Spotted fever group *Rickettsia* infecting ticks (Acari: Ixodidae) in the state of Santa Catarina, Brazil

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*During 2006-2008, a total of 260 adult ticks were collected from domestic and wild animals in different regions of the state of Santa Catarina (SC), Brazil, including areas where human cases of Brazilian spotted fever have been reported. Collected ticks belonging to nine species (*Amblyomma aureolatum, Amblyomma cajennense, Amblyomma dubitatum, Amblyomma longirostre, Amblyomma ovale, Amblyomma tigrinum, Dermacentor nitens, Rhipicephalus microplus *and* Rhipicephalus sanguineus*) were tested by polymerase chain reaction (PCR) for rickettsial infection. Overall, eight (3.1%) ticks were found to be infected with* Rickettsia *species. After sequencing the PCR products, we determined that the sequences generated from three* A. aureolatum, *one* A. ovale *and one* R. sanguineus *from the municipality of Blumenau, one* A. ovale *from the municipality of Águas Mornas and one* A. ovale *from the municipality of Urussanga were identical to the corresponding partial rickettsial* ompA *gene sequence of* Rickettsia parkeri *strain Atlantic rainforest. The sequence generated from one* A. longirostre *from Blumenau was 100% identical to the corresponding partial rickettsial* ompA *gene sequence of* Rickettsia amblyommii *strain AL. Because* R. parkeri *strain Atlantic rainforest was recently shown to have caused two cases of human spotted fever in other states of Brazil, the role of this rickettsial agent as a possible etiological agent of spotted fever in SC is discussed.*

Key words: *Rickettsia* - spotted fever - ticks - *Amblyomma* - *Rhipicephalus* - *Dermacentor*

Until recently, the only spotted fever group (SFG) rickettsiosis known to occur in Brazil was Brazilian spotted fever, caused by *Rickettsia rickettsii*, the most pathogenic SFG species in the world. This pathogen is endemic in the southeastern states [Espírito Santo, Minas Gerais, Rio de Janeiro, São Paulo (SP)] of the country, where it is transmitted to humans chiefly by the tick *Amblyomma cajennense* but is also transmitted by *Amblyomma aureolatum* in a few areas of SP (Labruna 2009). From 1990-2009, 627 confirmed cases of Brazilian spotted fever were reported in these southeastern states; the fatality rates varied from 20-33% among the four states (data available from the Brazilian Ministry of Health site: saude.gov.br). Because most of these cases were diagnosed solely using serological assays employing *R. rickettsii* antigens and because serological cross-reactions between all members of the SFG are known to occur (Philip et al. 1978, Parola et al. 2005), technically these cases could have been caused by any SFG species. Therefore, these cases cannot be considered confirmed cases of *R. rickettsii* infection. However, the severe clinical signs, high fatality rate and epidemiological data suggest that most of these cases were caused by *R. rickettsii* (Lemos et al. 2001, Angerami et al. 2006a, b). In fact, a few of these infections were investigated more thoroughly using molecular methods and were confirmed to be caused by *R. rickettsii* (Nascimento et al. 2005, Rozental et al. 2006, Lamas et al. 2008, Nascimento et al. 2009).

In 2003, cases of Brazilian spotted fever were reported for the first time in the state of Santa Catarina (SC), southern Brazil. Since then, a total of 139 cases have been confirmed in that state alone (from 2003-2009); none of these cases was fatal (data available from the Brazilian Ministry of Health site: saude.gov.br). A recent study compared the clinical pictures of Brazilian spotted fever in SP (southeastern Brazil) and SC (Angerami et al. 2009). These authors called attention to the more severe disease in SP (usually associated with severe haemorrhagic manifestations) and to the frequent occurrence of lymphadenopathy in patients with this disease in SC. Lymphadenopathy was only rarely observed among the cases in SP, as expected for disease caused by *R. rickettsii* (Walker et al. 2008). The SFG disease in SC has been officially treated as Brazilian spotted fever because the laboratory diagnosis has relied upon the seroconversion to *R. rickettsii* antigens. However, because of the absence of lethal cases and the clinical differences with the disease in southeastern Brazil, it has been suggested that a different, currently unidentified SFG *Rickettsia* species has caused the disease in SC (Angerami et al. 2009, Labruna 2009).

Financial support: CNPq, FAPESP
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Received 7 February 2011
Accepted 12 September 2011

In the present study we used molecular methods to investigate the presence of *Rickettsia* species in ticks in different regions of SC, including regions were Brazilian spotted fever cases were recently reported.

MATERIALS AND METHODS

During 2006-2008, a total of 260 adult ticks were collected from domestic and wild animals in five out of the six official administrative regions of SC (Norte, Região Serrana, Vale do Itajaí, Grande Florianópolis, Sul) as part of an ongoing study on the tick fauna of SC. Due to logistical reasons, we did not sample the region Oeste Catarinense, where no human cases of spotted fever have been reported (Silva 2009). Ticks were identified to the species level according to Barros-Battesti et al. (2006). After being collected from the animals, ticks were preserved in plastic vials containing absolute ethanol until they were processed.

In the laboratory, the ticks were left for at least 1 h on a sterile paper towel to allow the ethanol to evaporate. Thereafter, the ticks were individually submitted to DNA extraction using the guanidine isothiocyanate-phenol technique, as previously described (Sangioni et al. 2005). For every 10 individual ticks, a blank tube was included in the DNA extraction. Samples were tested individually by polymerase chain reaction (PCR) using primers CS-78 and CS-323 targeting a 401-bp fragment of the rickettsial gene *gltA*, as previously described (Labruna et al. 2004). In each set of reactions, negative control tubes containing water and a positive control tube containing DNA of the strain NOD of *Rickettsia parkeri* were included (Ogrzewalska et al. 2009). Samples that yielded visible amplicons of the expected size by the *gltA*-PCR were further tested by a second PCR assay using primers Rr190.70p and Rr190.602n targeting a 532-bp fragment of the rickettsial gene *ompA*, as previously described (Regnery et al. 1991). All *ompA*-PCR amplicons of the expected size were submitted to direct DNA sequencing using an automated ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). The BLAST program (National Center for Biotechnology Information, Bethesda, MD) was used to determine the similarities of the partial rickettsial sequences generated in the current study.

RESULTS

The numbers of ticks per species and per locality collected in the present study are shown in Table. The 260 adult ticks belonged to nine species: 125 *A. aureolatum*, 82 *Amblyomma ovale*, 10 *Amblyomma tigrinum*, 30 *Rhipicephalus sanguineus* and one *Rhipicephalus microplus* were collected from domestic dogs, two *A. cajennense* and five *Dermacentor nitens* were collected from horses, four *Amblyomma dubitatum* were collected from capybaras (*Hydrochaeris hydrochoerus*) and one *Amblyomma longirostre* was collected from the grounds of a house.

Eight ticks (3.1%) were found to be infected with *Rickettsia* (Table). These eight ticks yielded amplicons of the expected size for both the *gltA* and *ompA* PCRs. The products of the *ompA* PCR were sequenced. The sequences generated from the three *A. aureolatum*, one *A. ovale* and one *R. sanguineus* from Blumenau; the one *A. ovale* from Águas Mornas and the one *A. ovale* from Urussanga were all the same and were 100% identical (450/450) to the corresponding *ompA* sequence of the Atlantic rainforest strain of *R. parkeri* (GQ855237). Each *R. parkeri*-infected tick was collected from different individual dogs. The sequence generated from one *A. longirostre* from Blumenau was 100% identical (450/450) to the corresponding *ompA* sequence of the AL strain of *Rickettsia amblyommii* (EU274656). We excluded any cross-contamination of our tick samples with DNA from our positive control sample (strain NOD) because the NOD strain of *R. parkeri* differs from the Atlantic rainforest strain by 10 nucleotides in the segment of *ompA* that was amplified by the primers.

DISCUSSION

The presence of the Atlantic rainforest strain of *R. parkeri* is reported in SC for the first time. This strain is considered to be pathogenic for humans and it was recently found to be the causative agent of two cases of spotted fever in humans in Brazil, one in SP (Spolidorio et al. 2010) and another in the state of Bahia (BA) (Silva et al. 2011). In addition, in SP, Sabatini et al. (2010) reported that the Atlantic rainforest strain was present in 13.6% and 1.9% of *A. ovale* and *R. sanguineus* ticks, respectively, collected from dogs, and in 8.8% of *A. ovale* ticks collected from vegetation. Because *A. ovale* is an important human-biting tick (Guglielmone et al. 2006), it was suggested to be a vector for the transmission of the Atlantic rainforest strain to humans (Sabatini et al. 2010).

The two cases of human spotted fever caused by *R. parkeri* strain Atlantic rainforest in SP (Spolidorio et al. 2010) and BA (Silva et al. 2011) were clinically similar to the dozens of spotted fever cases that have been reported in SC (Angerami et al. 2009). These cases were characterised by milder haemorrhagic manifestations and no lethality. The BA case also presented with lymphadenopathy, as was also seen in most cases in SC. Therefore, our results highlight the possibility that the spotted fevers in SC have been caused by *R. parkeri* strain Atlantic rainforest, a hypothesis yet to be confirmed by identification of this agent in human clinical samples. Although this hypothesis has not been confirmed, it is noteworthy that we found the pathogenic strain Atlantic rainforest in SC, where it is potentially transmitted to humans by the ticks *A. ovale* and *A. aureolatum*; this second species is also an important human-biting tick in Brazil (Guglielmone et al. 2006). Interestingly, another pathogenic strain of *R. parkeri* is transmitted by the tick *Amblyomma triste* in Uruguay and Argentina (Venzal et al. 2004, Romer et al. 2011); however, this tick species is not known to be present in SC.

Most of the spotted fever cases of SC have been reported in the Vale do Itajaí region, especially in Blumenau (Silva 2009), where we found most of the ticks infected by this agent in the present study. However, we also found this rickettsia in *A. ovale* ticks from two other municipalities (Águas Mornas and Urussanga), where human spotted fever has never been confirmed. Thus, clinicians should be alert to the possible occurrence of human spotted fever in these two areas.

TABLE

Ticks collected in the state of Santa Catarina, Brazil during 2006-2008 and tested by polymerase chain reaction for rickettsial infection

Region	Municipality	Geographic coordinates	Ticks			
			Species	Tested (n)	Infected n (%)	*Rickettsia* species
Norte	Araquari	-26.37S -48.72W	*Amblyomma aureolatum*	1	0	-
			Amblyomma ovale	1	0	-
			Dermacentor nitens	5	0	-
	Joinvile	-26.30S -48.84W	*A. aureolatum*	3	0	-
			Amblyomma tigrinum	1	0	-
Região Serrana	Bom Retiro	-27.79S -49.48W	*A. aureolatum*	4	0	-
	Capão Alto	-27.93S -50.51W	*A. tigrinum*	4	0	-
	Coxilha Rica	-28.12S -52.29W	*A. tigrinum*	2	0	-
	Curitibanos	-27.28S -50.58W	*A. aureolatum*	4	0	-
	Lages	-27.81S -50.32W	*A. aureolatum*	37	0	-
			A. tigrinum	3	0	-
	Monte Castelo	-26.46S -50.23W	*A. aureolatum*	2	0	-
	Otacílio Costa	-27.48S -50.12W	*A. aureolatum*	3	0	-
	Santa Isabel	-28.10S -49.98W	*A. aureolatum*	2	0	-
	Urubici	-28.01S -49.59W	*A. aureolatum*	1	0	-
Vale do Itajaí	Barra Velha	-26.63S -48.68W	*Amblyomma cajennense*	1	0	-
			Rhipicephalus sanguineus	2	0	-
	Blumenau	-26.91S -49.06W	*A. aureolatum*	28	3 (10.7)	*Rickettsia parkeri*
			A. ovale	34	1 (2.9)	*R. parkeri*
			Amblyomma longirostre	1	1 (100)	*Rickettsia amblyommii*
			R. sanguineus	25	1 (4.0)	*R. parkeri*
			Rhipicephalus microplus	1	0	-
	Itapema	-27.09S -48.61W	*A. cajennense*	1	0	-
			R. sanguineus	1	0	-
	Rio do Sul	-27.21S -49.64W	*A. aureolatum*	1	0	-
	Salete	-26.98S -50.00W	*A. aureolatum*	1	0	-
	Taió	-27.11S -49.99W	*A. aureolatum*	5	0	-
			A. ovale	3	0	-
			Amblyomma dubitatum	4	0	-
Grande Florianópolis	Águas Mornas	-27.69S -48.82W	*A. aureolatum*	12	0	-
			A. ovale	5	1 (20)	*R. parkeri*
	Florianópolis	-27.59S -48.54W	*R. sanguineus*	2	0	-
	São João Batista	-27.27S -48.84W	*A. aureolatum*	2	0	-
			A. ovale	1	0	-
Sul	Urussanga	-28.51S -49.32W	*A. aureolatum*	19	0	-
			A. ovale	38	1 (2.6)	*R. parkeri*
Total				260	8 (3.1)	

In the study of Sabatini et al. (2010) in an Atlantic rainforest area of SP, the tick *A. ovale* was found to occur only at low-elevation areas (< 100 m above sea level), whereas the tick *A. aureolatum* tick was found to occur only at higher elevation areas (> 700 m). Interesting, Sabatini et al. (2010) found no *A. aureolatum* infected by *R. parkeri* strain Atlantic rainforest, whereas at low elevation, this rickettsia was found in *A. ovale* and in one *R. sanguineus* tick collected from a dog. In the present study, we report for the first time *A. aureolatum* ticks infected with the Atlantic rainforest strain. However, in contrast to the situation reported by Sabatini et al. (2010) in SP, in

Blumenau (elevation ≈150 m) both *A. ovale* and *A. aureolatum* occurred sympatrically, many times infesting the same individual hosts (data not shown). In contrast, many *A. aureolatum* and no *A. ovale* were collected from dogs in the municipality of Lages, a high-elevation area (≈900 m) of SC. Because we found no *A. aureolatum* infected with the Atlantic rainforest strain in Lages or in other areas of SP where this tick does not occur sympatrically with *A. ovale* (Pinter & Labruna 2006, Moraes-Filho et al. 2009), it is possible that the *Rickettsia*-infected *A. aureolatum* ticks of the present study acquired the infection through horizontal transmission while feeding on the same dogs as infected *A. ovale* ticks. However, this mechanism must be evaluated in future studies encompassing larger samples of questing and feeding ticks.

A single *A. longirostre* adult tick, collected on the ground, was shown to be infected by *R. amblyommii* strain AL. This rickettsial strain was recently found in the immature stages (5 larvae and 1 nymph) of *A. longirostre* collected from passerine birds in SP (Ogrzewalska et al. 2008). Although different strains of *R. amblyommii* have been reported to infect different tick species of the New World (Labruna 2009, Castellaw et al. 2010, Ogrzewalska et al. 2010, Bermúdez et al. 2011), the pathogenicity of *R. amblyommii* in humans remains to be demonstrated. Interestingly, there is serological evidence suggesting that it is a human pathogen in the United States (Apperson et al. 2008).

Clinical cases of Brazilian spotted fever have been reported in SC since 2003, but the *Rickettsia* species involved in these cases has never been identified. We report for the first time the *Rickettsia* species infecting human-biting ticks in SC. Although it is possible that the *R. parkeri* strain Atlantic rainforest is the causative agent of some of the human spotted fever cases that have been diagnosed by serology in that state, it is possible that other strains of *R. parkeri* or even other *Rickettsia* species are also causing spotted fever in SC. Further studies are warranted to better understand the pathogenic rickettsiae in southern Brazil.

Herein, we considered the Atlantic rainforest strain to belong to the species *R. parkeri* based on the gene sequence-based criteria recently proposed for identification of new *Rickettsia* isolates (Fournier et al. 2003), as discussed by Spolidorio et al. (2010). However, we are aware that the species definition for *Rickettsia* remains a controversial issue, without a major consensus among rickettsiologists (Walker & Ismail 2008, Fournier & Raoult 2009, Goddard 2009).

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