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Short communication

# Molecular detection of the human pathogen *Rickettsia parkeri* strain Atlantic rainforest in *Amblyomma ovale* ticks in Argentina

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ABSTRACT

Human rickettsioses caused by *Rickettsia parkeri* strain Atlantic rainforest in Brazil motivated the analysis of *Amblyomma ovale* ticks in Misiones province, a similar ecological region in northeastern Argentina. During 2010–2017, 393 *A. ovale* ticks were collected from domestic and wild animals and from vegetation, and 177 were pooled for rickettsial detection by PCR targeting the *gltA*, *ompA* and *ompB* genes. A sample consisting of a pool of two *A. ovale* adults collected on *Nasua nasua* was positive for *Rickettsia parkeri* strain Atlantic rainforest. Since the Atlantic rainforest areas in Brazil share environmental characteristics with Misiones province, the transmission cycle of *R. parkeri* strain Atlantic rainforest observed in Brazil should be extrapolated to Argentina, where awareness on the possibility of occurrence of rickettsiosis cases caused by this strain should be raised.

## 1. Introduction

In Argentina, fatal cases of tick-borne rickettsiosis caused by *Rickettsia rickettsii* were reported in the northwestern province of Jujuy (Paddock et al., 2008). Nonfatal cases of human disease by infection with *Rickettsia parkeri* sensu stricto have been reported mainly in the Delta Paraná region (East Argentina); nevertheless, some cases have been reported in patients from Córdoba and La Rioja provinces (Central and West-central Argentina) (Romer et al., 2011, 2014). The only human case of *Rickettsia massiliae* infection from Argentina was reported in a patient from Buenos Aires (East Argentina), and was diagnosed in Spain (García-García et al., 2010).

Human tick-borne rickettsioses associated with infection with a spotted fever group (SFG) agent, described as *Rickettsia* sp. strain Atlantic rainforest, were reported in Brazil (Spolidorio et al., 2010; Silva et al., 2011; Krawczak et al., 2016a). Recently, this strain closely related to *Rickettsia africae*, *R. parkeri* s.s. and *Rickettsia sibirica,* was classified as a strain of *R. parkeri,* and it is currently named *R. parkeri* strain Atlantic rainforest which forms a group with *R. parkeri* s.s., *R. parkeri* strain NOD and *R. parkeri* strain Parvitarsum (Nieri-Bastos et al., 2018). In Brazil, the agent caused an acute febrile illness although fatal cases were not recorded (Spolidorio et al., 2010; Silva et al., 2011; Krawczak et al., 2016a), and the vector involved in its transmission is the tick *Amblyomma ovale,* a species that bites humans regularly (Guglielmone et al., 2006; Szabó et al., 2013; Barbieri et al., 2014; Krawczak et al., 2016b). Although *Amblyomma aureolatum* and *Rhipicephalus sanguineus* sensu lato have been found infected with *R. parkeri* strain Atlantic rainforest, their vector competence have not yet been established; but since *A. aureolatum* is a recognized anthropophilic tick, it could be an alternative vector for *R. parkeri* strain Atlantic rainforest (Medeiros et al., 2011; Barbieri et al., 2014). *Rickettsia parkeri* strain Atlantic rainforest has also been found infecting *A. ovale* ticks in Colombia, Belize and possibly Nicaragua (confused with *R. africae*) (Londoño et al., 2014; Lopes et al., 2016; Vogel et al., 2018).

A single record of *R. parkeri* strain Atlantic rainforest exists for Argentina (Monje et al., 2015), but the tick species (*Amblyomma dubitatum*) and the ecological area (margin of the Paraná river in the Humid Chaco eco-region) where this rickettsia was detected are not related to the epidemiological conditions described in Brazil where cases of human disease by *R. parkeri* strain Atlantic rainforest were reported (Szabó et al., 2013; Barbieri et al., 2014; Krawczak et al., 2016b). On the other hand, the province of Misiones in northeastern Argentina, which contains the largest remnants of Atlantic rainforest in the country, presents similar ecological features to those described for the *R. parkeri* strain Atlantic rainforest endemic area in southern Brazil. Moreover, Misiones province shares borders with the states of Paraná, Santa Catarina and Rio Grande do Sul, where this *Rickettsia* has been found infecting *A. ovale* ticks (Barbieri et al., 2014; Krawczak et al., 2016b; Nieri-Bastos et al., 2016). The aim of this work is to report the detection of *R. parkeri* strain Atlantic rainforest in *A. ovale* ticks

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collected in the Atlantic rainforest in Misiones Province, northeastern Argentina, in order to provide relevant epidemiological data which could be useful for the diagnostic and detection of spotted fever-diseases in an area where this information is lacking.

## 2. Materials and methods

Misiones province is characterized by large areas of highly endangered Atlantic rainforest contacting rural environments and peri-urban settlements, which determines a high contact rate between domestic and wild animals and man. Furthermore, this province contains the Iguazú National Park (INP) which receives more than 1 million tourists each year, and borders the neighboring countries of Brazil and Paraguay. During 2010–2017, *A. ovale* ticks were collected from vegetation and from wild and domestic animals in Misiones province, Argentina, and the samples were screened for SFG rickettsiae (ethical approval by the Advisory Committee on Ethics and Safety (CAES) of the Faculty of Veterinary Sciences of the National University of the Litoral: 194/14). Collection sites included the INP (25°41′S, 54°27′W), the Puerto Península (25°43′S, 54°32′W), Urugua-í (25°52′S, 54°11′W), Piñalito (26°29′S, 53°48′W), Cruce Caballero (26°27′S, 53°58′W) and Moconá (27°9′S, 53°54′W) Provincial Parks, and rural areas of Puerto Iguazú (25°39′37″S, 54°33′30″W), Andresito (25°44′2″S, 53°57′21″W), Libertad (25°56′38″S, 54°32′37″W) and Montecarlo (26°34′7″S, 54°47′54″W) cities (Fig. 1).

Questing ticks were collected on vegetation in active animal trails using white cloth flags of 1 × 1.5 m, and more than 15 sampling sessions were carried out to capture birds, rodents, medium and large mammals. In addition, dead animals that had been run over on roads were examined, and domestic animals were also examined in urban, rural and natural forest areas. All ticks were preserved in 96% ethanol and identified by taxonomic keys and descriptions (Barbieri et al., 2008; Nava et al., 2017). A total of 177 selected ticks were pooled for DNA extraction. DNA was extracted using Proteinase K digestion and purified with a phenol-chloroform-isoamyl alcohol solution. DNA extracts were tested via a PCR to amplify fragments of the citrate synthase rickettsial gene (*gltA*) with primers CS-239 and CS-1069 (Labruna et al., 2004a), using *R. massiliae* as positive control. PCR-positive samples were further tested by two PCR to amplify fragments of the outer membrane protein genes *ompA* with primers Rr190.70p and Rr190.602n (Regnery et al., 1991), and *ompB* with primers 120-M59 and 120–807 (Roux and Raoult, 2000), using *R. parkeri* s.s. and *R. massiliae* as positive controls. Amplicons of *ompA* and *ompB* were purified and sequenced. The sequences were edited using BioEdit Sequence Alignment Editor (Hall, 1999), aligned with Clustal W (Thompson et al., 1994), and compared with sequences of *Rickettsia* species available in GenBank.

**Table 1**
*Amblyomma ovale* ticks collected on host and on vegetation in Misiones province, Argentina, 2010–2017. In parentheses, number of tick infested hosts and number of tested ticks.

| Hosts (n) | *Amblyomma ovale* (tested) | | |
|---|---|---|---|
| | Larvae | Nymphs | Adults |
| *Tachyphonus coronatus* (1) | 1 (1) | | |
| *Akodon* cf. *A. montensis* (3) | 5 (0) | 2 (2) | |
| *Oligoryzomys* cf. *O. nigripes* (1) | | 1 (0) | |
| *Dasyprocta azarae* (1) | 2 (2) | | |
| *Didelphis aurita* (3) | 21 (9) | 1 (0) | |
| *Nasua nasua* (44) | 7 (5) | 2 (1) | 134 (46) |
| *Cerdocyon thous* (5) | | | 12 (9) |
| *Puma concolor* (5) | | | 48 (16) |
| *Panthera onca* (6) | | | 51 (22) |
| *Puma yagouaroundi* (2) | | | 2 (1) |
| *Tapirus terrestris* (2) | | | 3 (0) |
| Domestic dog (30) | | | 61 (31) |
| Environment | 2 (0) | 6 (1) | 32 (31) |
| Total | 38 (17) | 12 (4) | 343 (156) |

## 3. Results and discussion

A total of 393 *A. ovale* specimens were collected, of which 100 pools of 1–5 adults, 1–2 nymphs, and 1–5 larvae, totaling 177 ticks, were selected for the detection of *Rickettsia* (Table 1). A sample consisting of a pool of two adults (1.1%) collected on *Nasua nasua* in the INP was positive in the *gltA* PCR. This sample was used to amplify ca. 550-bp and 800-bp fragments of the *ompA* and *ompB* genes, respectively. The sequences of both genes (GenBank accession numbers: *ompA*: MH247927; *ompB*: MH247926) showed 100% identity with the corresponding sequences of *R. parkeri* strain Atlantic rainforest (named in GenBank as *Rickettsia* sp. strain Atlantic rainforest) from southern Brazil deposited in GenBank (*ompA*: KX137902; *ompB*: KX034218). In addition, 24 pools consisting of 53 ticks (29.9%) amplified a fragment of the *gltA* gene but did not amplify the *ompA* gene (the primers are specific for SFG rickettsiae). Two of these samples (DNA from six ticks) were purified and sequenced. Sequences (GenBank accession numbers: MH282857; MH282858) showed very high identities to each other and to *Rickettsia bellii* sequences deposited in GenBank (GenBank accession numbers: AY375161; AY362703).

During the sampling of active animal trails, 40 *A. ovale* ticks were collected (32 adults and 8 immature). Of these individuals, eight adults

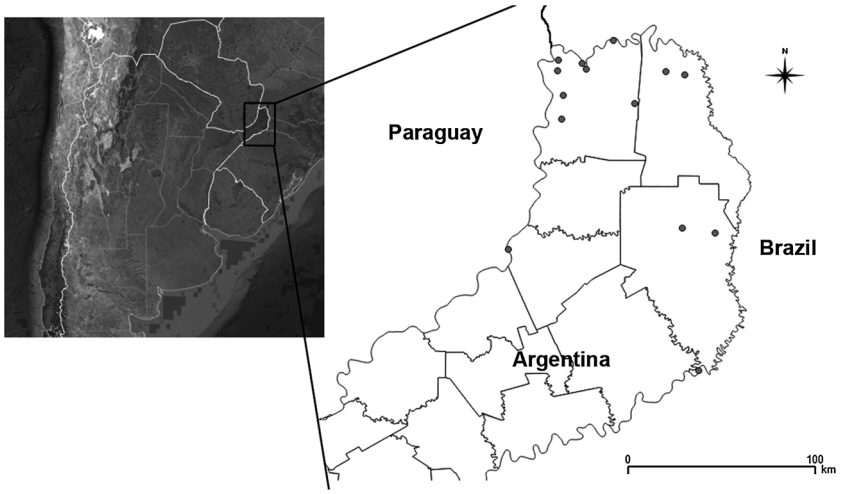


**Fig. 1.** *Amblyomma ovale* collection sites in Misiones province, Argentina.

(20%) bit the researchers and were removed immediately.

This work provides evidence on the infection of *A. ovale* with *R. parkeri* strain Atlantic rainforest in Argentina. The epidemiological nexus between *A. ovale*, *R. parkeri* strain Atlantic rainforest and human cases of rickettsiosis has been established in Brazil in areas where domestic dogs get infested with *A. ovale* when accessing Atlantic forest patches (Barbieri et al., 2014). These zones share environmental characteristics with Misiones province, where there are dwellings in rural areas in close contact with remnant forest areas, and canines have permanent access to the forest where they get infested with *A. ovale* ticks, which are thus transported to homes. Therefore, the transmission cycle of *R. parkeri* strain Atlantic rainforest observed in Brazil could be extrapolated to Argentina. The tick eco-epidemiological scenario in Misiones province resembles even more that of Brazil, considering that *A. ovale* populations are also usually found infected with *R. bellii* (Labruna et al., 2004b; Szabó et al., 2013).

Taking into account that 20% of the *A. ovale* ticks found on animal trails in this study bit humans, the risk of pathogen transmission is high. All features mentioned above define a high rate of exposure of rural workers, peri-urban dwellers and tourists from around the world to *A. ovale* tick bites, which determines a high risk of transmission of *R. parkeri* strain Atlantic rainforest and highlights the need for further study of tick eco-epidemiology and its public health relevance in Misiones province, where no official data about human cases of eschar-associated rickettsiosis exist. It is also necessary to raise public awareness about the risk of tick bites and infection with pathogens, and to inform about protection methods against ticks. Knowledge about human rickettsiosis by clinical practitioners in Misiones is limited, and this *Rickettsia* in particular produces mild symptoms, so it is possible that human cases are being underdiagnosed. The possibility of occurrence of rickettsiosis cases caused by this strain should be taken into account by public health institutions involved in the diagnosis and treatment of these diseases.

## Conflicts of interest

The authors declare to have no conflicts of interest.

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