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Original Article

Off-host development and survival of *Rhipicephalus* (*Boophilus*) *microplus* in the Brazilian semiarid

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ABSTRACT

Rhipicephalus (*Boophilus*) *microplus* is the most economically important tick of the world, including Brazil. Whereas the epidemiology of *R. microplus* is well known in most of the Brazilian land, virtually nothing is known from the Brazilian semiarid region, the Caatinga biome. Considering the relevance of *R. microplus* for the cattle industry within the Caatinga, this study aimed to evaluate for the first time the off-host development of this tick species under natural conditions of the Caatinga. During 2011–2015, engorged females of *R. microplus* were exposed to field conditions of native Caatinga, where female oviposition, egg incubation and hatching, and larval survival were quantified. In parallel, counterpart ticks (control group) were evaluated under optimal conditions in the laboratory. During the study, nearly 100% of the engorged females of the control group successfully oviposited fertile egg masses, from which most of the eggs yielded larvae (mean % hatching usually > 90%). Under field conditions, while almost 100% of the engorged females oviposited, in most of the times no larvae hatched from these eggs. Furthermore, when larvae hatched, mean % hatching was most of the times < 50%. Soil temperatures did not oscillate drastically through the study, with mean temperature between 30 and 35 °C in most of the time. In contrast, rainfall was irregular throughout the study. Correlation analyses indicated that either the number of egg masses that yielded larvae, or the mean % hatching of the egg masses were positively associated with higher rainfall months. Our results showed that in most of the time, field conditions were unfavorable for *R. microplus*, indicating that cattle would have a low exposure to *R. microplus* infestations when raised in Caatinga fields like those of the present study.

1. Introduction

The cattle tick, *Rhipicephalus* (*Boophilus*) *microplus*, is the most economically important tick of the world, affecting the cattle industry in tropical and subtropical areas of America, Africa, and southeastern Asia (Estrada-Peña et al., 2012). Only in Brazil, annual economic losses due to *R. microplus* were estimated at US$3.2 billion (Grisi et al., 2014). Besides causing direct losses such as decreased milk and meat production, cattle tick infestations are also responsible for transmitting major cattle fever agents, such as the protozoa *Babesia bigemina* and *Babesia bovis*, and the bacterium *Anaplasma marginale* (Cordovés, 1997; Campos Pereira et al., 2008).

The magnitude of losses due to cattle fever agents is generally dependent on the climatic conditions of each region. For example, babesiosis or anaplasmosis outbreaks followed by high fatality rates are rare in most of the Brazilian country, where environmental conditions provide *R. microplus* infestations on pastured cattle throughout the year. In this case, cattle has the chance to become infected by *B. bigemina*, *B. bovis*, and *A. marginale* during the first weeks or months after birth, and then remain infected without suffering severe disease for the rest of their lives (Ribeiro and Reis, 1981; Madruga et al., 1984, 1985; Patarroyo et al., 1987; Santos et al., 2001; Costa et al., 2013). On the other hand, environmental conditions in some areas are not suitable for *R. microplus* throughout the year, as for example, in southern Brazil, where lower temperatures inhibit the off-host development of *R. microplus* during the autumn-winter months. Consequently, many animals do not come into contact with infected ticks during their first months, and when they first become infected later on, severe disease with high lethality is a common feature (Martins et al., 1994; Marana et al., 2009; Costa et al., 2013).

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The Brazilian semiarid region is located in the northeastern part of the country, where the Caatinga biome prevails, covering 12% of the Brazilian territory. Two formations predominate in the Caatinga: plateaus with altitudes averaging between 650 and 1000 m, and large depressions with altitudes averaging 200–400 m (http://www.invivo.fiocruz.br). Caatinga is characterized by xeric shrub land and thorn forest that consist primarily of small, thorny trees that shed their leaves seasonally. Cacti, thick-stemmed plants, thorny brush and arid-adapted grasses make up the ground layer; however, during the dry periods there is no ground foliage or undergrowth (Costa et al., 2013; Andrade-Lima, 1981). A recent study reported a number of highly lethal outbreaks of cattle tick fever (caused by *A. marginale* or *Babesia* spp.) in farms within the Caatinga biome (Costa et al., 2011). A subsequent study reported that the prevalence of the infection by *A. marginale*, *B. bovis*, or *B. bigemina* is usually low (< 50%), or sometimes zero, among adult cattle that have been raised in the Caatinga biome (Costa et al., 2013). These authors argued that such low prevalence is related to the general low exposure of cattle to *R. microplus* ticks, due to the characteristic weather (long drought period) and vegetation type (Caatinga) of the region; this condition is likely to postpone cattle primary infection by tick fever agents during adulthood, when animals are highly susceptible to develop clinical and severe disease (Costa et al., 2011, 2013).

Despite the unfavorable conditions – semiarid climate, scarcity of artificial pastures - that the Caatinga biome provides for livestock, cattle breeding is an important activity in the region, since nearly 10 million cattle are reared in the area (www.ibge.com.br), and almost the totality of beef and milk consumed by the Caatinga-inhabited population is produced by local, family-employed farmers (unpublished data). Considering the relevance of *R. microplus* for the cattle industry within the Caatinga biome, the present study aimed to evaluate for the first time the off-host development of this tick species under natural conditions of the Caatinga biome.

2. Materials and methods

2.1. Study site

This study was performed in Patos municipality (07°00′25″S, 37°16′41″W, elevation 260 m), state of Paraíba, northeastern Brazil. The area belongs to the Caatinga biome. The weather is characterized by a hot and semiarid climate, with temperatures averaging 27 °C throughout the year, and the mean annual rainfall typically of ≈ 500 mm. There are typically two seasons: a rainy season from February to May, and a long drought period from June to January or occasionally longer than one year (Batista et al., 2007; Costa et al., 2013). For the present study, engorged females of *R. microplus* were exposed to field conditions in three paddocks of the Federal University of Campina Grande at Patos Municipality. All three paddocks, free of domestic animals, were composed by native Caatinga, characterized by low forest composed mostly of small trees and shrubs (mostly Leguminosae, Euphorbiaceae and Cactaceae), with an ephemeral herbaceous layer present only during the rainy season. Paddocks 1 and 2 were close to each other (100 m apart), whereas paddock 3 was ≈ 1.2 Km apart from the formers.

2.2. Ticks

Engorged females of *R. microplus* were collected from naturally infested cattle in a farm at Patos municipality, and used to establish a laboratory colony. Free-living stages of the tick colony (preoviposition, oviposition, egg incubation, and unfed larvae) were held in an incubator at 27 °C and 80% relative humidity. For production of engorged females throughout the study, one or two young calves (1–2 years old) were infested with 10,000 to 15,000 larvae of *R. microplus* at monthly intervals. Infested calves were held in individual stalls (12 m^2), which had an elevated, slatted floor. Then, naturally detached engorged females were conveniently collected by flushing them carefully from beneath the elevated, slatted floor.

Table 1
Reproductive performance of engorged females of *Rhipicephalus microplus* in the incubator (control group).

Date[a]	Engorged female mean weight (g)	No. engorged females that oviposited (%)	No. egg masses that yielded larvae	Mean % hatch of egg masses (range)[b]
15 Sep. 11	0.2169	10 (100)	10	92.5 (85–95)
10 Oct. 11	0.2325	10 (100)	10	92 (85–95)
3 Nov. 11	0.1890	10 (100)	10	92 (85–95)
28 Nov. 11	0.2096	10 (100)	10	85 (30–95)
28 Dec. 11	0.2342	10 (100)	10	89.5 (75–95)
20 Jan. 12	0.2440	10 (100)	10	76 (15–95)
12 Feb. 12	0.2156	10 (100)	10	68 (10–95)
14 Mar. 12	0.2298	10 (100)	10	92 (75–95)
13 Apr. 12	0.1458	10 (100)	8	38.1 (5–70)
15 May 12	0.2417	10 (100)	10	90.5 (80–95)
10 Jul. 12	0.2395	10 (100)	10	92.5 (85–95)
10 Aug. 12	0.2583	10 (100)	10	91.5 (80–95)
2 Sep. 12	0.1879	10 (100)	10	88 (70–95)
3 Oct. 12	0.2419	10 (100)	10	90 (85–95)
31 Oct. 12	0.2346	10 (100)	10	89.5 (75–95)
24 Nov. 12	0.2241	10 (100)	10	91 (80–95)
16 Dec. 12	0.2173	10 (100)	10	90.5 (85–95)
29 Jan. 13	0.2333	10 (100)	10	90 (80–95)
21 Feb. 13	0.2098	10 (100)	10	90.5 (70–95)
15 Mar. 13	0.2187	10 (100)	10	92 (70–95)
8 Apr. 13	0.2540	10 (100)	10	89 (55–95)
4 May 13	0.2108	10 (100)	10	89 (50–95)
4 Jun. 13	0.2434	10 (100)	10	77.2 (45–95)
1 Jul. 13	0.2030	10 (100)	10	43.2 (20–60)
23 Jul. 13	0.2556	10 (100)	9	86.1 (70–95)
19 May 14	0.2033	10 (100)	10	92 (85–95)
23 Jun. 14	0.2442	10 (100)	10	92 (85–95)
18 Jul. 14	0.2771	10 (100)	10	85 (30–95)
16 Aug. 14	0.2630	10 (100)	10	89.5 (75–95)
11 Sep. 14	0.2235	10 (100)	10	76 (15–95)
7 Oct. 14	0.2234	10 (100)	10	68 (10–95)
3 Nov. 14	0.2146	10 (100)	10	92 (75–95)
8 Dec 14	0.2160	10 (100)	10	92.5 (85–95)
7 Jan. 15	0.2122	10 (100)	10	91.5 (85–95)
3 Feb. 15	0.1920	10 (100)	10	88 (70–95)
3 Mar. 15	0.2244	10 (100)	10	90 (85–95)
30 Mar. 15	0.2216	10 (100)	10	89.5 (75–95)
26 Apr. 15	0.2412	10 (100)	10	87.2 (45–95)
21 May 15	0.2656	10 (100)	10	43.5 (20–60)
Total (%)	–	390 (100)	387 (99.2)	–

N.A.: not acquired.
[a] Date when 10 engorged females were released in the paddock.
[b] Only for egg masses that yielded larvae.

2.3. Experimental groups of ticks

Engorged female ticks were monthly exposed to field experimental conditions from September 2011 to July 2013, and then from May 2014 to May 2015. In each month, 40 engorged females collected in the stalls were selected to be exposed to experimental conditions, while the remaining engorged females were used to keep the tick colony. The 40 selected females were individually weighed in an electronic balance, divided into four groups of 10 engorged females, and exposed to experimental conditions the same day. For this purpose, each engorged female was held inside a stainless wire-gauze cylindrical tube (60 mesh/cm^2, 61 mm long, 17 mm diameter), which was closed with a rubber cork. In each month, 3 groups of 10 cylindrical tubes (10 engorged females/group) were each placed in one of the 3 paddocks; tubes were placed horizontally in the soil surface under plant leaves next to plant stems, in order to be totally protected from direct sunlight. A forth group of 10 cylindrical tubes, comprising the control group, was