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Survival and moulting of *Amblyomma variegatum* nymphs under cold conditions of the Malagasy highlands

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ABSTRACT

Although *Amblyomma variegatum* is now regularly recorded up to 1600 m in altitude in the Malagasy highlands, where it was previously reported not to persist without a constant supply of ticks introduced from lower infested regions, some parts of the highlands remain tick-free. Studies were carried out to verify whether the cold climate prevailing in these areas in June–September could prevent the survival and moulting of nymphs, the tick life stage present in the environment at this period. Cohorts of engorged *A. variegatum* nymphs were released from June to August in six different sites (three in 2010, altitudes 1200–1415 m; three in 2011, altitudes 1585–1960 m) which were reported to be either tick-infested (two in 2010, one in 2011) or tick-free. The ticks were placed in cages driven into the soil and open at the bottom so that they could hide in the soil or root network. Of the 1975 nymphs released in 2010 and the 1494 released in 2011, 86% and 85% were recovered, respectively. Twenty to 23% of the recovered ticks were dead, and some of them were obviously predated; predation also likely contributed to the disappearance of the non-recovered ticks. When the rainy season started in October, 59% of the newly moulted adults were still alive in the cages. The moulting period lasted up to 20 weeks, depending on the site and release period. As verified in 2011, unfed nymphs could also survive the cold season. Various *A. variegatum* life stages are thus able to survive the adverse cold and/or dry seasons: unfed nymphs, engorged nymphs in developmental diapause, moulted adults in behavioural diapause as observed previously. Strong variation in mortality and recovery rates was observed between cages, highlighting the importance of the micro-environment and micro-climate for tick survival. The minimum temperature recorded in the field sites varied from 1.1 °C to 6.8 °C, but the tick-free sites were not the coldest ones; they were, however, those for which the temperature remained below 10 °C for the longest time over the study period. Recovery and mortality rates in the tick-free sites were similar to those of the tick-infested sites: the temperatures recorded during the study periods did not prevent ticks from surviving and moulting although it did delay the metamorphosis. Low temperature alone can therefore not explain the persistence of tick-free areas in the highlands. To further monitor survival, cohorts of engorged nymphs were also maintained in an incubator at 3.6 °C, 6.2 °C or 12.8 °C. More than 50% mortality was observed after 6 days at 3.6 °C, and after 15 days at 6.2 °C, whereas 18 days at 12.8 °C only delayed moulting. The collected survival, moulting and climatic data presented in this study should help to develop a predictive model to assess the distribution of *A. variegatum* according to climate characteristics.

1. Introduction

Amblyomma variegatum is a tick of ruminants with a large distribution in tropical and equatorial areas of Africa. It has successfully invaded various other regions of the world over the last few centuries, in particular Guadeloupe, Marie-Galante, Antigua, Martinique and other islands of the West Indies, along with Madagascar, Comoros and the Mascarene Islands (Mauritius, La Réunion) in the Indian Ocean (Barré and Uilenberg, 2010). *A. variegatum* is also one of the most harmful species in the tropics: adults can cause considerable direct losses (reduced growth (Pegram et al., 1989) and milk production, serious wounds especially to the udder which is one of the attachment predilection sites (Stachurski, 2000), nymphs and adults can transmit cowdriosis (Daubney, 1930), and adults are

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associated with dermatophilosis, the presence of the ticks leading to more pronounced lesions of this cutaneous disease (Plowright, 1956).

A. variegatum was first described in 1899 in Madagascar (Uilenberg et al., 1979) but was probably present on the island long before as cattle bones dating from the V to VIIth centuries have been found during archaeological digs (Andrianaivoarivony, 1987) and since it is unlikely that cattle were introduced from Africa without their ectoparasites. It was mentioned in 1979 that the tick could be found in the country at an altitude of 1100–1400 m "along the trails used by cattle herds coming from the western lowlands towards the slaughterhouses of the capital city" but that it was not sure that the species "could persist in the Antananarivo area (*average altitude:* 1250–1300 m*)* without the permanent supply of ticks brought from the western regions" by infested cattle (Uilenberg et al., 1979). Twenty-five years earlier, Hoogstraal (1953) simply stated that it was uncommon on the Plateau.

In 2008, surveys revealed that cattle reared in various villages located between 1250 m and 1450 m altitude were infested by *A. variegatum*, but that the Mangamila area (1400 m), situated 50 km N-E from Antananarivo, remained tick-free (unpublished data). As this town and its surroundings are reputed to be very cold during the dry season (June to August), it was hypothesised that the local climate could prevent tick survival even if engorged ticks brought in with infested animals detached in the pastures. To ascertain this assumption, trials were carried out during the 2010 cold season in the Mangamila area as well as in tick-infested villages of the same region. During the next cold season, new studies were implemented in areas at higher altitudes (and therefore supposedly colder) to complete the first observations. Finally, laboratory trials were carried out in 2012 to verify some of the field results and to obtain survival data at controlled temperatures and humidity. The collected data will be used to develop predictive models in order to assess the possible spread and establishment of the tick to currently tick-free areas, according to the future global climate change predictions.

In regions with tropical climate like the Malagasy highlands, the life cycle of *A. variegatum* shows important seasonal variation (Petney et al., 1987): adults infest their hosts during the rainy season (October to April) with the infestation peak occurring 1–2 months after the onset of the rains, larvae are present at the end of the rainy season, and nymph infestation is observed during the dry season (May to September). The coldest months are recorded during this dry season. In the study, we therefore only estimated the survival of *A. variegatum* nymphs as it is this tick stage which is exposed to low temperatures under natural conditions.

2. Materials and methods

2.1. Experimental sites

In 2010, the first study was carried out between the beginning of June and mid-November in three sites situated north-east of Antananarivo (Fig. 1): site A (altitude: 1415 m) where cattle were not infested by *A. variegatum*, according to local farmers; site M (1200 m) where the tick was present but where cattle infestation was very low; site T (1415 m) where the animals were regularly highly infested for several decades. To verify the claims of cattle owners, 5 animals from different herds in each village were examined every three weeks for tick infestation. At each site, cages with ticks (see below) were placed on a natural bushy savannah. Trees (mainly eucalyptus) were also present in the chosen plots, providing temporary shade to some of the cages.

In 2011, a similar study was implemented from the end of June to mid-November in three sites located west of Ambatolampy

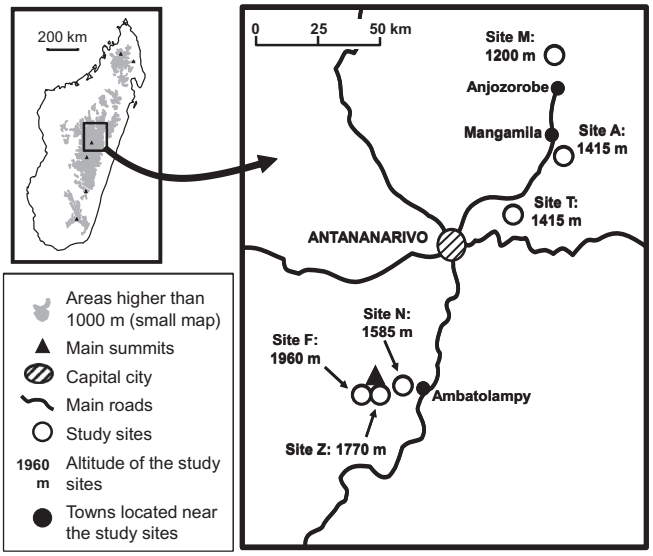

Fig. 1. Location of the study sites (white dots; respective altitude indicated in metres). The three 2010 study sites were north-east of the capital, and the three 2011 sites were west of Ambatolampy.

(Fig. 1): site N (1585 m) where farmers claimed that *A. variegatum* was present; site Z (1770 m) and site F (1960 m) where the tick was not known. More precisely, the herdsmen in site F mentioned that they knew the species, that cattle infested by *A. variegatum* were from time to time introduced to the farm, but that the tick never established.

2.2. Experimental schedules

Ticks used for these studies were bred on animals kept in the FOFIFA-DRZV[1] facilities in Antananarivo and are issued from females collected in 2009 in the Tsiroanomandidy region (west from the capital city). Unfed nymphs maintained in an incubator at 25–28 °C and 80–95% RH were placed in cloth bags stuck on ears of sheep, goats and rabbits, 8 days before the scheduled release date of engorged ticks in the field (the nymphal blood meal of *A. variegatum* lasts 6.25 days on average (Stachurski, 2000). The first detached nymphs were kept in the laboratory at room temperature until a sufficient number of ticks were available. Tick batches were prepared just prior to release, mixing nymphs engorged on different host types and at different times.

In 2010, four different tick cohorts were placed in the three sites on June 3rd, July 1st and 29th and August 29th, respectively.[2] Each cohort consisted of 4–7 cages depending on the number of scheduled weeks of observation (see below). Each cage contained 30 engorged nymphs except in a few cases when an insufficient number of ticks were available: 5 of the 18 cages of cohort 2 contained 29 ticks and were randomly assigned to either site A or M. The cages were similar to those used in previous studies (Stachurski et al., 2010): 50 cm-side metal-framed cubes with the four sides covered by mosquito netting tied at the top after the ticks were put in. The bottom of each frame was driven into the ground to a depth of 3–5 cm with the base open so that ticks could dig into the soil or root network at will. Each cage was placed on an area with at least 2–3 grass tufts and plant and leaf debris. Ticks were introduced into the installed cages between 15h30 and 17h30 in order to mimic the natural drop-off

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[2] For the three first series, the ticks were placed two days earlier in site T for logistic reasons.