Survival of *Theileria parva* in its nymphal tick vector, *Rhipicephalus appendiculatus*, under laboratory and quasi natural conditions

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SUMMARY

Groups of nymphal *Rhipicephalus appendiculatus* Muguga, having a mean of 1 or 9 *Theileria parva* Muguga-infected salivary gland acini per tick, were kept under quasi-natural conditions at an altitude of 1950 m or 20 °C at a relative humidity of 85% in the laboratory and their survival and infection prevalence and abundance determined over time. *Theileria parva* infections for both categories of ticks survived in the nymphal ticks for 50 or 26 weeks post salivary gland infection under quasi-natural or laboratory conditions respectively. There was a distinct decline in infections in the more heavily infected nymphae under both conditions of exposure, reflecting an apparent density dependence in parasite survival. Nymphal ticks having an average infection level of 1 infected salivary gland acinus per tick, survived for up to 69 or 65 weeks post-repletion under quasi-natural or the laboratory conditions respectively. Nymphae having an average infection level of 9 infected salivary gland acini per tick survived for a similar duration under each of the 2 conditions. The infection level of 9 infected salivary gland acini per tick did not seem to significantly affect the survival of the tick vector compared to those having an average of 1 infected salivary gland acinus per tick.

Key words: *Theileria parva*, *Rhipicephalus appendiculatus*, nymphae, survival, quasi-natural conditions, laboratory conditions.

INTRODUCTION

The sporozoan parasite, *Theileria parva*, is the causative agent of East Coast fever (ECF), January disease and Corridor disease in cattle. Theileriosis due to *T. parva* is restricted to eastern, central and southern Africa and is one of the major constraints to the improvement of the cattle industry in this region. The total regional loss due to theileriosis has been estimated at US$ 168 million per year (Mukhebi, Perry & Kruska, 1992). The principal field vector of *T. parva* is the brown ear tick, *Rhipicephalus appendiculatus*. This is a 3 host ixodid tick that becomes infected by feeding on infected cattle or buffalo as a larva or nymph and transmits the parasites in the next tick instar, that is, as nymph or adult respectively.

Different climatic conditions exist throughout the range of *R. appendiculatus* and *T. parva* in Africa (Perry *et al*. 1990). However, little progress on the understanding of the effect of ambient climatic conditions on the development and survival of *T. parva* within its tick vector under natural conditions has been made since Theiler (1905) reported that *T. parva* infections of adult *R. appendiculatus* in paddocks in South Africa would die out within 15 months. Yet, ambient climatic conditions, particularly humidity and temperature, are known to have a major effect on the development and survival of *T. parva* in the tick and on the tick itself (Young & Leitch, 1981; Fielden & Rechav, 1996; Billiouw *et al*. 1999). Field studies on the survival of *T. parva* and the infected tick vector in Kenya have all been undertaken using adult ticks (Branagan, 1973; Young *et al*. 1983, 1987; Newson *et al*. 1984). In these studies it was shown that *T. parva* survived in adult ticks for at least 19 months in cooler climate at 2100 m altitude and for 15 months in warmer climate at 1600 m altitude.

There have been, to our knowledge, no studies on the survival of *T. parva* in nymphal ticks even though it has been demonstrated that nymphae play a very important role in the transmission dynamics of *T. parva*. For example, apart from being able to effectively transmit *T. parva* to cattle (Purnell, Boarer & Peirce, 1971; Koch *et al*. 1993; Ochanda *et al*. 1996), nymphae also feed on cattle at an approximate ratio of 10 nymphae to 1 adult (Short & Norval, 1981). Thus, their potential role in transmission can not be underestimated. In addition, in the absence of adult ticks during the dry season, nymphae transmit subclinical infections to cattle

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thereby generating immune carrier cattle. These cattle infect the adult ticks to a sufficient degree to cause clinical cases during the wet season (Koch *et al.* 1993). In this way, nymphae contribute to the maintenance of *T. parva* infection. However, despite the potential role of nymphal transmission, very little is known about it. Detailed information on the survival of *T. parva* in the nymphal tick and the infected nymphal tick vector itself would thus be useful in understanding the epidemiology of and in the control of the parasite. Transmission models, which would ultimately be used to help in the design of viable tick and tick-borne disease control methods, would also benefit from this information (Randolph, 1999).

In this study, nymphal *R. appendiculatus* ticks infected with *T. parva* were exposed to laboratory or quasi-natural conditions at a typical climate for enzootic ECF to obtain survivorship curves for the parasite and the infected tick vector.

MATERIALS AND METHODS

Infection of ticks with Theileria parva

The origin, characterization and subsequent storage of the Muguga stock of *T. parva* has been documented by Bailey (1960) and Radley *et al.* (1974). One ml of the parasite stabilate, equivalent to a suspension derived from 1 tick, was inoculated subcutaneously below and in front of the right parotid lymph node of each of 4 Boran steers which were sero-negative for *T. parva* schizont antigen using the indirect fluorescent antibody test (Goddeeris *et al.* 1982). Development of the parasite in the cattle was then monitored as described in Ochanda *et al.* (1996). Uninfected larvae of the *R. appendiculatus* Muguga stock (Bailey, 1960) were subsequently applied on the backs of the steers in separate cloth patches and allowed to feed to repletion during the parasitaemic phase of infection. The replete ticks were collected daily and kept separately at 24 °C and approximately 85% relative humidity (RH) for 21 days to complete their moult before being transferred to 20 °C and approximately 85% RH for 14 days to complete post-moult development.

Assessment of ticks for Theileria parva *infection levels*

Five weeks after repletion, equivalent to 2 weeks post-salivary gland infection (Ochanda, 1994), 60 nymphae from each batch (a daily harvest of ticks after feeding to repletion on an animal) were fed on the ears of rabbits for 3 days for *Theileria* sporoblasts to mature into sporozoites. The salivary glands were dissected from the nymphae then spread individually on glass slides and assessed for salivary gland infection after Feulgen's staining (Büscher & Otim, 1986). After staining, the whole salivary glands were examined under 100× magnification to assess the number of acini infected with *T. parva* per individual tick.

Two groups of infected nymphae were selected for use in these experiments. The first group moulted from larvae dropping replete from steer 1 on day 17, 18 and 19 post-infection, when the steer had a piroplasm parasitaemia of 25·6, 34·3 and 60·2% respectively. These nymphae were pooled and the median day of 18 was taken as the representative day of repletion for the group. These nymphae had an average infection level of about 9 infected acini each and were referred to as having 'high' infections. The second group moulted from larvae dropping replete from steer 2 on day 24 post-infection when the steer had a piroplasm parasitaemia of 4·3%. These nymphae had an average infection level of about 1 infected acinus each and were categorized as having 'low' infections. *Theileria parva* infection levels in nymphal *R. appendiculatus* under field conditions have not been recorded before and the 2 categories selected were based on that found in adult ticks. Naturally infected *R. appendiculatus* ticks in endemic areas have an average infection abundance of about 1 infected acinus per adult (Moll *et al.* 1986). Use of the terms 'low' and 'high' in this context was thus arbitrary. Each of the 2 categories of ticks were thereafter divided into 2 groups.

Exposure of ticks to quasi-natural and laboratory conditions

The first group of ticks from each of the 2 categories was enclosed, in cohorts of 200, in glass tubes measuring 8 cm in length × 2 cm in diameter and then kept in an incubator set at 20 °C and approximately 85% RH. The second group of ticks was enclosed, also in cohorts of 200, in tubes 50 cm long × 4 cm in diameter, made from nylon bolting silk material of 400 μm meshes. The tubes were sealed at the top and kept standing upright in the field in naturally growing vegetation at the International Livestock Research Institute (ILRI), Nairobi, Kenya (altitude 1950 m). On this day and at monthly intervals thereafter, the number of nymphs still alive, in a cohort from each group, was recorded. Sixty of them were then routinely assessed for *T. parva* salivary gland infection. Climatic conditions in the field at the study site were also recorded. This type of sampling, whereby a new cohort of ticks in the group was assessed, as opposed to a longitudinal study whereby a single cohort is sampled throughout the study, was important to this study in two aspects. First, there was no disturbance to the remaining ticks, thereby minimizing any extraneous influence on the tick and parasite survival. Unfed ticks become active and move up vegetation to quest for a host in response to any activity within its vicinity (Short *et al.* 1989). In so doing, they expend their energy

reserves, thereby shortening their survival in the absence of a bloodmeal. Secondly, it is necessary to kill the ticks to assess their infection with *T. parva*.

Statistical analysis

Differences in the decline of abundance of infection of *T. parva*, being measured by simple count data, and proportions of surviving ticks between those kept under different climatic conditions were analysed using the Poisson regression analysis or logistic regression analysis respectively. A log-linear model structure was used for the abundance of infection data. The various variables were analysed separately for their effect on the fit of the respective models using the GLIM software. The analysis was performed using the statistical software SAS/STAT® (SAS Institute Inc., SAS Campus Drive, Cary, NC 27513, USA).

RESULTS

Climatic conditions

Climatic conditions during the period of exposure of the ticks to quasi-natural conditions are shown in Fig. 1. During the first half of the exposure period, the normal bimodal rainfall pattern of the long and short rains, was not experienced. Sustained rainfall fell throughout the year even though the total amount during the long rainy season period was far below the usual average. The second half of the experimentation period was more typical, however, with the bimodal pattern of the long and short rains distinct. In general, humidity patterns were closely correlated with the rainfall patterns. The mean monthly relative humidity at 15.00 h varied from about 40 to 70%. The mean monthly maximum and mean monthly minimum temperatures remained relatively constant during the exposure period.

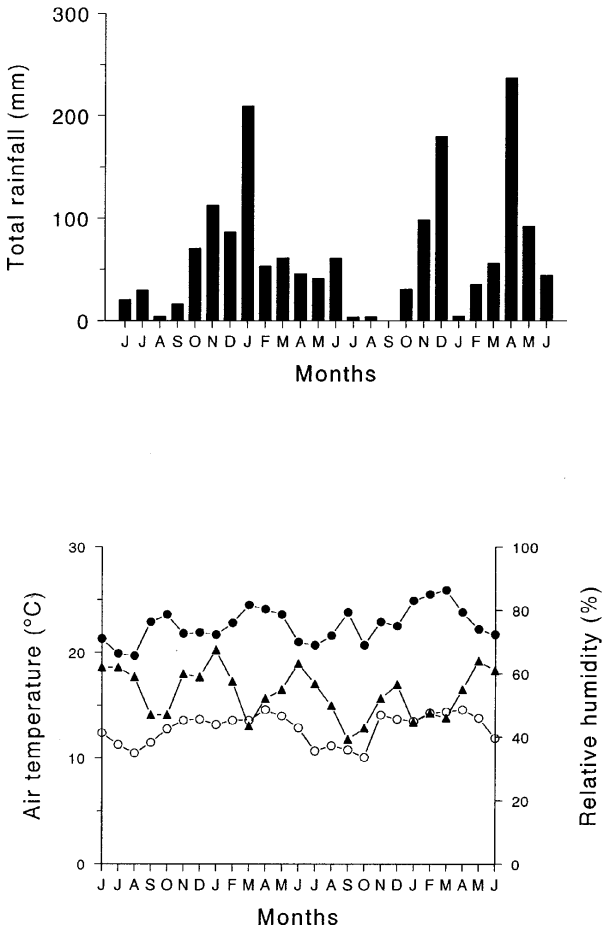

Fig. 1. Total monthly rainfall, mean maximum (—●—) and mean minimum (—○—) monthly air temperatures and relative humidity at 15.00 h (—▲—) recorded at the study site during the period of exposure of ticks to quasi-natural conditions.

Survival of Theileria parva

Survival of *T. parva* infection in the highly and lowly infected ticks exposed to quasi-natural conditions in the field or to a constant temperature of 20 °C and 85% RH in the laboratory is shown in Fig. 2. *Theileria parva* survival in ticks kept under field conditions was significantly longer than that of those kept under laboratory conditions ($P<0{\cdot}01$) for the respective infection levels.

Decline in the level of infection in the highly infected nymphae kept under laboratory conditions fell dramatically from about 9 per tick to 0 in just 26 weeks post-salivary gland infection (24 weeks post-exposure to the different conditions). Infection levels in highly infected nymphae but exposed to field conditions initially remained high until week 14 post-salivary gland infection when the levels also fell dramatically. However, the general slope of the fall

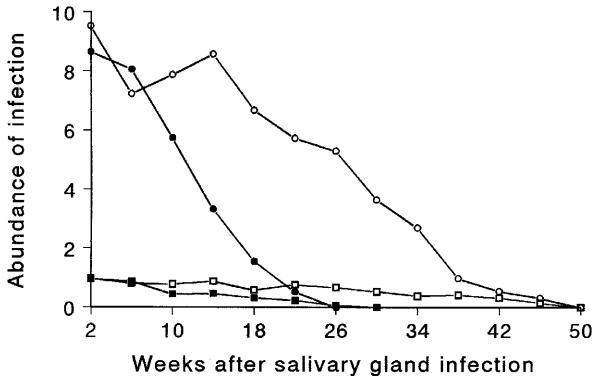

Fig. 2. Survival of *Theileria parva* Muguga in the highly infected nymphal *Rhipicephalus appendiculatus* Muguga after exposure to 20 °C and approximately 85% RH (—●—) or quasi-natural conditions (—○—) and that in the lowly infected nymphae exposed to 20 °C and approximately 85% RH (—■—) or quasi-natural conditions (—□—). Sixty ticks from each cohort were assessed at monthly intervals.

in infection level was not as steep as that of the highly infected nymphae kept under laboratory conditions. Further, there was a relatively longer drawn out period of *T. parva* survival in the ticks

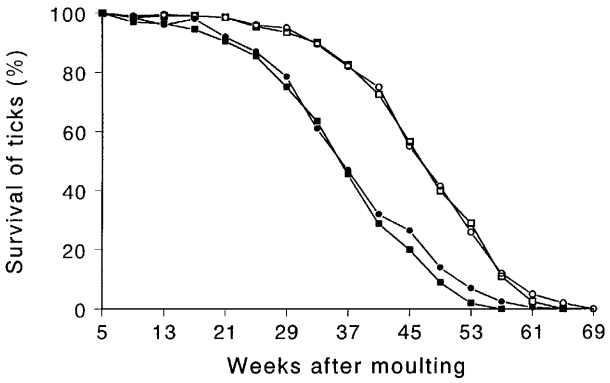

Fig. 3. Survival of *Theileria parva* Muguga-infected nymphal *Rhipicephalus appendiculatus* Muguga having high infections after exposure to 20 °C and approximately 85% RH (—●—) or quasi-natural conditions (—○—) and those having low infections exposed to 20 °C and approximately 85% RH (—■—) or quasi-natural conditions (—□—). Surviving ticks were counted at monthly intervals ($n = 200$).

kept in the laboratory once infection levels had dropped to about an average of 1 per tick.

Contrary to the pattern of decline in infection levels in the highly infected ticks, decline in the lowly infected ticks was very gradual under both conditions of exposure. Infections in ticks kept under laboratory conditions died out at about the same time (week 26 post-salivary gland infection) irrespective of the infection levels. This was also true for infections in ticks maintained under field conditions where infections died out by week 50 post-salivary gland infection in both cases.

Survival of the infected nymphal vector

Survival of the highly and lowly infected ticks under laboratory or field conditions is shown in Fig. 3. Survival patterns of the ticks were almost identical for both categories of infection levels under each condition of exposure. Ticks kept under field conditions survived for a significantly longer time than those kept under laboratory conditions for the respective categories of infection levels ($P < 0{\cdot}05$). No appreciable mortality occurred in the highly or lowly infected ticks exposed to field conditions until about 33 weeks after moulting of the ticks when it increased rapidly. At 20 °C and approximately 85% RH, appreciable mortality in both categories of ticks developed at approximately 17 weeks after moulting of the ticks.

DISCUSSION

Although there have been several studies on the survival of *R. appendiculatus* and *T. parva* within adult ticks under various climatic conditions, this is the first to consider the survival of *T. parva* in nymphae under laboratory and field conditions. In addition, it is the first to study the influence of infection levels on the survival of the parasite and the nymphal tick.

Parasite survival in nymphae kept under field conditions was much better than under laboratory conditions whether the ticks had high or low infections. Similar results were noted for survival of the nymphae. Indeed, the distinct decline in parasite survival in highly infected nymphae was less marked than in that of ticks exposed to laboratory conditions. Ticks are able to select microhabitats in the field that are best suited for their survival. They do this by their up and down movement in vegetation. It was noted that, unless questing in response to human activity in their vicinity (they are particularly responsive to human breath), ticks rest quiescent deep down in vegetation during the hot mid-day period but move to the upper heights on the vegetation during the cool hours of the early morning or late afternoon periods of the day. This behaviour has been noted in ticks in the field (Short *et al.* 1989). Ticks kept in the laboratory were permanently under constant conditions of temperature and humidity, hence could not manipulate their ambient conditions. This may partly explain the discrepancies in the parasite and tick survival under the two conditions. High relative humidity and low temperatures alone do not adequately explain longevity of the parasites and the infected tick vector as these conditions under the laboratory failed to favour parasite and tick survival. However, nymphal ticks exposed to the laboratory condition of 20 °C and 85% RH humidity showed a comparable survival pattern to those exposed to field conditions. Differences in survival of the ticks kept under the two conditions was not as wide as that of parasites kept under the two conditions. A question arises as to how survival of the ticks can be similar in the laboratory and the field but the survival of the parasite is much better under field conditions. Further investigations have to be undertaken to determine the factors which allow better survival of *T. parva* in the salivary glands of ticks exposed to field conditions than in laboratory maintained ticks.

The majority of infected ticks in the field naturally harbour low infection levels while a minority harbour high infection levels. This overdispersed distribution of *T. parva* infections have been shown to be the norm in adult ticks (Büscher & Otim, 1986; Ochanda *et al.* 1998). The dramatic decline in parasite loads seen in the ticks would appear to imply that the conditions in ticks with high infections are not as favourable for parasite survival as in ticks with low infections. In other words, survival of *T. parva* in ticks seems to be density dependent. This is further supported by the fact that there was a very gradual decrease in the intensity of infection in ticks harbouring low infections throughout the period of exposure irrespective of whether the ticks were kept under natural or laboratory conditions. Further

analysis is, however, required to consider how overdispersed distributions change subject to mortality.

Even though it has been reported elsewhere that *T. parva* may have a detrimental effect on the survival of *R. appendiculatus* (Watt & Walker, 2000), the parasite did not appear to lower survival in *R. appendiculatus* nymphae significantly. This could have been due to the relatively low *T. parva* infection in the ticks (9 infected acini per tick). Except for the last few weeks of tick survival, climatic conditions were relatively favourable for tick survival from the sustained rainfall and mild temperatures experienced throughout the period of exposure. The resulting survivorship curves of the ticks kept under quasi-natural conditions were thus of the typical sigmoidal shape. As expected, nymphal survival would be more closely correlated with ambient RH patterns compared to that of the adult tick. The adult tick, whose body size is larger than that of nymphae (about 10 times), would be better suited to survive unfavourable dry ambient conditions than nymphae (Billiouw *et al*. 1999).

As expected, *T. parva* survived for almost as long as the nymphal tick vector itself under field conditions. This has been shown to be also true for *T. parva* in adult ticks (Young *et al*. 1983; Newson *et al*. 1984).

The results obtained here showing that *T. parva* infections in nymphal ticks survive for about 1 year are new data. This information is very important as it goes some way in elucidating whether maintenance of *T. parva* in endemic areas is due to the cattle host in the form of carrier animals or the tick vector which harbours infection during non transmission. This, and earlier work (Ochanda, 1994) demonstrates that it is quite possible for the adult and nymphal tick to act as reservoirs of infection. Previous work on the development of *Theileria* parasites within tick salivary glands of the tick vectors has been carried out mostly in the adult instar, with that in the nymphal instar receiving scant attention. In view of the fact that more larval and nymphal ticks feed on cattle compared to adult ticks, and that larval/nymphal transmission cycle of *T. parva* is shorter than in the nymphal/adult cycle, it is important that development of *Theileria* in the nymphal instar should be given more attention than it has been given in the past.

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