

RESEARCH COMMUNICATION

Larval biology of *Rhipicephalus (Boophilus) decoloratus* (Acarina: Ixodidae) in Free State Province, South Africa

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ABSTRACT

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The objective of this study was to determine certain aspects of the biology of *Rhipicephalus* (*Boophilus*) *decoloratus* larvae under laboratory and field conditions. Larvae allowed 48 h to select a vertical questing substrate preferred 90 cm rods in length to those of 60 or 30 cm, while in a separate experiment migration from rods 5 cm or 25 cm in length to rods 45 cm in length continued between 48 h and 72 h after larval release. Hatching of the larval progeny of engorged female ticks exposed to ambient field temperatures during the period June to August, occurred synchronously during the third or fourth week of November. With a single exception, larvae that hatched during November and between April and July survived for 38 days or longer, while those that hatched from throughout the year, with most being recovered during January and February. Parasitic larvae were present on cattle from October to May with most being collected during January and February.

Keywords: Free State Province, larval survival, parasitic larvae, questing larvae, *Rhipicephalus* (Boophilus) decoloratus, seasonality

INTRODUCTION

The blue tick, *Rhipicephalus* (*Boophilus*) *decoloratus*, is one of the most widely distributed ixodid ticks in South Africa (Howell, Walker & Nevill 1978), and is the principal vector of *Babesia bigemina*, the causative organism of African redwater in cattle (De Vos & Potgieter 1994). It has consequently been the subject of several laboratory and field studies (Londt 1974, 1977; Robertson 1981; Spickett & Heyne 1990; Horak, Spickett & Braack 2000). Its

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larvae are most numerous on the vegetation in the Eastern Cape Province during the period February to June (Rechav 1982), while infestation on cattle in this Province and in KwaZulu-Natal is most intense from spring or early summer to autumn or early winter (Robertson 1981; Rechav 1982; Baker, Ducasse, Sutherst & Maywald 1989). However, in a recent study in the eastern region of Free State Province, an area not previously surveyed, *R.* (*B.*) decoloratus was most numerous on cattle from late summer to mid-winter, a period during which average daily temperatures decreased from 18–8 °C, with the largest numbers present in the coldest month, namely June (Dreyer, Fourie & Kok 1998).

The present study was designed to determine the preference of R. (B.) decoloratus larvae for vertical substrates of various lengths in the laboratory, as well as their hatching and survival under field condi-

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tions. In addition, the relationship between the numbers of larvae on the vegetation and those parasitic on cattle was examined.

Laboratory

Identical numbers of 1.5 mm diameter copper rods (30, 60, or 90 cm long) were equally spaced vertically in alternating sequence in a square metal arena (16 x 16 x 6 cm) with a wooden base covered with moist vermiculite. Fifty milligrams of 7-day-old R. (B.) decoloratus larvae were released into the centre of the arena, and prevented from escaping by double-sided adhesive tape along the edges of the square. The arena was incubated for 48 h at 24 ± 2 °C with a photoperiod of 14 h light and 10 h dark, after which the larvae on each rod were collected and counted and the counts for rods of identical length pooled. Within 48 h of release 57.5% of unfed larvae clustered at the tips of the 90 cm long vertical rods compared to 10.1 % on the tips of the 30 cm long rods.

In a follow-on study rod lengths of 5, 25 and 45 cm were used and observations were made 24, 48 and 72 h after the release of 50 mg of larvae into the centre of the arena. At each occasion the numbers of larvae on the rods were categorized on a subjective scale without their being disturbed. Larvae observed at 24 h intervals over a 72 h period continued to migrate from 5 cm and 25 cm long rods to rods 45 cm in length.

The heights at which adult ticks quest on the vegetation may relate to the size of their preferred hosts (Fourie, Kok & Van Zyl 1991; Goddard 1992). The preference of *R. (B.) decoloratus* larvae for taller substrates in our experiments possibly reflects an adaptation by this life stage to expedite infestation of their favoured hosts, which are generally large animals such as domestic cattle, wild bovids and domestic and wild equids (Mason & Norval 1980). The presence of questing larvae on the tips of tall grass would facilitate their contact with the chest and abdomen of these animals.

Month of tick placement	Pre-oviposition period (days)	Oviposition period (days)	Date of first larval hatching	Larval survival (days)
March 1998	11	30	Eggs dried out before eclosion	
April	31	31	Eggs dried out before eclosion	
Мау	No oviposition took place			
June	74	8	21 Nov. 1998	56
July	33	58	20 Nov. 1998	44
August	17	70	23 Nov. 1998	38
September	No engorged female ticks collected			
October	20	22	22 Dec. 1998	31
November	8	23	12 Jan. 1999	30
December	8	19	19 Jan. 1999	24
January 1999	7	24	4 Mar. 1999	26
February	21	27	5 Apr. 1999	84
March	7	29	2 May 1999	56
April	36	21	20 Jul. 1999	42
May	89	13	Eggs dried out and no larvae hatched	
June	67	15	Eggs dried out and no larvae hatched	
July	No engorged female ticks collected			
August	20	53	19 Nov 1999	28

TABLE 1 Pre-oviposition period, oviposition period and subsequent survival of the larval progeny of engorged female *Rhipicephalus* (Boophilus) decoloratus exposed to ambient outdoor climatic conditions

Free-living ticks

When procurable, six engorged female R. (B.) decoloratus, each weighing more than 150 mg, were collected at monthly intervals from March 1998 to August 1999 from cattle at Botshabelo (29°15' S, 26°43' E), a semi-urban township 55 km east of the city of Bloemfontein, Free State Province. These ticks were placed in separate 15 x 30 mm cylindrical vials, gauze-stoppered at both ends, and exposed to prevailing climatic conditions in a field on the premises of the University of the Free State in Bloemfontein. The vials were placed approximately 2 cm deep in the soil and were loosely covered with a mixture of grass litter and sand. A 1 m squarewire mesh cage was positioned over the placement site to prevent rodents from disturbing the vials, which were examined daily for the onset of oviposition, egg eclosion and survival of larvae. Larval survival was calculated as the period between eclosion and the date on which all larvae were considered dead

Atmospheric temperature and rainfall were measured on the campus of the university. The pre-oviposition periods of female ticks exposed during the cooler autumn and winter months (April to July) exceeded 30 days, while those exposed from November to January and during March were 8 days or less (Table 1). The first larval progeny of ticks exposed from June to August 1998 hatched more or less synchronously from 20–23 November 1998, and those of ticks exposed during August 1999 hatched on 19 November 1999. Larvae that hatched during November 1998 and from April to July 1999 survived for the greatest length of time. Less than 3 mm of rain per month was measured from June to August and none during September.

Londt (1974, 1977) noted that the pre-oviposition and oviposition periods of *R*. (*B*.) decoloratus females and the incubation period of their eggs were temperature dependent, becoming shorter as the temperature increased, whereas the effects of humidity appeared to be negligible. He recorded mean pre-oviposition periods of 37–42 days for females placed in a Stevenson's screen from late July to September when calculated mean daily temperatures varied between 13.0 and 14.5 °C, compared to 28 days for females maintained at 15 °C in the laboratory, decreasing to 5 days or less for ticks maintained at 25 °C or higher.

Robertson (1981) exposed engorged female ticks in Stevenson's screens on two coastal farms in the Eastern Cape Province over a period of 45 consec-

utive months. The pre-oviposition periods of these ticks varied between approximately 8 days for ticks exposed during January to 28 days for those exposed during June, and the incubation periods of their eggs varied from approximately 41 days for females exposed during January to 134 days for those exposed during June. The mean maximum survival times of larvae hatching from these eggs varied between 175-245 days, with no clear seasonal pattern of survival. She stated, "While these data highlight the tremendous survival capacity of these larvae, their survival under true field conditions may be considerably reduced. It should also be remembered that maximum survival periods have been recorded with no attention being paid to the rate of mortality, or the ability of the ageing larvae to attach and feed."

Spickett & Heyne (1990) placed engorged female R. (B.) decoloratus in gauze containers at the base of grass stems in a field covered throughout the year with approximately 50-100 cm tall indigenous grass. They found that high temperature at the onset of oviposition and rapid temperature accumulation thereafter during the summer months resulted in an incubation period as short as 40 days for eggs laid in December, whereas a low initial temperature and slow temperature accumulation thereafter lead to an incubation period of 138 days for eggs laid during August. They suggested that the critical minimum temperature below which hatching does not take place appears to be 10 °C. Larvae survived for approximately 10-35 weeks after hatching and the duration of the survival period was inversely dependent on increased temperature accumulation. In their experiments, larvae that hatched during December and January survived for the shortest period, while larvae that hatched during the third week of April, just prior to the onset of low winter temperatures, survived the longest. The viability of surviving larvae, determined by percentage attachment to cattle, was remarkably good, and not much shorter than their total survival period. The maximum survival time of 84 days for larvae exposed to the prevailing climatic conditions in the present study is thus considerably shorter than that recorded by other authors.

Questing larvae

Small-scale cattle farmers residing at Botshabelo make communal use of natural pastures along the Modder River on the western periphery of the township. At monthly intervals from September 1998 to August 1999 questing tick larvae were collected by



FIG. 1 The seasonal occurrence of larvae of *Rhipicephalus* (*Boophilus*) *decoloratus* on vegetation and on cattle in an eastern region of Free State Province

dragging ten weighted flannel strips (each 100 x 10 cm), attached adjacent to each other on a wooden spar, over the vegetation alongside the river. On each occasion a single 100 m long drag-sample was taken between 10:00 and 12:00 from the vegetation of three localities along the river. The larvae so collected were identified and counted under a stereoscopic microscope and only the data pertaining to *R*. (*B.*) *decoloratus* were used. Larvae were collected from the vegetation during each month of the survey, with peak numbers present during January and February 1999 and very few from May to August 1999 (Fig. 1).

The peak in larval numbers on the vegetation near Botshabelo occurred about 1 month earlier than that recorded on vegetation at three localities in the Eastern Cape Province (Rechav 1982) and 2–3 months later than that recorded in the Lowveld of Mpumalanga Province, where temperatures are considerably higher than in the eastern Free State (Horak *et al.* 2000). The decline in larval numbers on the vegetation near Botshabelo from March onwards could have resulted from depletion by attachment to grazing cattle, accidental ingestion by these animals, predation, or age-related mortality, and was probably due to a combination of these. Their increase on the vegetation in September can be ascribed to the hatching of eggs laid by females that had detached during March or April (late summer and autumn) in response to a rise in temperature in spring (Robertson 1981).

Parasitic larvae

At approximately 4-week intervals from September 1998 to August 1999 ticks were collected from ten cattle of mixed-breed and older than 1 year, belonging to a single owner at Botshabelo. Except when an animal was sold or had died, and replaced by the owner with another of similar age, the same animals were examined throughout the study. No acaricides were used on the animals during the study period. Ticks were collected from 20 x 20 cm areas of the skin, delineated by a wire square, on the neck, abdomen and iguinal region of each animal. The left and right hand sides of the cattle were sampled in alternate months. After the collection of all visible ticks from one of these areas it was combed with a flea-comb followed by thorough scraping with a serrated edged knife blade. At the laboratory the ticks were separated from the hair under a stereoscopic microscope, identified and counted. For the purpose of this study only the numbers of R. (B.) decoloratus collected were considered.

Although larvae were collected from the vegetation throughout the year, they were recovered from cattle only from October to May, with a peak in intensity of infestation during January and February 1999 (Fig. 1). There was otherwise a significant similarity in the seasonality of larvae collected from the animals and from the vegetation (P < 0.01) (Pearson Correlation Co-efficient test). Adult ticks were present throughout the year. The peak in total numbers of R. (B.) decoloratus on the cattle during April, 2 months after peak numbers of larvae had been collected from them and from the vegetation may signify a slight delay in development on the host during the cooler months. This would imply that although larvae were still being acquired in small numbers, the adults from earlier infestations were not detaching at the same tempo.

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