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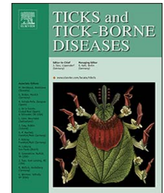

Short communication

Experimental infection of *Rickettsia parkeri* in the *Rhipicephalus microplus* tick

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ABSTRACT

This study aimed to evaluate, by means of artificial feeding, the interaction between a pathogenic rickettsia and the hard tick *R. microplus*. We used partially engorged females fed on calves free of *Rickettsia* spp. Group 1 (G1), containing 20 ticks, was fed bovine blood only. Group 2 (G2), containing 20 ticks, was fed blood containing uninfected VERO cells, and group 3 (G3), containing 40 ticks, was fed blood containing VERO cells infected with *Rickettsia parkeri*. Biological parameters of the non-parasitic phase and a possible bacterial transmission to the tick eggs and to guinea pigs were evaluated. At the end of oviposition, all G3 females were PCR-positive for genes specific for the genus *Rickettsia*. Although no guinea pigs were infected, the experimental infection of *R. microplus* by *R. parkeri* caused a deleterious effect on the oviposition and provided the first report of transovarian transmission of rickettsia in this tick.

1. Introduction

Rhipicephalus microplus is a one-host tick that can be found worldwide in subtropical and tropical regions. It is considered to be the most important tick parasite of livestock in the world (Estrada-Pena, 2008). The *Rhipicephalus microplus* tick is frequently found in regions where spotted fever is endemic, and in some cases, it is revealed by polymerase chain reaction (PCR) to be infected with pathogenic rickettsiae (Moura-Martiniano et al., 2014).

In South America, *Rickettsia parkeri* was first detected in *Amblyomma triste* ticks in Uruguay (Venzal et al., 2004). According to the authors, there was an epidemiological association between this finding and a reported case of human rickettsial disease.

It is possible that the acquisition of pathogenic rickettsia by *R. microplus* may occur through infection mechanisms such as co-feeding with an infected tick and by feeding on a rickettsiemic host (Socolovschi et al., 2009). Thus, the aim of this study was to evaluate, by means of artificial feeding, the interaction between a pathogenic rickettsia and the *R. microplus* tick using the *R. parkeri* bacterium as a model.

2. Materials and methods

For acquisition feeding, first-generation pathogen-free larval progeny (1 pool of ≈2 g) were allowed to feed on pathogen-free dairy cattle (*Babesia* spp., *Anaplasma* spp. and *Rickettsia* spp.). On the 21 st day post-infestation, partially engorged female ticks were carefully detached from the skin of the cattle by hand.

A frozen stock of the At24 strain of *R. parkeri* (22th passage) was obtained. The rickettsial strain was thawed and inoculated into a 75 cm^2 flask containing a monolayer of Vero cells. When the monolayer was close to 100% infected, it was harvested and centrifuged at $6000 \times g$ for 10 min. The supernatant (DMEM medium) was completely removed, and the pellets were used to prepare a fresh homogenate in dairy cattle blood at a concentration of $\approx 2 \times 10^6$ cells/ml for artificial feeding.

The ticks were infected using an artificial feeding system with plastic tips, as previously described by Ribeiro et al. (2014).

A total of 80 partially engorged female ticks weighing 40–71 mg were divided into 3 homogeneous weight groups. Group 1 (G1) ticks ($n = 20$) were fed with blood only. Group 2 (G2) ticks ($n = 20$) were fed with blood containing $\approx 2 \times 10^6$ uninfected Vero cells/ml. Group 3 (G3) ticks ($n = 40$) were fed with blood containing $\approx 2 \times 10^6$ *R. parkeri*-infected Vero cells/ml. All groups were fed for 12 h in an incubator with a controlled temperature between 30 °C and 32 °C (86 °F – 89.6 °F) and a relative humidity of 80–90%. Work on the three groups was carried out at different times to avoid contamination by aerosols.

At the end of the artificial feeding periods, the females were

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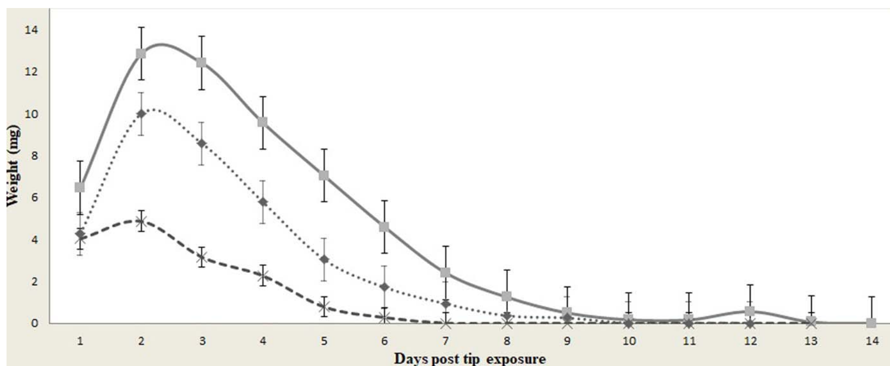

Fig. 1. Daily rate of oviposition obtained by partially engorged *Rhipicephalus microplus* females fed artificially: (■—■) with only bovine blood; (♦…♦) with bovine blood add uninfected VERO cells; and (×—×) with bovine blood add VERO cells infected with *Rickettsia parkeri*.

weighed to check their blood intake and later placed in Petri dishes to perform oviposition. Biological parameters were then analysed, including the pre-laying and laying periods, the egg mass and nutritional efficiency, and the reproductive efficiency indexes, calculated according to Bennett (1974) and Meléndez et al. (1998). The non-gaining ticks and the weight gainers with artificial feeding were analysed separately in the three study groups. During the non-parasitic phase, the ticks were kept in an incubator at 27 ± 1 °C (80.6 °F) and a relative humidity index of 80–90%. After hatching, larvae from half of the G2 and G3 pools were preserved in RNA Later® at −20 °C (−4 °F) until DNA was extracted for evaluating transovarial transmission; the other half of the larvae were used for guinea pig infestation. Larval pools of approximately 100 mg of eggs (combined oviposition of five *R. microplus* females) were applied to each guinea pig (adult males approximately 90 days old). Four male guinea pigs received G3 larvae, and one guinea pig received G2 larvae. Tick infestation on guinea pigs was performed inside white cotton sleeves (10–15 cm diameter), as previously described by Labruna et al. (2011b). Rectal temperature was measured daily from day 0 to day 20 post-infestation (DPI). To detect the presence of anti-*R. parkeri* antibodies, blood samples were collected (45th DPI) and tested by the indirect immunofluorescence assay (IFA), according to Horta et al. (2007), and adapted using a fluorescent-linked anti-Guinea pig antibody (KPL®).

The experiment was conducted according to the protocols of ethical principles adopted by the *Conselho Nacional de Controle de Experimentação Animal* and approved by the internal ethical committee of the institution (under report number 062/2014).

DNA was extracted from the ticks according to McIntosh et al. (2015). For every 20 individual samples, a blank tube was included in the DNA extraction to ensure that there was no contamination in this step.

DNA samples were individually processed via PCR assay using the primers CS-78/CS-323, which amplify the rickettsial *glt*A gene, and the primers *Rr*190.70p/Rr190.602n, which amplify the rickettsial *Omp*A gene (Labruna et al., 2004). PCR mixtures and thermal conditions were used as previously described by McIntosh et al. (2015). One positive control (DNA from the *R. parkeri* strain NOD) and two negative controls (water) were included for each reaction. A PCR product from one larval tick pool from a G3 infected female underwent DNA sequencing, and the resultant sequence was compared to GenBank data by Blast analysis.

For the statistical analysis of the biological aspects of the non-parasitic phase, we used the Tukey test and the Student–Newman–Keuls (SNK) test with a significance level of 5% to compare means.

3. Results

Weight gain was observed in 60% (12/20) of the females in G1 (Negative Blood), 58% (11/19) in G2 (Blood + VERO negative) and 55% (22/40) in G3 (blood + VERO positive).

The detection of genes specific for *Rickettsia* spp. (*glt*A and *omp*A) confirmed that all females artificially fed with blood + *R. parkeri* remained infected until the end of the oviposition (up to approximately 20 days). The infected females presented a darkening of the exoskeleton from the second post-feeding day.

The oviposition rhythm (Fig. 1) revealed that the presence of VERO cells negatively influenced blood ingestion and thus directly affected the amount of daily oviposition. In contrast, *R. parkeri* infection caused a drop in the number of eggs from the first day of laying (Fig. 1). The viability of the eggs was negatively affected, with the hatching rate being approximately 80% in the infected group but above 90%, on average, in the control group.

Upon evaluating the biological parameters, it was evident that *R. parkeri* infection caused a damaging effect in the non-parasitic phase of *R. microplus* females (Table 1). Analysis of non-gaining ticks and weight gainers allowed us to evaluate whether there was any damage to ticks caused by *R. parkeri*, excluding the influence of weight gain.

A total of 11 of the 20 pools of larvae tested were positive for the *glt*A and *omp*A genes. None of the 10 pools of larvae from the G1 and G2 groups showed positivity for the genes tested. DNA sequences were successfully obtained from G3-derived larval ticks, resulting in sequences that were 100% identical to the corresponding sequence of *R. parkeri* strain At24 in GenBank (EF102236).

Regarding the infestation of guinea pigs by *R. parkeri*-infected *R. microplus* larvae, no guinea pigs presented symptoms characteristic of rickettsiosis caused by *R. parkeri*, such as fever, scabs or scrotal reaction (edema and scrotal necrosis), and none of the five animals (G2 and G3) were reactive to IFAT.

4. Discussion

This is the first experimental study that evaluates the biological aspects involving the hard tick *R. microplus* and a species of pathogenic rickettsia (*R. parkeri*). This was done using an artificial feeding technique. The experimental infection of ticks by pathogenic rickettsiae is usually performed using a mammalian host susceptible to amplification of the pathogen (Sakai et al., 2014). The scarcity of data from mammals amplifying *R. parkeri* makes it difficult to carry out infections of this rickettsia in ticks for studies on their interaction with mammalian hosts. In addition, this species of monoxeno tick is extremely specific in choosing a host, making it even more difficult to infect hosts that are not of its predilection.

The *Rhipicephalus microplus* tick has been found infected with a rickettsiae species of the Spotted Fever Group (Bermúdez et al., 2009; Moura-Martiniano et al., 2014; Pesquera et al., 2015). To date, the involvement of this tick in the epidemiology of rickettsiae has not been well elucidated, but it is possible that the acquisition of pathogenic rickettsia by *R. microplus* may occur through infection mechanisms such as co-feeding with an infected tick and by feeding on a rickettsiemic host (Socolovschi et al., 2009).

The *R. parkeri* strain used in the present study was isolated from *A. triste* collected from a flooded area of the Paulicéia-São Paulo

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