Journal of Medical Entomology, 53(3), 2016, 660–665
doi: 10.1093/jme/tjv250
Advance Access Publication Date: 21 January 2016
Research article

OXFORD

Dynamics of Exposure to *Rickettsia parkeri* in Cattle in the Paraná River Delta, Argentina

L. D. Monje,[1,2,] F. B. Costa,[3] V. C. Colombo,[1] M. B. Labruna,[3] L. R. Antoniazzi,[1] I. Gamietea,[4] S. Nava,[5] and P. M. Beldomenico[1]

[1]Laboratorio de Ecología de Enfermedades, Instituto de Ciencias Veterinarias del Litoral (ICiVet-Litoral), Universidad Nacional del Litoral (UNL)/Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Esperanza, Santa Fe, S3080, Argentina (lmonje@fcv.unl.edu.ar; valecc1983@hotmail.com; leandro_raul_antoniazzi@hotmail.com; pbeldome@fcv.unl.edu.ar), [2]Corresponding author, e-mail: lmonje@fcv.unl.edu.ar, [3]Departamento de Medicina Veterinária Preventiva e Saúde Animal, Faculdade de Medicina Veterinária e Zootecnia, São Paulo, 05508-270, Brazil (franc.borges@yahoo.com.br; labruna@usp.br), [4]Instituto Nacional de Tecnología Agropecuaria, Estación Experimental San Pedro, B2930, Argentina (igamietea@yahoo.com), and [5]Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Rafaela, S2300, Argentina (nava.santiago@inta.gob.ar)

Received 1 September 2015; Accepted 21 December 2015

Abstract

Several cases of human rickettsiosis caused by *Rickettsia parkeri* were recently documented in the Paraná River delta of Argentina, where the tick vector is *Amblyomma triste* Koch. As cattle suffer recurrent *A. triste* infestations, they are at risk of becoming infected with *R. parkeri*. Herein we investigated the dynamics of *R. parkeri* and its *A. triste* vector in a herd of beef cattle. Cattle were followed for 18 mo and samples were analyzed for the presence of antibodies against four *Rickettsia* species (*R. parkeri*, *Rickettsia bellii*, *Rickettsia amblyommii*, and *Rickettsia felis*) and also for the presence of rickettsial DNA. Additionally, cattle were examined for attached ticks and questing adult ticks were collected. All ticks were analyzed for the presence of rickettsial DNA. No evidence of rickettsemia was found in any cow, but the high *R. parkeri* infection rate documented in *A. triste* both questing in the study area (13.9%) and feeding on cattle (19.8%) and the identification of antibodies against *R. parkeri* antigen in 90% of cattle are evidence that infection is taking place. Altogether, our data suggest that *A. triste* ticks are capable of naturally exposing cattle to *R. parkeri*. However, the progress of *R. parkeri* infection and its impact on bovine health and production remain to be established.

Key words: Rickettsia parkeri, Amblyomma triste, cattle, Argentina

In South America, clinical cases of human rickettsioses caused by tick-borne rickettsiae have been reported in Argentina, Brazil, Colombia, and Uruguay (reviewed in Parola et al. 2013). In Argentina, seven species of *Rickettsia* were found infecting ticks, namely, *Rickettsia rickettsii*, *Rickettsia parkeri*, *Rickettsia massiliae*, *Rickettsia* sp. strain Atlantic Rainforest, 'Candidatus *Rickettsia amblyommii*', 'Candidatus *Rickettsia andeanae*', all belonging to the spotted fever group rickettsiae (SFGR), and the ubiquitous endosymbiont *Rickettsia bellii* (Labruna et al. 2007a, b; Nava et al. 2008; Paddock et al. 2008; Tomassone et al. 2010; Cicuttin et al. 2014; Monje et al. 2015). Of these, *R. rickettsii*, *R. parkeri*, *R. massiliae*, and *Rickettsia* sp. strain Atlantic Rainforest have been associated with human diseases (Paddock et al. 2008, Garcia-Garcia et al. 2010, Spolidorio et al. 2010, Romer et al. 2014), and in Argentina these pathogens were found infecting the ticks *Amblyomma cajennense* sensu lato, *Amblyomma triste* Koch and *Amblyomma tigrinum* Koch, *Rhipicephalus sanguineus* sensu lato, and *Amblyomma dubitatum* (Paddock et al. 2008, Nava et al. 2008, Cicuttin et al. 2014, Romer et al. 2014, Monje et al. 2015), respectively.

The alpha-proteobacterium *R. parkeri* causes a disease similar to, but milder than, that caused by *R. rickettsii* and was first reported as pathogenic in the United States in 2004 (Paddock et al. 2004). In Argentina, cases of human rickettsiosis caused by *R. parkeri* were documented in the provinces of Buenos Aires, Entre Rios, and Chaco (Romer et al. 2011), all in the vicinity of Parana River where the alleged vector is *A. triste*, and also in the inland provinces of Cordoba and La Rioja where the suspected vector is *A. tigrinum* (Romer et al. 2014). Moreover, human disease allegedly caused by *R. parkeri* has been reported in Uruguay since 1990 but just recently a case of *R. parkeri* infection in a human was confirmed (Portillo et al. 2013).

Adults of *A. triste* can use cattle as hosts (Nava et al. 2011), and it has been reported that cattle are susceptible to infection by several members of SFGR, such as *Rickettsia slovaca* (Ortuño et al. 2012), *R. rickettsii* (Gazeta et al. 2009), *Rickettsia conorii* (Kelly et al. 1991), *Rickettsia sibirica* (Liu et al. 1995), and *Rickettsia africae* (Parola et al. 1999). A recent study in domestic animals from China showed molecular evidence of infection with SFGR in dogs, goats,

and cattle, with the one in cattle having the highest bacterial loads (Liang et al. 2012). Additionally, it has been demonstrated that *R. parkeri*-infected *Amblyomma maculatum* ticks are capable of transmitting this SFGR to cattle, although no evidence of persistent systemic infection and no clinical signs associated with the infection were reported (Edwards et al. 2011).

The Paraná River delta constitutes a vast human–domestic–wildlife interface where the risk of pathogen transmission across species is substantial. In this region, farming beef cattle is on the rise largely because beef cattle breeding in the Pampas region have been replaced by agricultural crops, and thus cows have been gradually displaced to marginal fields. Recent studies conducted in the Paraná River delta region reported a prevalence of *R. parkeri* in *A. triste* ranging from 8 to 20% (Nava et al. 2008, Monje et al. 2014). As cattle are infested by *A. triste* (Nava et al. 2011), they are at risk of becoming infected with *R. parkeri* and could act as amplifier hosts of the infection. To contribute to our knowledge on the ecology of this pathogen in the region, we investigated the dynamics of *R. parkeri* and its *A. triste* vector in a herd of beef cattle that was followed for 18 mo.

Materials and Methods

Study Area

The study was conducted in fields of an Experimental Station belonging to Instituto Nacional de Tecnología Agropecuaria (INTA Delta), Campana (34° 9.5′ S, 58° 51.8′ W) in Buenos Aires Province, Argentina. This site corresponds to the lower Paraná River delta region. The region is affected by human activities, mainly by the development of Salicaceae plantations, recreation activities, and silvopastoral systems. The climate of the region is temperate, characterized by mean annual temperature of 16.7°C (minimum 6°C, maximum 30°C) and a mean annual rainfall of ∼1,000 mm without seasonality.

Sample Collection

A herd of beef cattle consisting of 21 Aberdeen Angus cows grazing in a mixed system consisting of natural pasture and Salicaceae plantations in INTA Delta were repeatedly bled from the tail vein every 5 wk from December 2010 to May 2012. The grazing area was managed avoiding the use of agrochemicals and no tick control program was applied before nor during the study period. In total, 16 sampling sessions were carried out and all the 21 cows were sampled every time with the exception of September 2011 when cattle were not available due to internal management of the INTA Experimental Station. All cattle studied was born and raised in the study area, with an age range of 24–30 mo by the time of the first bleeding. Blood samples were transported refrigerated to a field laboratory where they were centrifuged (1,500 × *g* for 10 min) and both, serum and blood clots were aliquoted into labeled microtubes, transported in liquid nitrogen, and stored at –20°C until further use. As observed for *Amblyomma maculatum* adult ticks (Edwards et al. 2011), adults of *A. triste* prefer feeding on the ears of cattle. Since only the left side of immobilized animals was easily available to the investigator in the cattle squeeze, the left ear of each cow was thoroughly examined in search of ticks in parallel to blood collection. Ticks were counted and classified according to their stage and sex. Male *Amblyomma* ticks can stay on hosts for >12 wk compared to females, which complete their feeding period in <2 wk (Labruna et al. 2002, 2003; Pinter et al. 2002). Therefore, only female ticks (FF) were considered in the analysis to avoid overestimation of the number of ticks per animal. A few specimens per sampling session were removed at random and kept in ethanol 96%. Simultaneously, questing adult ticks were collected from the vegetation by drag-sampling using a 1- by 1.50-m white cloth sheet from eight sampling sites distributed across the range where cattle grazed. This procedure was conducted consistently in the same way by the same investigator in every sampling session. All the ticks collected were brought to the laboratory, where they were identified by using standard taxonomic keys and stored in 96% ethanol.

Serology

The indirect immunofluorescence assay (IFA) used crude antigens derived from *R. parkeri* strain At24, *R. bellii* strain Mogi, *R. amblyommii* strain Ac37, and *Rickettsia felis* strain Pedreira. While *R. parkeri* is known to occur in the study site (Nava et al. 2008, Monje et al. 2014), the other three *Rickettsia* species are known to occur elsewhere in Argentina. For antigen preparation, each *Rickettsia* was cultivated in Vero cells (except for *R. felis*, which was cultivated in C6/36 cells) and slides were prepared as previously described (Labruna et al. 2007a, b). Bovine sera were serially diluted in twofold increments with PBS starting from 1:64, as indicated by international reference laboratories for the diagnosis of rickettsiosis (Brouqui et al. 2004). Slides were incubated with fluorescein isothiocyanate-labeled rabbit anti-bovine IgG (Sigma, USA, cat. F7887) diluted 1:2000. Reactive sera were titrated at twofold dilutions in order to determine endpoint titers. In each slide, a bovine serum previously shown to be nonreactive (negative control) and a known reactive bovine serum (positive control) were tested at the 1:64 dilution.

DNA Extraction and Real-Time PCR

The presence of rickettsial DNA was assessed in all cows showing an event of seroconversion, and in questing and feeding ticks. For this purpose, both, the sample that seroconverted and the sample previously obtained from the same cow (seronegative) were analyzed. Total genomic DNA from each cow sample was obtained using 200 µl of blood clot. The blood clot was mixed with 400 µl of lysis buffer (10 mM Tris-Cl, 100 mM EDTA, 0.5% SDS, pH 8.0) and proteinase K was added to a final concentration of 200 µg/ml. Samples were then incubated in 50°C water bath overnight. After incubation, proteinase K was inactivated by boiling samples for 5 min. The following DNA extraction steps were performed according to standard phenol/chloroform methods. The DNA pellet was washed, air-dried, and resuspended in 50 µl of sterile TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 8.0). DNA from ticks was obtained as previously described (Monje et al. 2014). Genomic DNA concentration and purity was assessed using the SPECTROstar Nano and the MARS Data Analysis Software (BMG Labtech, Germany). Each set of DNA extractions included a negative control that used molecular grade water instead of blood or tick.

All DNA samples were then analyzed by SYBR Green quantitative real-time PCR. The integrity of the DNA extracted from blood and ticks was first assessed using a real-time PCR that amplifies bovine 18S rRNA gene and tick 16S rRNA, respectively. In this respect, primers 18sF/18sR and T16S5/T16S3 designed to anneal to highly conserved nucleotide sequences were used as previously described (Eberhardt et al. 2014, Monje et al. 2014). For the detection of rickettsial DNA the gene *glt*A was targeted using primers CS-5/CS-6 which amplify a 147-bp fragment of this gene. These primers have shown sensitivity down to one copy of the *glt*A gene of SFGR (Labruna et al. 2004). Rickettsial DNA was quantified by

Table 1. Count of questing ticks recovered by systematic dragging and mean abundance of females (FF) on the left ear of repeatedly sampled cattle

	Dec.-10	Jan.-11	Feb.-11	Mar.-11	April-11	May-11	July-11	Aug.-11
Questing	27	4	2	0	0	0	0	82
Attached FF	1.14	1.6	0	0	0	0.05	0	1.67
Seroprevalence[a]	0.33	0.57	0.52	0.2	0.14	0.1	0.2	0.67
	Sept.-11	Oct.-11	Nov.-11	Dec.-11	Jan.-12	Mar.-12	April-12	May-12
Questing	30	38	49	46	17	0	0	0
Attached FF	—	0.52	2	0.62	1.33	0.05	0	0.05
Seroprevalence[a]	—	0.38	0.33	0.29	0.38	0.4	0.14	0.14

[a] Number of cows seroreactive to *R. parkeri* antigen/number of tested cows.

comparison with 10-fold serial dilutions of known plasmids standards in independent assays, as previously described (Monje et al. 2014).

Real-time PCRs were performed in an Applied Biosystems (CA, USA) Step One thermocycler with 20 µl per reaction, which contained 4 µl of 5× Phire reaction buffer, 200 µM dNTP, 0.4 pM of each primer, 2 µl of 10× SYBR Green I (Invitrogen, USA), 150 ng of total DNA, and 0.4 µl of Phire Hot Start II DNA polymerase (Thermo Scientific, USA). For *gltA* real-time PCR each run consisted of 3 min at 98°C for initial denaturation, 40 cycles of 5 s at 98°C and 20 s at 53°C followed by extension 20 s at 60°C and included a positive control (100 ng of DNA of *R. parkeri* strain NOD-infected Vero cells). For bovine 18S rRNA gene real-time PCR, each run consisted of 3 min at 98°C for initial denaturation, 40 cycles of 5 s at 98°C and 20 s at 58°C followed by extension 20 s at 72°C. Tick 16S rRNA real-time PCR was made as previously described (Monje et al. 2014). All PCR runs included a negative control with the blood- or tick-free DNA extraction respectively. Product purity was confirmed by dissociation curves, and random samples were subjected to agarose gel electrophoresis. Randomly selected ticks positive for *gltA* real-time PCR were subjected to further amplification of *ompA* gene by routine PCR using primers RR190.70 and RR190.701 as previously described (Monje et al. 2014). PCR products were column purified and sequenced directly in both directions using amplifying primers.

Statistical Analysis

Associations between counts of ticks on ears, vegetation, and seroprevalence for a given sampling session were assessed by Spearman's Rho Correlation tests. To test whether prevalence was associated with tick count at the individual level, we used a Generalized Linear Mixed Model (GLMM) with a binary response (seropositivity) and tick count as the independent variable, using "cow ID" and "trapping session" as random effects. All analyses were conducted using the statistical package R (www.r-project.org).

Results

Tick Counts

Along the entire study, *A. triste* was the only tick species found both on the environment and feeding on cattle. Coincidently with previous reports (Nava et al. 2008, 2011) no immature stages of *A. triste* were collected with drags or feeding on cattle. Adults of *A. triste* in the environment were found only from August to February (116 males, 179 females), with a peak in August (Table 1, Fig. 1). *A. triste* adult ticks were found in the ears of cattle (391 males, 177 females). As expected, the count of female ticks (FF) on the left ear largely reflected the seasonal pattern observed for questing ticks (Table 1, Fig. 1). Both counts were highly correlated (Spearman's Rho correlation coefficient = 0.813, $P = 0.0002$).

IFA of Bovine Samples

Of the 313 serum samples analyzed by immunofluorescence, all were negative for the presence of antibodies against *R. amblyommii* and *R. felis*, meanwhile 99 samples tested positive for *R. parkeri*, two of which were also positive for *R. bellii*. Both samples presenting antibodies against *R. bellii* were from the same cow (12-03-10, 05-28-11) and the endpoint titers of these two samples were of 1:64 for *R. bellii* and 1:128 for *R. parkeri*. Eighteen sera, from eight different animals, showed titers to *R. parkeri* at least fourfold higher than any of the other three antigens tested, with endpoint titers ranging from 1:128 to 1:512. These sera were considered to be homologous to *R. parkeri* or a very closely related serotype.

The temporal pattern of seroprevalence for *R. parkeri* matched that of tick exposure (Fig. 1), showing a peak in August. Seroprevalence was positively correlated with total count of questing ticks (Spearman's Rho coefficient = 0.612; $P = 0.015$) and mean count of FF on the left ear (Spearman's Rho coefficient = 0.546; $P = 0.0353$). At the individual level, the GLMM showed that every female tick attached on the left ear increased the odds of seropositivity in 37.7% (Odds Ratio = 1.377, Table 2). During the entire study, two of twenty-one bovids were never seroreactive to the *R. parkeri* antigen. On the other hand, one cow presented antibodies to *R. parkeri* in all the samples taken during the study and other three bovids in most of the samples analyzed (12 samples out of 15). The rest of the herd presented at least one episode of seroconversion, which in most cases was only transient, but in three individuals seropositivity was maintained from three to five consecutive months.

Real-Time PCR of Bovine Blood Clots

No rickettsial DNA was detected by *gltA* real-time PCR in the blood of cattle showing events of seroconversion, neither in the blood clot corresponding to the serum sample that seroconverted nor in the blood clot obtained from the same animal in the previous sampling session (seronegative at 1:64 dilution). All bovid blood DNA samples were positive for 18S rRNA gene real-time PCR (data not shown), indicating that no PCR inhibitors were present.

Real-Time PCR of Ticks

A total of 295 adults of *A. triste* were collected from the vegetation where cattle grazed and 86 ticks were collected feeding on cattle along the entire study. Forty one ticks from vegetation (13.9%) were found to contain DNA of the rickettsial gene *gltA* by real-time PCR, 34 of which presented high rickettsial concentration (from 1.9×10^7 to 7.9×10^5 *gltA* copies per tick), while the remaining 7

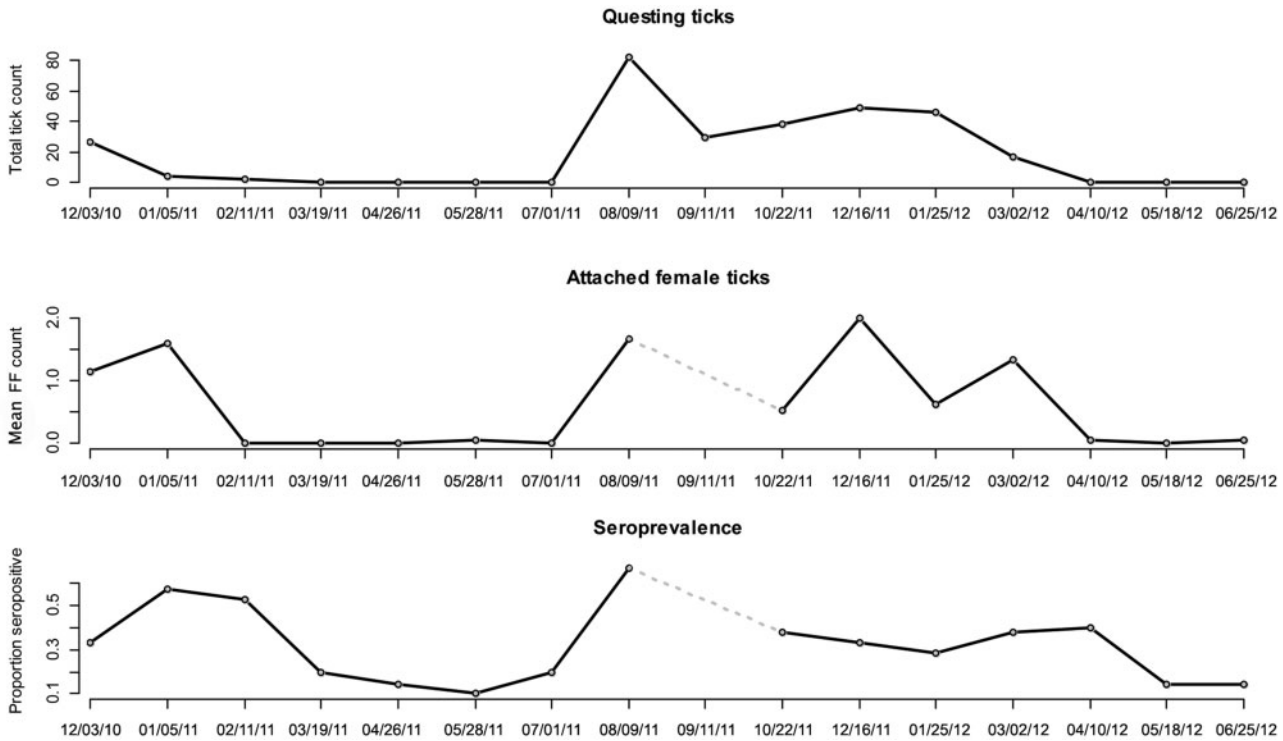

Fig. 1. Temporal relationship between tick exposure and reactivity to *Rickettsia parkeri* antigen.

Table 2. Generalized linear mixed model with a binomial response showing the association between the probability of seropositivity and the count of female ticks on the left ear

Model: Response = Seropositivity
Independent variable = Number of female ticks on the left ear
Random intercepts = trapping session and cow ID

Term	Coefficients	Standard error	*P*-value
Intercept	−1.5667	0.5894	0.0079
Females	0.3197	0.1589	0.0442

presented much lower rickettsial levels (from 2.0×10^3 to 1.1×10^2 *gltA* copies per tick). Regarding ticks feeding on cattle, 17 (19.8%) were found to contain DNA of the rickettsial gene *gltA* by real-time PCR, where 12 presented high rickettsial concentration (from 7.0×10^6 to 3.6×10^5 *gltA* copies per tick) and 5 presented lower rickettsial levels (from 4.0×10^4 to 1.4×10^2 *gltA* copies per tick). A fragment of the *ompA* gene was amplified and sequenced from eight randomly selected *gltA*-positive ticks. The resulting nucleotide sequences were in all cases 100% identical to the *ompA* sequence of *R. parkeri* (KF782320). All samples were positive for 16S rRNA real-time PCR, and no amplification was observed in the negative controls (Ct > 50).

Discussion

In the present study, we followed a small cattle herd from the Paraná River delta to investigate the dynamics of exposure to *R. parkeri* in these animals. Although no evidence of rickettsemia was detected by real-time PCR, antibodies against *R. parkeri* were identified in 90% of cattle (19 out of 21). This coupled with the common occurrence of *A. triste* parasitizing this herd, and the high *R. parkeri*-infection rate documented in *A. triste* both questing in the study area (13.9%) and feeding on cattle (19.8%), are evidence that infection is taking place. However, with the apparent high rate of exposure to infected ticks, it may be expected that all animals should be permanently seropositive. Instead, 15 animals (71%) were seropositive during 5 mo or less. We hypothesize that the pattern observed might be due to two reasons. Firstly, at least for nonnaïve cattle, not all exposures to *R. parkeri*-infected ticks would result in infections of a sufficient magnitude to elicit a detectable humoral immune response. At the same time, the intermittent periods of seropositivity observed and the close match between tick exposure and seroprevalence strongly suggest that the humoral immune response against *R. parkeri* is relatively short lived, and that re-infection is occurring each year. In this regard, a recent study reported that dogs experimentally infested with *R. conorii*-infected *Rh. sanguineus* ticks produced an antibody response that reached its maximum 2–3 wk post infestation that quickly declined below diagnostic level within the next 3 wk (Levin et al. 2014). Moreover, re-infestation of these SFGR-exposed dogs with *R. conorii*-infected *Rh. sanguineus* ticks 40 wk after the first SFGR-challenge elicited a new humoral immune response (Levin et al. 2014).

Other two traits that are relevant to the patterns observed are the level of antibody production and the distribution of infection intensity in ticks. The highest antibody titer to *R. parkeri* reported herein was 1:512, which is in agreement with previous reports of cattle developing relatively low antibody titers to SFGR throughout the world (Kelly et al. 1991, Edwards et al. 2011, Ortuño et al. 2012). Moreover, the infection intensity by *R. parkeri* in *A. triste* ticks presents a bimodal distribution (Monje et al. 2014, this study) and the rate of seroconversion to SFGR in cattle was found to be related to the infective dose (Kelly et al. 1991). Therefore, not only the numbers of infected ticks feeding on the cattle but also the rickettsial infection intensity of these ticks are determinant for seropositivity to *R. parkeri*.

Recently, Nieri-Bastos et al. (2013) reported that *A. triste* can act as a natural reservoir for *R. parkeri*. They demonstrated that *R. parkeri* is preserved by transstadial maintenance and transovarial transmission in *A. triste* ticks. However, marked deleterious effects were caused by *R. parkeri* on engorged nymphs (Nieri-Bastos et al. 2013), for which amplification by vertebrate hosts or other transmission mechanism would be necessary for *R. parkeri* to persist in the long term. Our data suggest that *A. triste* ticks are capable of naturally exposing cattle to *R. parkeri*. However, we failed to detect the bacterium in the blood, for which either the length of the rickettsemic period is short or the rickettsemic levels in cattle blood are not high enough to allow real-time PCR detection, suggesting that *R. parkeri* replication in cattle might not be efficient. In addition, repeated exposures to infected ticks might boost immunity of cattle against *R. parkeri*, rapidly clearing bacterium from blood and limiting their role as amplifier host. In this scenario, naïve cattle (e.g., calves, translocated cattle) could act as amplifier hosts following a primary infection; however, further research in this respect is warranted. Our observations are in agreement with previous studies reporting short periods of rickettsemia in experimentally *R. parkeri*-infected animals (Edwards et al. 2011, Moraru et al. 2013). Notwithstanding, a feasible role for cattle in the ecology of *R. parkeri* could be providing a bloodmeal to a large number of *A. triste* adult ticks. This could increase tick population. In fact, in the study area, rodents captured in sites with cattle had higher burdens of immature stages of *A. triste* than those captured where cattle was absent (Colombo et al. 2015). In addition, cattle might also facilitate tick infections via cofeeding as demostrated for other SFG rickettsiae (Matsumoto et al. 2005, Levin et al. 2014).

Finally, none of the animals that presented antibodies to *R. parkeri* developed any apparent clinical signs of systemic illness in the duration of our study. However, further research is warranted to find out whether these recurrent periods of infection have any effect over the health and the productive performance of the animals.

Acknowledgments

We would like to thank all personnel of INTA Delta and particularly to Ariel Mauro Fernandez, Cristian Ortiz, and Lucio "Morcilla" Ortiz for fieldwork assistance, Natalia Fracassi and Gerardo Mujica for logistic support, and Jonas Moraes-Filho and Fernanda A. Nieri-Bastos (University of São Paulo) for production of rickettsial antigens for the present study. This study was supported by Agencia Nacional de Promoción Científica y Tecnológica (PICT-2008-0090), Universidad Nacional del Litoral (CAI+D 501-2011-0100498LI), and International Cooperation Project MINCyT-CAPES (BR/11/08). All procedures involving animals were approved by the Ethics and Biosafety Committee of the Facultad de Ciencias Veterinarias - Universidad Nacional del Litoral.

References Cited

Brouqui, P., F. Bacellar, G. Baranton, R. J. Birtles, A. Bjoersdorff, J. R. Blanco, G. Caruso, M. Cinco, P. E. Fournier, E. Francavilla, et al. 2004. Guidelines for the diagnosis of tick-borne bacterial diseases in Europe. Clin. Microbiol. Infect. 10: 1108–1132.

Cicuttin, G. L., D. F. Brambati, J. I. Rodriguez Eugui, C. G. Lebrero, M. N. De Salvo, F. J. Beltran, F. E. Gury Dohmen, I. Jado, and P. Anda. 2014. Molecular characterization of *Rickettsia massiliae* and *Anaplasma platys* infecting *Rhipicephalus sanguineus* ticks and domestic dogs, Buenos Aires (Argentina). Ticks Tick Borne Dis. 5: 484–488.

Colombo, V. C., S. Nava, L. R. Antoniazzi, L. D. Monje, A. L. Racca, A. A. Guglielmone, and P. M. Beldomenico. 2015. Factors affecting patterns of *Amblyomma triste* (Acari: Ixodidae) parasitism in a rodent host. Vet. Parasitol. 211: 251–258.

Eberhardt, A. T., L. D. Monje, D. A. Zurvera, and P. M. Beldomenico. 2014. Detection of *Trypanosoma evansi* infection in wild capybaras from Argentina using smear microscopy and real-time PCR assays. Vet. Parasitol. 202: 226–233.

Edwards, K. T., J. Goddard, T. L. Jones, C. D. Paddock, and A. S. Varela-Stokes. 2011. Cattle and the natural history of *Rickettsia parkeri* in Mississippi. Vector Borne Zoonotic Dis. 11: 485–491.

Garcia-Garcia, J. C., A. Portillo, M. J. Nunez, S. Santibanez, B. Castro, and J. A. Oteo. 2010. A patient from Argentina infected with *Rickettsia massiliae*. Am. J. Trop. Med. Hyg. 82: 691–692.

Gazeta, G. S., E. R. Souza, A. E. Abboud-Dutra, M. Amorim, P. R. Barbosa, A. B. Almeida, V. Gomes, F. S. Gehrke, M. T. Marrelli, and T. T. Schumaker. 2009. Potential vectors and hosts of rickettsia spp: Epidemiological studies in the Vale do Paraiba, state of Rio de Janeiro/Brazil. Clin. Microbiol. Infect. 15: 269–270.

Kelly, P. J., P. R. Mason, T. Manning, and S. Slater. 1991. Role of cattle in the epidemiology of tick-bite fever in Zimbabwe. J. Clin. Microbiol. 29: 256–259.

Labruna, M. B., N. Kasai, F. Ferreira, J. L. Faccini, and S. M. Gennari. 2002. Seasonal dynamics of ticks (Acari: Ixodidae) on horses in the state of Sao Paulo, Brazil. Vet. Parasitol. 105: 65–77.

Labruna, M. B., E. Y. Fugisaki, A. Pinter, J. M. Duarte, and M. J. Szabo. 2003. Life cycle and host specificity of *Amblyomma triste* (Acari: Ixodidae) under laboratory conditions. Exp. Appl. Acarol. 30: 305–316.

Labruna, M. B., M. C. Horta, D. M. Aguiar, G. T. Cavalcante, A. Pinter, S. M. Gennari, and L. M. Camargo. 2007a. Prevalence of *Rickettsia* infection in dogs from the urban and rural areas of Monte Negro municipality, western Amazon, Brazil. Vector Borne Zoonotic Dis. 7: 249–255.

Labruna, M. B., R. C. Pacheco, S. Nava, P. E. Brandao, L. J. Richtzenhain, and A. A. Guglielmone. 2007b. Infection by *Rickettsia bellii* and *Candidatus* "Rickettsia amblyommii" in *Amblyomma neumanni* ticks from Argentina. Microb. Ecol. 54: 126–133.

Labruna, M. B., T. Whitworth, M. C. Horta, D. H. Bouyer, J. W. McBride, A. Pinter, V. Popov, S. M. Gennari, and D. H. Walker. 2004. *Rickettsia* species infecting *Amblyomma cooperi* ticks from an area in the state of Sao Paulo, Brazil, where Brazilian spotted fever is endemic. J. Clin. Microbiol. 42: 90–98.

Levin, M. L., G. E. Zemtsova, M. Montgomery, and L. F. Killmaster. 2014. Effects of homologous and heterologous immunization on the reservoir competence of domestic dogs for *Rickettsia conorii* (israelensis). Ticks Tick Borne Dis. 5: 33–40.

Liang, C. W., J. B. Zhao, J. Li, L. T. Chang, H. L. Yu, L. X. Zhang, L. J. Zhang, and X. J. Yu. 2012. Spotted fever group *Rickettsia* in Yunnan Province, China. Vector Borne Zoonotic Dis. 12: 281–286.

Liu, Q. H., G. Y. Chen, Y. Jin, M. Te, L. C. Niu, S. P. Dong, and D. H. Walker. 1995. Evidence for a high prevalence of spotted fever group rickettsial infections in diverse ecologic zones of Inner Mongolia. Epidemiol. Infect. 115: 177–183.

Matsumoto, K., M. Ogawa, P. Brouqui, D. Raoult, and P. Parola. 2005. Transmission of *Rickettsia massiliae* in the tick, Rhipicephalus turanicus. Med. Vet. Entomol. 19: 263–270.

Monje, L. D., S. Nava, L. R. Antoniazzi, V. C. Colombo, and P. M. Beldomenico. 2014. In vitro isolation and infection intensity of *Rickettsia parkeri* in *Amblyomma triste* ticks from the Parana River Delta region, Argentina. Ticks Tick Borne Dis. 5: 924–927.

Monje, L. D., S. Nava, A. T. Eberhardt, A. I. Correa, A. A. Guglielmone, and P. M. Beldomenico. 2015. Molecular detection of the human pathogenic *Rickettsia* sp. strain Atlantic rainforest in *Amblyomma dubitatum* ticks from Argentina. Vector Borne Zoonotic Dis. 15: 167–169.

Moraru, G. M., J. Goddard, C. D. Paddock, and A. Varela-Stokes. 2013. Experimental infection of cotton rats and bobwhite quail with *Rickettsia parkeri*. Parasit. Vectors 6: 70.

Nava, S., Y. Elshenawy, M. E. Eremeeva, J. W. Sumner, M. Mastropaolo, and C. D. Paddock. 2008. *Rickettsia parkeri* in Argentina. Emerg. Infect. Dis. 14: 1894–1897.

Nava, S., A. J. Mangold, M. Mastropaolo, J. M. Venzal, N. Fracassi, and A. A. Guglielmone. 2011. Seasonal dynamics and hosts of *Amblyomma triste* (Acari: Ixodidae) in Argentina. Vet. Parasitol. 181: 301–308.

Nieri-Bastos, F. A., M. P. Szabo, R. C. Pacheco, J. F. Soares, H. S. Soares, J. Moraes-Filho, R. A. Dias, and M. B. Labruna. 2013. Comparative evaluation of infected and noninfected *Amblyomma triste* ticks with *Rickettsia parkeri*, the agent of an emerging rickettsiosis in the New World. Biomed. Res. Int. 2013: 402737.

Ortuño, A., I. Pons, M. Quesada, S. Lario, E. Anton, A. Gil, J. Castella, and F. Segura. 2012. Evaluation of the presence of *Rickettsia slovaca* infection in domestic ruminants in Catalonia, Northeastern Spain. Vector Borne Zoonotic Dis. 12: 1019–1022.

Paddock, C. D., S. Fernandez, G. A. Echenique, J. W. Sumner, W. K. Reeves, S. R. Zaki, and C. E. Remondegui. 2008. Rocky Mountain spotted fever in Argentina. Am. J. Trop. Med. Hyg. 78: 687–692.

Paddock, C. D., J. W. Sumner, J. A. Comer, S. R. Zaki, C. S. Goldsmith, J. Goddard, S. L. McLellan, C. L. Tamminga, and C. A. Ohl. 2004. *Rickettsia parkeri*: A newly recognized cause of spotted fever rickettsiosis in the United States. Clin. Infect. Dis. 38: 805–811.

Parola, P., C. D. Paddock, C. Socolovschi, M. B. Labruna, O. Mediannikov, T. Kernif, M. Y. Abdad, J. Stenos, I. Bitam, P. E. Fournier, and D. Raoult. 2013. Update on tick-borne rickettsioses around the world: A geographic approach. Clin. Microbiol. Rev. 26: 657–702.

Parola, P., G. Vestris, D. Martinez, B. Brochier, V. Roux, and D. Raoult. 1999. Tick-borne rickettiosis in Guadeloupe, the French West Indies: Isolation of *Rickettsia africae* from *Amblyomma variegatum* ticks and serosurvey in humans, cattle, and goats. Am. J. Trop. Med. Hyg. 60: 888–893.

Pinter, A., M. B. Labruna, and J. L. Faccini. 2002. The sex ratio of *Amblyomma cajennense* (Acari: Ixodidae) with notes on the male feeding period in the laboratory. Vet. Parasitol. 105: 79–88.

Portillo, A., C. Garcia-Garcia, M. M. Sanz, S. Santibanez, J. M. Venzal, and J. A. Oteo. 2013. A Confirmed Case of *Rickettsia parkeri* Infection in a Traveler from Uruguay. Am. J. Trop. Med. Hyg. 89: 1203–1205.

Romer, Y., S. Nava, F. Govedic, G. Cicuttin, A. M. Denison, J. Singleton, A. J. Kelly, C. Y. Kato, and C. D. Paddock. 2014. *Rickettsia parkeri* rickettsiosis in different ecological regions of Argentina and its association with *Amblyomma tigrinum* as a potential vector. Am. J. Trop. Med. Hyg. 91: 1156–1160.

Romer, Y., A. C. Seijo, F. Crudo, W. L. Nicholson, A. Varela-Stokes, R. R. Lash, and C. D. Paddock. 2011. *Rickettsia parkeri* Rickettsiosis, Argentina. Emerg. Infect. Dis. 17: 1169–1173.

Spolidorio, M. G., M. B. Labruna, E. Mantovani, P. E. Brandao, L. J. Richtzenhain, and N. H. Yoshinari. 2010. Novel spotted fever group rickettsiosis, Brazil. Emerg. Infect. Dis. 16: 521–523.

Tomassone, L., P. Nunez, L. A. Ceballos, R. E. Gurtler, U. Kitron, and M. Farber. 2010. Detection of "*Candidatus* Rickettsia sp. strain Argentina" and *Rickettsia bellii* in *Amblyomma* ticks (Acari: Ixodidae) from Northern Argentina. Exp. Appl. Acarol. 52: 93–100.