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SHORT COMMUNICATION

Questing activity of *Rhipicephalus appendiculatus* (Acari: Ixodidae) nymphs: a random process?

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Abstract. In Zambia, an experiment under quasi-natural conditions indicated that *Rhipicephalus appendiculatus* nymphs react to prevailing climatic conditions. Higher temperatures and higher vapour pressure deficits lead to decreased activity. The majority of nymphs (>75%) were recorded at ground level. Simulations showed that larval phenology and temperature during the nymphal premoulting period largely explain the seasonal abundance patterns of nymphs, as observed on cattle, given the absence of a behavioural diapause. Consequently, the effect of climate, as observed in our studies, is masked. However, the results of the present study indicate that daily climatic conditions probably have a much larger effect on the transmission dynamics of *Theileria parva*. The vertical distribution of questing instars is a function of temperature and humidity. In years of unfavourable conditions, nymphs might feed mainly on hosts other than cattle, and this could govern the infection prevalence in the adult population. This suggestion is supported by previous epidemiological studies.

Key words. Activity, modelling, *Rhipicephalus appendiculatus*, *Theileria parva*.

Introduction

Studying activity patterns of ticks in their natural environment during the period of questing is essential when attempting to understand the ecology of these important disease vectors and their control. Mulumba *et al.* (2000) showed that during years with below average rainfall in southern Zambia, nymphs of *Rhipicephalus appendiculatus* play a considerable role in the transmission of *Theileria parva*, which is the causal agent of East Coast fever, one of the most important cattle diseases in eastern, central and southern Africa (Norval *et al.*, 1992). The probability that cattle pick up nymphs depends, among other things, on the ticks' host-seeking activity. Hence, understanding (and being able to predict) nymphal activity patterns is of epidemiological importance and essential for modelling purposes (Floyd, 1987; Randolph & Rogers, 1997). Rechav (1979), Short *et al.* (1989) and Short & Norval (1981a,b) reported on the activity patterns of the different stages of *R. appendiculatus*. Each concluded that the general nymphal activity pattern in time was not influenced by prevailing climatic conditions, although the activity of nymphs did show a diurnal pattern. Rechav (1979) found a lower activity around midday and demonstrates that nymphs were reaching peak levels at around 18.00 hours, as confirmed by Short *et al.* (1989) in short grass during the cool season. Pegram and Banda (1990) also observed that *R. appendiculatus* nymphs were questing early in the morning and afternoon and that from approximately 10.00 until 14.00 hours, the nymphs descended into the shade of ground-level vegetation. However, behavioural observations in the field show that the unfed stages of *R. appendiculatus* may actively avoid extreme high temperatures and low humidity levels (Short *et al.*, 1989). The present study aimed to analyse nymphal activity patterns under quasi-natural conditions in southern Zambia. The epidemiological importance of these findings is discussed by weighing the effect of nymphal activity against the influence of length of developmental periods on the abundance of nymphs on cattle.

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Materials and methods

Nymphal activity experiment

The experiment was carried out at Mazabuka (16°10′S, 27°44′E) in southern Zambia. On 15 May 1997, freshly moulted, incubator-reared *R. appendiculatus* nymphs of Zambian origin were released into three circular, gauze columns (height 40 cm, diameter of 5 cm) (Nylon mesh PES243, 243μ pores, Swiss Bolting Company, Switzerland). This timing coincides with peak nymphal densities on cattle in southern Zambia (Matthysse, 1954; MacLeod, 1970; Colbo & MacLeod, 1976; MacLeod & Colbo, 1976; MacLeod *et al.*, 1977; Pegram *et al.*, 1986). The columns were set up under the cover of a shaded experimental plot covered with dry grass. In each column, 35 nymphs were released. Water was sprinkled close to the columns to reduce nymphal mortality. Daily checking of the number of nymphs present in the column was limited to three occasions (08.00, 10.00 and 17.00 hours) due to the reported diurnal activity patterns (Rechav, 1979; Pegram & Banda, 1990), and also to minimize disturbance. Observations were made until no nymphs were observed for at least 1 month. Ambient temperature and relative humidity were recorded continuously, 3 cm above ground level, using a Rolog® Climatic Device (Kritech, Belgium). Average daily vapour pressure deficits were calculated according to Rosenberg *et al.* (1983):

$$\mathrm{VPD} = 610.6164 * \left(1 - \frac{\mathrm{RH}}{100}\right) * \mathrm{e}^{\left(\frac{17.269 * \mathrm{T}}{\mathrm{T}+237.30}\right)}$$

where VPD = vapour pressure deficit in Pa; RH = % relative humidity; and T = temperature in °C.

A forward stepwise logistic regression was used to investigate the influence of covariates on activity patterns ($P_{\text{adding a variable}} = 0.05$, $P_{\text{removing a variable}} = 0.1$). The response variable is a presence or absence in the gauze column. The covariates that were considered included: time (day), hour (08.00, 10.00 and 17.00 hours), relative humidity (%) and temperature (°C). Robust variance estimates were obtained by clustering for column, a covariate not under investigation but whose variability must be accounted for. This allowed for the fact that observations in columns were not independent. The analysis, performed until nymphs were recorded for the last time, was carried using the statistical software, Stata 7 (Stata Corp., College Station, TX).

Simulation of development period

A simulation model, computing development periods from engorged larva to nymph, was used to determine the role of the developmental period on the abundance of *R. appendiculatus* nymphs on cattle. Magoye Research Station, situated near Mazabuka (Fig. 1), was chosen as representative for the areas studied. The temperature data used were the averages obtained from dekadal records collected between 1950 and 1980 (Muchinda & Venkataraman, 1987). Development times were calculated based on the principle of fractional development as described by Gardiner and Gettinby (1981). Development rates, reported by Branagan (1973), were used to make predictions. For the nymphs, a constant hardening period of 14 days was assumed.

Results and discussion

Nymphal activity patterns

Figures 1 and 2 show the average daily humidity and temperature and a fitted moving average (bandwidth = 7) in the experimental tick-plot at ground level. Although the variable ‘sprinkling’ influenced the humidity, a general trend of decreasing humidity in time was present. Temperature and humidity were strongly correlated, as expected. The study period could be split into four periods with respect to temperature (Fig. 1): (i) a first period with decreasing temperatures; (ii) a second period with increasing temperatures; (iii) a third period with stable temperatures; and (iv) a fourth period, again with increasing temperatures. Figure 3 presents the daily numbers of ticks active as well as the temperature pattern. The stepwise logistic modelling revealed that the following factors significantly contributed to nymphal activity: (i) hour [ticks more active at 10.00 hours ($P = 0.018$) and 17.00 hours ($P = 0.002$) compared to 08.00 hours]; (ii) temperature (higher temperatures led to lower activity, $P = 0.006$); (iii) VPD (higher vapour pressure deficit led to lower activity, $P = 0.029$); and (iv) day (borderline positive effect, $P = 0.055$). Humidity was highly significant in the model but was dropped because of colinearity with temperature. Nymphs were last seen on day 98 (20 August) and a maximum activity of 34% was observed. Sprinkling was increased after day 98 (Fig. 2) (increasing humidity) but this did not stimulate any nymphs. When examining the tubes 12 days later, all nymphs were recovered and were dead.

Simulations

Figure 4 demonstrates that the length of nymphal premoulting period, as a function of date of drop-off of the *R. appendiculatus* larvae, first increases and then decreases. This can result in more than one wave of nymphs, as indicated by the shortening of the intervals between dates of questing near September.

These results indicate that, by contrast to the observations in other studies (Rechav, 1979; Short & Norval, 1981a; Short *et al.*, 1989), nymphal activity is influenced by climatic conditions. Higher temperatures led to decreased activity ($P = 0.006$) and, additionally, higher vapour pressure deficit also led to lower activity. The latter variable indicates that humidity and temperature do not influence nymphal activity patterns independently. Activity

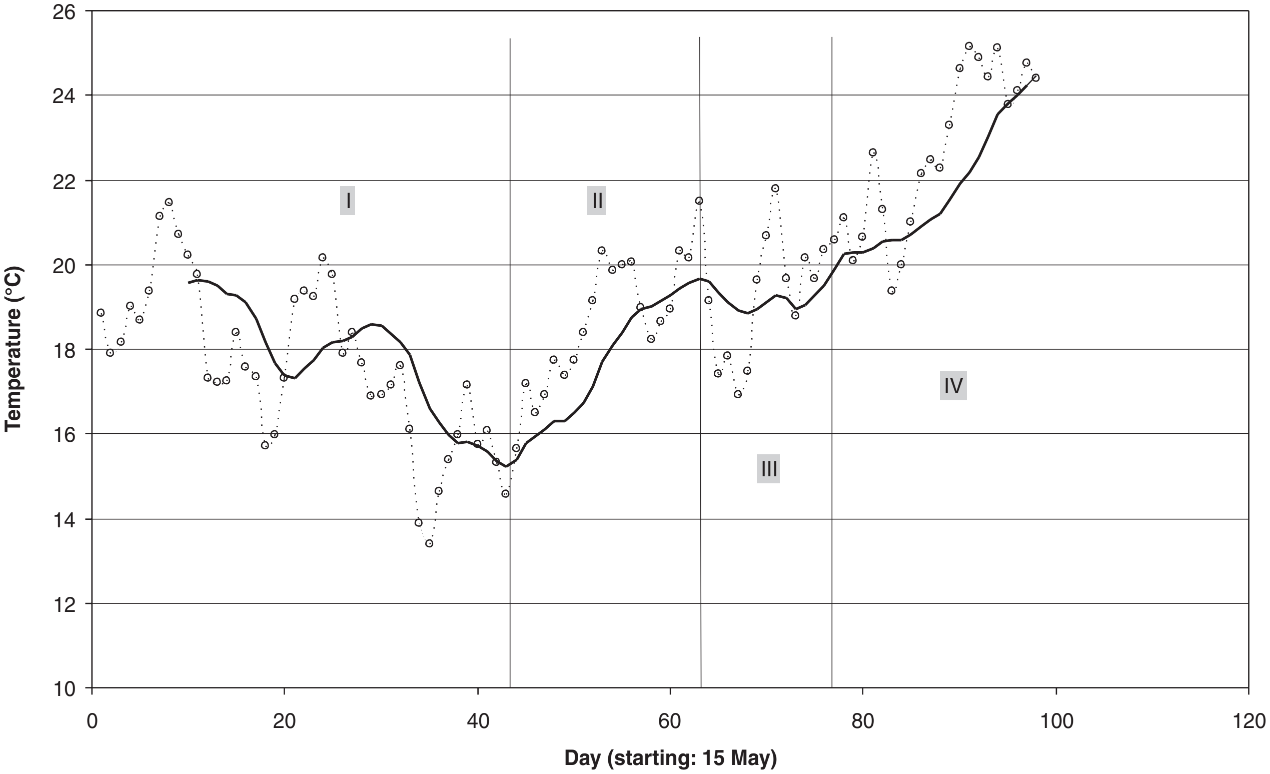

Fig. 1. Temperature as a function of time. Dotted line, observations; full line, moving average (bandwidth = 7).

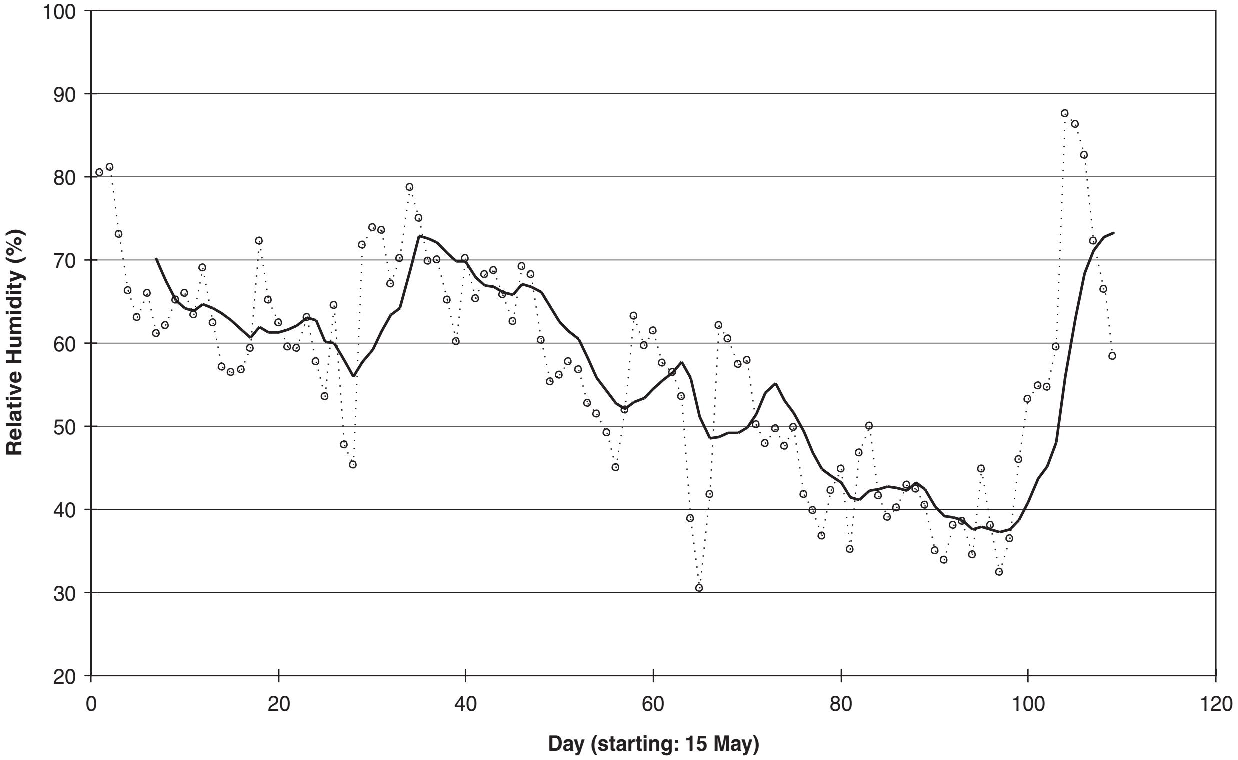

Fig. 2. Humidity as a function of time. Dotted line, observations; full line, moving average (bandwidth = 7).

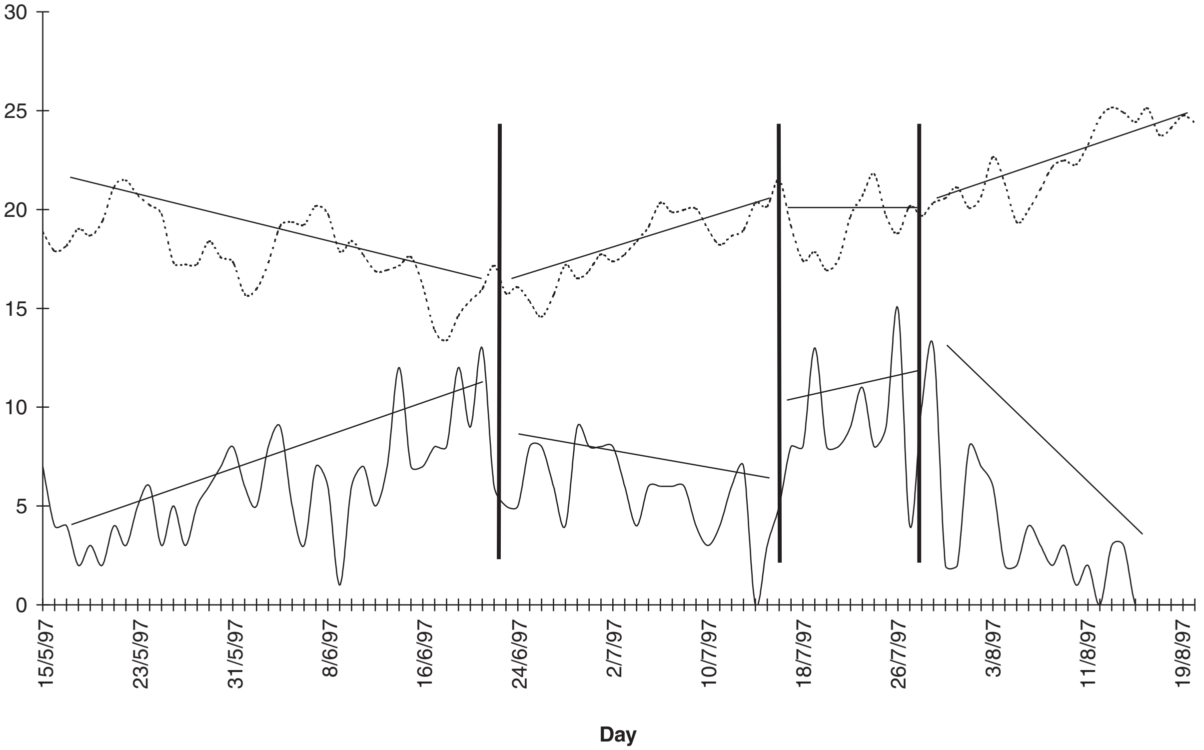

Fig. 3. Daily numbers of active *Rhipicephalus appendiculatus* nymphs (full line), temperature (dotted line) and visual trends (straight lines).

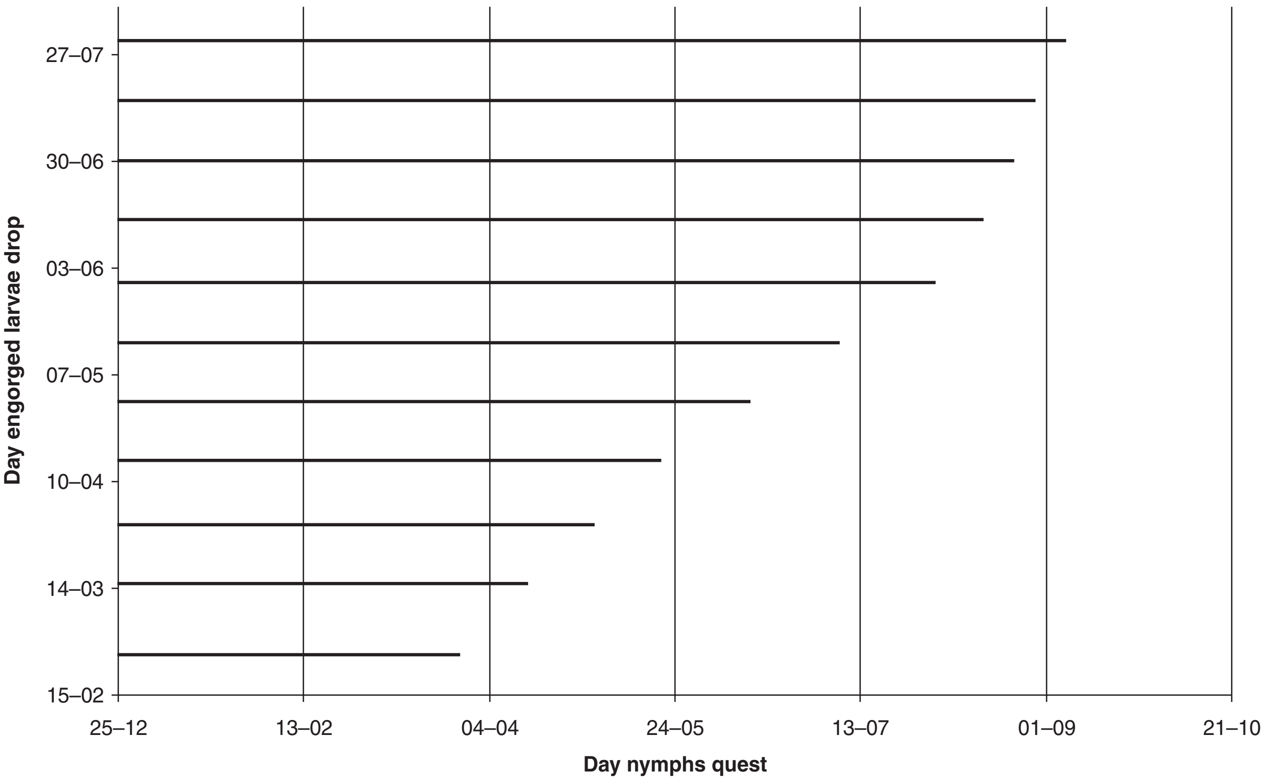

Fig. 4. Results of simulating the development from engorged larvae to questing nymphs in function of day of dropping (y-axis), based on climatic data (Muchinda & Venkataraman, 1987) and development parameters (Branagan, 1973).

started to increase immediately after releasing the nymphs. This was also found by Short *et al.* (1989) and indicates that a diapause in the sense of retarded questing is probably not present. Our findings of diurnal patterns [highest activity seen at 10.00 and 17.00 hours ($P < 0.01$) compared to 08.00 hours] are not in agreement with earlier studies (Rechav, 1979; Pegram & Banda, 1990; and Short *et al.*, 1989). Rechav (1979) indicated that, unlike larvae, the majority (70–80%) of nymphs were recorded at ground level. This is confirmed by the results of our study, with the maximum nymphal activity being 34%. MacLeod (1970) already demonstrated that *R. appendiculatus* nymphs feed on hosts other than cattle. The lower proportion of nymphs questing near the top of the vegetation might thus help to explain the lower number of nymphs found on cattle compared to that expected from the resulting numbers of adults recorded there (Speybroeck *et al.*, 2002). The lower questing activity during periods of lower humidity might also be of importance in the epidemiology of *T. parva*. If, during drier years, more nymphs are found at ground level, then feeding on other hosts than cattle would be dominant. Adult ticks with lower levels of infection would then be expected during the next rainy season. Obviously, the effect of the dry years might be aggravated by a higher mortality in the nymphal population.

The survival of nymphs in Zimbabwe was reported to range from 62 to 158 days in long grass and from 38 to 67 days in short grass (Short *et al.*, 1989). The survival period of 98 days in this study, and observed in the present trial, indicates that conditions were probably similar to the long-grass conditions of Short *et al.* (1989).

Short and Norval (1981a) did not detect an effect of climate on the abundance of nymphs on cattle, and this appears to contradict the observed effect of temperature on nymphal activity under our experimental conditions. At the other extreme, Pegram & Banda (1990) and Mulumba *et al.* (2000) reported two cohorts of nymphs in Zambia, the second of which is recorded on hosts when conditions for nymphal activity are suboptimal (August to September). This phenomenon is also unexplained by the present observations.

However, the latter observation can be explained by the results of the simulation of development periods. It was shown that the very low temperature during the cold season (June to August in southern Zambia) delays development, resulting in a second wave during a period of low humidity and higher temperatures, as suggested by Pegram and Banda (1990). The preceding larval phenology and temperature during the nymphal premoulting period thus largely explains the seasonal abundance patterns of nymphs, as observed on cattle, given the absence of a behavioural diapause. As a result, the effect of climate, as observed in the present study, is probably only secondary and very difficult to discern. However, the results of the present study indicate that daily climatic conditions could have a much larger effect on the transmission dynamics of *T. parva*, governing its infection prevalence in the tick population as a result of differences in host spectra, which themselves are a consequence of the vertical distribution of questing instars as a function of temperature and humidity, resulting in the dramatic differences in East Coast fever epidemiology observed by Mulumba *et al.* (2000, 2001). Ultimately, global warming may change host preferences, lower the infection prevalence in the tick population and upset any development or existence of East Coast fever endemicity in cattle populations.

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References

Branagan, D. (1973) The developmental periods of the ixodid tick *Rhipicephalus appendiculatus* Neum. under laboratory conditions. *Bulletin of Entomological Research*, **63**, 155–168.

Colbo, M.H. & MacLeod, J. (1976) Ecological studies of ixodid ticks (Acari, Ixodidae) in Zambia. II. Ticks found on small mammals and birds. *Bulletin of Entomological Research*, **66**, 489–500.

Floyd, R.B. (1987) Modelling tick populations. 3. Host-finding phase (ed. by R. W. Sutherst). *Ticks and Tick-Borne Diseases*. Proceedings of an International Workshop on the Ecology and Epidemiology of Tick-Borne Diseases, Nyanga, Zimbabwe, 17–21 February 1986, ACIAR Proceedings No. 17, Brisbane.

Gardiner, W.P. & Gettinby, G. (1981) Models based on weather for the development phase of the sheep tick Ixodes ricinus L. *Veterinary Parasitology*, **9**, 75–86.

MacLeod, J. (1970) Tick infestation patterns in the Southern Province of Zambia. *Bulletin of Entomological Research*, **60**, 253–274.

MacLeod, J. & Colbo, M.H. (1976) Ecological studies of ixodid ticks (Acari, Ixodidae) in Zambia. I. Cattle as hosts of the larvae of *Amblyomma variegatum* (F.) and *Rhipicephalus appendiculatus* Neum. *Bulletin of Entomological Research*, **66**, 65–74.

MacLeod, J., Colbo, M.H., Madbouly, M.H. & Mwanaumo, B. (1977) Ecological studies of ixodid ticks (Acari: Ixodidae) in Zambia. III. Seasonal activity and attachment sites on cattle, with notes on other hosts. *Bulletin of Entomological Research*, **67**, 163–173.

Matthysse, J.G. (1954) *Report on Tick-Borne Diseases*. Government Printer, Lusaka.

Muchinda, M.R. & Venkataraman, S. (1987) *Agrometeorological Report No. 12: Dekadal Climatic Normals*. Government Printer, Lusaka.

Mulumba, M., Speybroeck, N., Billiouw, M., Berkvens, D.L., Geysen, D.M. & Brandt, J.R. (2000) Transmission of theileriosis in the traditional farming sector in the southern province of Zambia during 1995–1996. *Tropical Animal Health and Production*, **32**, 303–314.

Mulumba, M., Speybroeck, N., Berkvens, D.L., Geysen, D.M. & Brandt, J.R. (2001) Transmission of Theileria parva in the traditional farming sector in the Southern Province of Zambia during 1997–1998. *Tropical Animal Health and Production*, **33**, 117–125.

Norval, R.A.I., Perry, B.D. & Young, A.S. (1992) *The Epidemiology of Theileriosis in Africa*. Academic Press, London.

Pegram, R.G. & Banda, D.S. (1990) Ecology and phenology of cattle ticks in Zambia: development and survival of free-living stages. *Experimental and Applied Acarology*, **8**, 291–301.

Pegram, R.G., Perry, B.D., Musisi, F.L. & Mwanaumo, B. (1986) Ecology and phenology of ticks in Zambia: seasonal dynamics on cattle. *Experimental and Applied Acarology*, **2**, 25–45.

Randolph, S.E. & Rogers, D.J. (1997) A generic population model for the African tick *Rhipicephalus appendiculatus*. *Parasitology*, **115**, 165–179.

Rechav, Y. (1979) Migration and dispersal patterns of three African ticks (Acari: Ixodidae) under field conditions. *Journal of Medical Entomology*, **16**, 150–163.

Rosenberg, N.J., Blad, B.L. & Verma, S.B. (1983) *Microclimate: The Biology Environment*, 2nd edn. John Wiley & Sons, New York.

Short, N.J. & Norval, R.A.I. (1981a) The seasonal activity of *Rhipicephalus appendiculatus* Neumann 1901 (Acarina: Ixodidae) in the highveld of Zimbabwe Rhodesia. *Journal of Parasitology*, **67**, 77–84.

Short, N.J. & Norval, R.A.I. (1981b) Regulation of seasonal occurrence in the tick *Rhipicephalus appendiculatus* Neumann, 1901. *Tropical Animal Health and Production*, **13**, 19–26.

Short, N.J., Floyd, R.B., Norval, R.A.I. & Sutherst, R.W. (1989) Survival and behaviour of unfed stages of the ticks *Rhipicephalus appendiculatus*, *Boophilus decoloratus* and *B. microplus* under field conditions in Zimbabwe. *Experimental and Applied Acarology*, **6**, 215–236.

Speybroeck, N., Madder, M., Van den Bossche, P., Mtambo, J., Berkvens, N., Chaka, G., Mulumba, M., Brandt, J., Tirry, L. & Berkvens, D. (2002) Distribution and phenology of ixodid ticks in southern Zambia. *Medical and Veterinary Entomology*, **16**, 430–441.

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