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Starvation and overwinter do not affect the reproductive fitness of *Rhipicephalus sanguineus*

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ABSTRACT

Rhipicephalus sanguineus is the most widespread tick in the world, being able to survive under different climate conditions. In this study, the longevity of *R. sanguineus* was investigated under laboratory and natural climate conditions in southern Italy. Moreover, the reproductive fitness of females after overwintering was assessed. In the environment, unfed larvae, nymphs and adults were able to survive for up to 34, 40 and 385 days, respectively. However, the longevity of larvae (44 days), nymphs (54 days) and adults (584 days) in the laboratory was longer. Adult ticks that had overwintered under natural climate conditions were able to feed and give rise to viable larvae, after 40 weeks of starvation. Thereby, it is demonstrated that *R. sanguineus* is able to overwinter in southern Italy, without any prejudice to its fitness. These findings advance our knowledge on the natural history of *R. sanguineus* in nature and provide new insights into the epidemiology of certain tick-borne diseases in southern Europe as well as into the risk for their introduction in northern European countries.

1. Introduction

Ticks are obligatory blood feeders, but they are able to survive for years without a bloodmeal. This is particularly true for argasid ticks (family Argasidae), which can survive for several years without feeding (Sonenshine, 1993). This outstanding capacity of living for a long time without taking a meal is one of the impressive evolutionary strategies adopted by argasid ticks (Oliver, 1989). Ixodid ticks (family Ixodidae) are not able to survive for that long (Sonenshine, 1993), even though adults of some ixodid species can persist without feeding for one year or so (Nuttall, 1915). Indeed, longevity depends on tick species and developmental stage (Nuttall, 1915), but it is also strongly linked to factors such as temperature, relative humidity (Elghali and Hassan, 2010), aggregation (Tsunoda, 2008) and also infection by certain pathogens (Levin et al., 2009). In practice, data on tick longevity might be helpful to clarify questions regarding the seasonal dynamics of tick-borne diseases.

The brown dog tick, *Rhipicephalus sanguineus*, is one of the most important tick species from the medical and veterinary standpoints (Dantas-Torres, 2008). Because of its great biological plasticity, *R. sanguineus* has a ubiquitous distribution and might be found on dogs throughout the entire year in some regions (Dantas-Torres, 2010). An early study demonstrated that larvae, nymphs and adults of *R. sanguineus* could survive for 253 (at 15 °C), 97 (at 22 °C), and 569 days (at 15 °C), respectively (Nuttall, 1915). Even though in the study above the relative humidity was not reported, the results suggested that *R. sanguineus* has "great powers of fasting", as the author stated. Another limitation of such study was that ticks were maintained in a laboratory environment (i.e., under relatively constant conditions), which is rather different from the field situation, where weather conditions are quite dynamic. In the present study we assessed the longevity of

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R. sanguineus under environmental conditions in southern Italy. Additionally, the reproductive fitness of *R. sanguineus* after starvation and overwintering was also studied. This study demonstrates that starvation and overwintering do not affect the reproductive fitness of *R. sanguineus*, which is able to feed and reproduce efficiently, even after 40 fasting weeks. These data provide new insights into the epidemiology of certain tick-borne diseases in the Mediterranean region as well as into the risk for their introduction in northern European countries.

2. Material and methods

2.1. Ticks

R. sanguineus ticks included in the present study were obtained from two colonies established during a previous study (Dantas-Torres et al., 2011). Accordingly, ticks used to establish these colonies were originally collected from dogs living in an urban area and identified as *R. sanguineus* stricto sensu, according to the keys and species re-description of Walker et al. (2000). In such study, part of the ticks was reared in the environment and part in the laboratory. In brief, unfed larvae ($n = 1163$) were obtained from wild females collected from the environment (e.g., on the ground or walls of dog kennels) in a dog shelter in southern Italy. Again, unfed nymphs ($n = 85$) and adults ($n = 100$) were obtained from larvae and nymphs, respectively, previously fed on rabbits. Finally, 16 first-generation engorged females reared in the environment and previously fed on a rabbit were also included.

2.2. Follow-up of ticks kept under environment and laboratory conditions

From 20 June 2009 to 7 March 2011, ticks were followed up under environmental and laboratory conditions. A detailed description of the environment in which this experiment was carried out is available elsewhere (Dantas-Torres et al., 2011). In brief, ticks were separated in groups and maintained in glass vials closed with a cotton plug and those vials were placed in a small metal framed insect cage covered with a rock and kept under natural climate conditions in a shadowed area at the Faculty of Veterinary Medicine of the University of Bari, in the municipality of Valenzano (41°3′N, 16°53′E). The suitability of this area for *R. sanguineus* rearing was inferred from the presence of tick-infested dogs and of free-living ticks (unpublished data).

Larvae were separated in groups of 25 and placed in the environment ($n = 538$) and in an incubator (26 ± 1 °C, $70 \pm 10\%$ RH, and scotophase) in the laboratory ($n = 625$). Nymphs were separated in groups (from 4 to 14 per vial) placed in the environment ($n = 71$) and in the incubator ($n = 14$). Again, 57 unfed adults were followed up in the environment and 43 in the incubator. Furthermore, 16 females (first generation) previously fed on a rabbit (Dantas-Torres et al., 2011) were also included in this study in order to evaluate their post-oviposition survival. Ticks were examined every day and the number of living individuals in each vial recorded.

2.3. Capacity of overwintered ticks to feed on rabbits

To assess the capacity of overwintered ticks to feed on rabbits, we used a subset of the ticks (10 males and 10 females) that have been kept in the environment since 5 August 2009. On 17 May 2010, these ticks were allowed to feed on a naive rabbit (*Oryctolagus cuniculus*), using cloth bags as described elsewhere (Srivastava and Varma, 1964). The rabbit was kept in a cage at the "Infectious Disease Unit" of the Animal Hospital, Faculty of Veterinary Medicine of Bari, water and food being provided *ad libitum*. During the experimental tick infestation, a mean temperature of 25.2 °C and a mean relative humidity of 49.4% were recorded by a data logger (HD226-1 Delta OHM, Padua, Italy), which was placed approximately 50 m far from the place where the rabbit was maintained. Experiments were carried out according to the guidelines for animal experimentation and were approved by the University of Bari (protocol no. 1115/10).

2.4. Biological parameters

Longevity (days of survival) was recorded for larvae, nymphs and adults (males and females) maintained in the environment and in the laboratory. Again, several biological parameters were recorded for overwintered adult ticks that were fed on a rabbit, as follows: tick yield (number of engorged ticks/total number of applied tick × 100); engorged female weight (mg); pre-oviposition period (number of days from detachment to the beginning of oviposition); oviposition period (number of days from the beginning to the end of oviposition); egg mass weight (mg); egg production efficiency (EPE) (weight of eggs/weight of the engorged female × 100); reproductive efficiency index (REI) (number of eggs/weight of the engorged female); reproductive fitness index (RFI) (number of eggs that hatch into larvae/weight of the engorged female); incubation period (number of days from the beginning of oviposition to the hatching of the first larva); egg hatch rate (mean value of visual evaluation performed by 3 examiners). Additionally, the LT_{50} (the time to kill 50% of the ticks) was also calculated (Koch and Tuck, 1986). The results are expressed as: mean ± standard deviation (minimum–maximum).

2.5. Statistical analysis

Biological parameters recorded were compared using Mann–Whitney *U*, being differences considered statistically significant when P was 0.05 or less ($P \leq 0.05$). Statistical analyses were carried out using BioEstat (version 5.0; Mamirauá/CNPq, Belém, PA, Brazil).

3. Results

Larvae were able to survive for up to 44 days (32.2 ± 6.7, 23–44) in the laboratory and for 34 days (23.4 ± 5.4, 17–34) in the environment (summertime, from 20 June to 23 July) (Fig. 1). However, the LT_{50} for larvae was 2 weeks in the laboratory and 3 weeks in the environment. Nymphs survived for up to 54 days (48.1 ± 5.1, 40–54) in the laboratory and 40 days (32.1 ± 2.2, 29–40) in the environment