-biting tick in South America (Guglielmone et al., 2006). It is noteworthy that the Tome's spiny rat has previously been found to be seropositive for spotted fever group rickettsiae in Colombia (Quintero et al., 2013), which suggests that this species could serve as an amplifier host.

In conclusion, our study shows 2 different rickettsiae cocirculating in the same region (Necocli, Colombia). To the best of our knowledge, this is the first report of the Atlantic rainforest strain in Colombia, which constitutes the second pathogenic spotted fever group *Rickettsia*, besides *R. rickettsii*, circulating in this country.

Conflict of interest

The authors declare that the disclosure of this paper will not generate or constitute any conflict of interest.

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