Ticks and Tick-borne Diseases xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Original article

Comparative biology of the tropical and temperate species of *Rhipicephalus sanguineus* sensu lato (Acari: Ixodidae) under different laboratory conditions

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ARTICLE INFO

Article history:
Received 15 August 2016
Received in revised form 23 October 2016
Accepted 23 October 2016
Available online xxx

Keywords:
Tick
Diapause
Brazil
South America

ABSTRACT

Recent studies have shown that the taxon *Rhipicephalus sanguineus* sensu lato (s.l.) is represented in Latin America by two distinct species, designated as 'tropical species' (distributed from Mexico to Brazil) and 'temperate species' (restricted to the southern cone of South America). Since both tropical and temperate species of *R. sanguineus* s.l. are parasites primarily of domestic dogs, the reasons for their distinct geographical distribution in South America could be related to particular requirements of abiotic conditions for off-host development. With the purpose to test this hypothesis, this study evaluated the off-host developmental stages (eggs, engorged larvae, nymphs and females) of both tick species simultaneously inside incubators with temperature and photoperiod regimens that simulated the summer and winter conditions of tropical Brazil (where the 'tropical species' occurs) and temperate Brazil (where the 'temperate species' occurs). Results showed that the temperate species had significantly higher survival rates than the tropical species, when engorged ticks (larvae, nymphs and females) and eggs were incubated at lower temperatures simulating winter seasons of many parts of the southern cone of South America, where the temperate species is known to occur. These results suggest that the absence of established populations of the tropical species in temperate areas of South America is related to the low overwinter capacity of the tropical species in those areas. Regarding the temperate species, unfed adults that molted from nymphs under summer conditions of either tropical or temperate Brazil remained dormant, at the state of behavioral diapause for at least 20 weeks. Contrastingly, when engorged nymphs of the temperate species were held at winter conditions for at least 3 months, and then transferred to summer conditions to complete molting, no diapause was observed in adult ticks. These results were corroborated by infestation trials, which showed that diapausing adult ticks took more days to attach to rabbits, and did in lesser numbers, when compared to nondiapausing adult ticks. Contextualization of our results in the current literature suggests that absence of established populations of the temperate species in tropical Brazil is linked to the fact that adult ticks would become inactive (diapause) right after molting from nymphs at any period of the year. On the other hand, absence of established populations of the tropical species in temperate Brazil is linked to the fact that this tick species would not enter diapause, and therefore, could not synchronize its life-cycle to avoid the lethal effects of a more severe winter on its developmental stages. Indeed, such assumptions should be corroborated by additional studies testing different populations of the tropical and temperate species, including more studies under natural conditions.

1. Introduction

Until recently, the taxon *Rhipicephalus sanguineus* was considered to represent a single tick species with a near cosmopolitan distribution, primarily associated with domestic dogs (Walker et al., 2000). In 2005, one South American study provided for the first time molecular and reproductive evidence that the taxon *R. sanguineus* could represent two distinct species in South America (Szabó et al., 2005). This study was corroborated by morphological analysis (Oliveira et al., 2005) and broader molecular analyses with ticks from different parts of Latin America (Burlini et al., 2010; Moraes-Filho et al., 2011). One of these studies proposed that the

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http://dx.doi.org/10.1016/j.ttbdis.2016.10.011
1877-959X/

taxon *R. sanguineus* is represented in Latin America by two distinct tick species, designated as 'tropical species' (distributed from Mexico to Brazil) and 'temperate species' (restricted to the southern cone of South America) (Moraes-Filho et al., 2011). Since there is no *Rhipicephalus* species native to the New World, it was proposed that the 'temperate species' is derived from ticks introduced from the Mediterranean region, whereas the 'tropical species' is derived from ticks introduced from sub-Saharan Africa (Szabó et al., 2005; Burlini et al., 2010; Moraes-Filho et al., 2011). Results of these studies were reinforced by further molecular analyses with ticks from different continents (Dantas-Torres et al., 2013; Zemtsova et al., 2016). Until the taxonomic status of *R. sanguineus* is solved, any tick specimen that is morphologically compatible with the taxon *R. sanguineus* should be named as *R. sanguineus* sensu lato (s.l.) (Nava et al., 2015).

Since both 'tropical' and 'temperate' species of *R. sanguineus* s.l. are parasites primarily of domestic dogs, the reasons for their distinct geographical distribution in South America could be related to particular requirements of abiotic conditions for off-host development. With the purpose to test this hypothesis, this study evaluated the off-host developmental stages (eggs, engorged larvae, nymphs and females) of both tick species in parallel inside incubators with temperature and photoperiod regimens that simulated the summer and winter conditions of tropical Brazil (where the 'tropical species' occurs) and temperate Brazil (where the 'temperate species' occurs).

2. Material and methods

2.1. Ticks

Engorged females of *R. sanguineus* s.l. representing the tropical species were collected from dogs in Chapada Gaúcha municipality (15°19′S, 45°36′W), state of Minas Gerais (MG), Brazil. Similarly, engorged females of *R. sanguineus* s.l. representing the temperate species were collected from dogs in Barra do Quaraí municipality (30°01′S, 57°32′W), state of Rio Grande do Sul (RS), Brazil. These engorged females were brought to the laboratory and held in an incubator set for 25 °C, photoperiod regimen of total darkness [0:24 (light:dark)], and 80% relative humidity (RH). The two species were reared in parallel, albeit separately, in the laboratory through F_1 and F_2 generations. For production of engorged ticks to be tested under different experimental conditions, unfed larvae, nymphs and adults were allowed to feed on tick-naïve rabbits (*Oryctolagus cuniculus*), as previously described (Labruna et al., 2011), whereas free-living ticks were held in the incubator mentioned above.

For each tick population (tropical and temperate species), two adult specimens of the F_1 ticks were processed molecularly in order to generate a portion of the mitochondrial 16S rDNA gene, as previously described (Moraes-Filho et al., 2011). DNA sequences generated from these ticks confirmed that ticks from Minas Gerais (MG) were the tropical species [DNA sequences 100% identical to haplotype A of the tropical species (GenBanK accession number GU553074)] and that ticks from Rio Grande do Sul (RS) were the temperate species [DNA sequences 100% identical to haplotype E of the temperate species (GenBanK accession number GU553084)].

2.2. Experimental groups with F_1 ticks

Within 24 h of natural detachment from rabbits, F_1 engorged larvae, nymphs or females were allocated into different experimental groups, which were held in different incubators simulating the temperature and photoperiod conditions of the summer or winter periods of Minas Gerais (MG) state (locality of the tropical species progenitors used in this study) or Rio Grande do Sul (RS) state (locality of the temperate species progenitors used in this study) (Tables 1–3). Engorged females were individually weighed before being placed in the incubators. Besides engorged ticks, we also evaluated the development of embryonated eggs under experimental conditions (Table 4). For this purpose, egg masses of 20 days of incubation under 25 °C and 0:24 photoperiod were used to form the experimental groups. These eggs were considered to be embryonated because after being 20 days of incubation under 25 °C, they clearly presented the vitelline sac and Malpighian tubules.

In each incubator set for a specific temperature and photoperiod, ticks were held inside glass tubes (14 mm diameter, 150 mm long) closed with cotton. Each glass tube contained 100 engorged larvae or 50 engorged nymphs or 1 engorged female or 100 mg of embryonated eggs (100 mg contained approximately 2000 eggs). For each experimental condition of each tick stage, there were replications varying from 3 to 10 glass tubes. Inside each incubator, glass tubes containing ticks were held inside a glass desiccator containing a saturated solution of sodium chloride (NaCl) at the bottom, in order to provide 76% relative humidity (RH) (Winston and Bates, 1960). Before starting and during the experiments, temperature and RH conditions inside desiccators were regularly calibrated by using electronic thermo-hygrometers (HOBO data loggers, model U23-002 Pro v2, Onset, Bourne, MA, USA). These calibrations showed that a 78% RH at 25 °C predominated inside our desiccators containing saturated NaCl solution. All incubators were of a single model purchased from the same company (germination cabinet model EL202/4, Eletrolab, São Paulo, Brazil) equipped with four internal fluorescent lamps (Philips TLT 75RS, Brazil) of 20W each, which were automatically switched on during the light period of each particular photoperiod regimen. Care was taken to synchronize the light period with major working hours in the laboratory; hence, incubators were opened for experimental procedures only during the light period inside the incubator.

Observations inside the incubators were performed weekly, when the following biological parameters were observed for glass tubes containing engorged larvae: larval premolt period (number of weeks between placement of engorged larvae and when the first and last nymphs ecdysed), larval molting success (percentage of engorged larvae that successfully molted to nymphs), and nymphal maximal survival (number of weeks between the first ecdysed nymph to the last week when at least one live unfed nymph was still observed). For glass tubes containing engorged nymphs, we observed: nymphal premolt period (number of weeks between placement of engorged nymphs and when the first and last adults ecdysed), nymphal molting success (percentage of engorged nymphs that successfully molted to adults), and adult maximal survival (number of weeks between the first ecdysed adult to the last week when at least one live unfed adult was still observed). For glass tubes containing engorged females, we observed: preoviposition period (number of weeks between placement of engorged females and when oviposited eggs were first observed), incubation period (number of weeks between start of oviposition and when hatched larvae were first observed), percentage of egg hatching [visually estimated following Labruna et al. (2000)], and larval maximal survival (number of weeks from initial hatching to the last week when at least one live unfed larva was still observed). Finally, the following parameters were observed for glass tubes containing embryonated eggs (observed only under winter conditions): incubation period (number of weeks between placement of embryonated eggs in the incubator and when hatched larvae were first observed), and larval maximal survival (number of weeks from initial hatching to the last week when at least one live unfed larva was still observed).

Please cite this article in press as: Labruna, M.B., et al., Comparative biology of the tropical and temperate species of *Rhipicephalus sanguineus* sensu lato (Acari: Ixodidae) under different laboratory conditions. Ticks Tick-borne Dis. (2016), http://dx.doi.org/10.1016/j.ttbdis.2016.10.011