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Short Communication

Field Sampling of Unfed Nymphs of *Amblyomma hebraeum*

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(Accepted 9 February 1987)

ABSTRACT

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INTRODUCTION

Amblyomma hebraeum Koch, 1844 is the main vector of heartwater (caused by *Cowdria ruminantium*) in southern Africa (Howell et al., 1978; Norval, 1983). *Cowdria ruminantium* is transmitted trans-stadially from larva to nymph and from nymph to adult. To understand the epidemiology of the disease, therefore, it is necessary to determine infection rates in the unfed stages in the field.

Unfed nymphs and adults of *A. hebraeum* are not collected by conventional sampling techniques such as blanket-dragging, flagging or carbon dioxide traps (Norval, 1974). However, it has recently been shown that adults respond to high concentrations of CO_2, as occurs in the breath of the large mammal hosts of this stage. Norval et al. (1987) found that, in the presence of high concentrations of CO_2 obtained either from the sublimation of dry ice or the breath of cattle or sheep, adults emerge from concealment in cracks in the soil, debris on the soil surface, and elsewhere, and engage in active host-seeking on the

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soil surface. The response to CO_2 is very rapid, but the ticks are not attracted to its source as is the case with several other tick species (Garcia, 1962; Wilson et al., 1972; Gray, 1985). As adults of *A. hebraeum* are large and conspicuous they can easily be collected as they run over the soil surface. Nymphs cannot be readily collected in this manner as they are much smaller, darkly coloured ticks not easily visible against the ground.

This paper reports the response of nymphs to CO_2 and methods that may be used to sample nymphs that are engaged in active host-seeking.

MATERIALS AND METHODS

The study was carried out at the Mbizi Quarantine Area (31°05′E, 21°25′S) in the southeastern low veld of Zimbabwe, where both *A. hebraeum* and heartwater are known to be endemic. Two thousand laboratory-reared nymphs were released into each of nine plots. The plots, which were marked with white string, were 1 m^2 in area, located approximately 100 m apart in mopane (*Colophospermum mopane*) woodland. The soil was covered with dead mopane leaves and twigs and tufts of short grass. The nymphs were released on 8 July 1986 and the plots were sampled between 16:00 and 18:00 h over the next 2 days. On the 1st day each plot was sampled with a single sweep of a 1-m^2 flannelette flag, with the operator approaching the plot from the downwind side. Each plot was then saturated with CO_2 for 2 min using a 1-kg block of dry ice suspended in a loose-mesh string bag, then resampled using the flag. Ticks collected on the flags were returned to the plots. The following day the plots were again saturated with CO_2 for 2 min, then sampled for 1 min using the vacuum apparatus described by Butler et al. (1984). The ticks, soil, and organic debris that accumulated in the vacuum canister were placed in plastic bags and later separated at the laboratory with the aid of sieves.

Other experiments were performed to test the response of nymphs to CO_2 traps. At three sites, 1-m squares of flannelette were placed on the ground and their margins covered with soil to prevent nymphs seeking shelter below them. A 0.5-kg block of dry ice (a strong source of CO_2) was placed on one square, a plastic dish in which 100 g sodium bicarbonate and 100 ml 5% acetic acid were mixed (a weak source of carbon dioxide) was placed on a second square, and the third square was used as a control. Two lots of 1000 nymphs were released at a distance of 1 m (north and south) from the edges of the squares. The nymphs that had migrated to the squares were counted after 1 h.

RESULTS

After their release, the nymphs soon disappeared from the soil surface but reappeared from beneath debris and loose soil when stimulated. There was an almost immediate response when the air above the soil surface was saturated

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TABLE 1

Numbers of nymphs of *Amblyomma hebraeum* that were recovered from nine seeded plots using different methods of sampling

Method of sampling	No. nymphs recovered	
	Mean	Range
Flag	10.0	1- 47
Flag following 2-min CO_2	39.4	1-231
Vacuum following 2-min CO_2	314.4	76-404

with CO_2 from sublimating dry ice. The ticks also responded to the presence of humans. Care was therefore taken when flag-sampling without prior stimulation with CO_2 to approach the marked plots from downwind.

The numbers of nymphs recovered from the plots using different sampling methods are given in Table 1. The numbers recovered by flagging without prior stimulation were low. The numbers recovered were higher after stimulation with CO_2 from dry ice. However, flagging was not an efficient method of collecting these ticks as large numbers of active nymphs (which could not be accurately counted) were still seen on the soil surface after sampling. Much higher numbers were recovered when the plots were sampled using the vacuum apparatus following CO_2 stimulation.

In the tests of CO_2 traps, where strong and weak sources of CO_2 were placed on flannelette squares on the ground, it was found that after 1 h the highest number of nymphal ticks was on the square with the strong CO_2 source (Table 2). Fewer ticks were present on the square with the weak CO_2 source, and very few were present on the control square. The ticks were not seen to be attracted to the CO_2 sources per se, but were distributed fairly evenly over the squares. It appeared that the strong CO_2 source had caused the nymphs to be most active and so more had walked onto this square than onto the square with the

TABLE 2

Numbers of nymphs of *Amblyomma hebraeum* counted on flannelette squares on which there was a strong source of CO_2 (from dry ice), a weak source of CO_2 (from sodium bicarbonate and acetic acid) and no CO_2 (control)

Treatment	No. nymphs
Strong CO_2	198
Weak CO_2	78
Control	14

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weak CO_2 source. In the control, where there was no CO_2 stimulation, few nymphs were active on the soil surface and hence few walked on to the square.

DISCUSSION

In this study we have shown that the behaviour of nymphs of *A. hebraeum* is similar to that of the adults (Norval et al., 1987), in that the ticks shelter beneath debris on the soil surface and emerge in response to CO_2.

Adults were found to respond only to high CO_2 concentrations from either sublimating dry ice or the breath of cattle or sheep, and it was suggested that this ensured that the ticks became active only in the presence of their large mammal hosts (Norval et al., 1987). Nymphs have a much wider host range that includes large and small mammals, birds and reptiles (Norval, 1983) and it is probably for this reason that nymphs are predisposed to respond to lower concentrations of carbon dioxide.

We showed that nymphs, like adults (Norval et al., 1987), are not attracted to CO_2 sources and hence cannot be collected in large numbers using CO_2 traps. However, active nymphs can be collected from the soil surface using flags. The normal absence of nymphs of *A. hebraeum* from drag samples from the field (Norval, 1974) is probably due to the absence of prior stimulation with CO_2 in the areas sampled.

Flagging proved to be an easy but inefficient method for collecting active nymphs from the soil surface (sampling efficiency approximately 2%). Vacuum sampling was more time-consuming but resulted in much higher sampling efficiency (approximately 15%). It would not be practical to sample large areas of pasture using CO_2 stimulation followed by flag or vacuum sampling, but it would be possible to sample certain foci where the ticks would be expected to occur. The engorged larvae of *A. hebraeum*, from which the unfed nymphs emerge, are known to drop from their hosts towards the end of the photophase or shortly after the onset of darkness (Rechav, 1978). Unfed nymphs would therefore be expected to be most abundant in areas where hosts rest in the late afternoon and evening. In the case of domestic cattle in Zimbabwe, the resting places are frequently small fenced 'kraals' in which the animals are kept overnight to protect them from predators and to keep them out of crops. The kraals obviously serve as important foci in the interaction between *A. hebraeum* and its domestic hosts and, for purposes of epidemiological sampling, could serve as a source of unfed nymphs. Carbon dioxide could be used to stimulate the nymphs, which could then be sampled using either flags or a vacuum apparatus.

ACKNOWLEDGEMENTS

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