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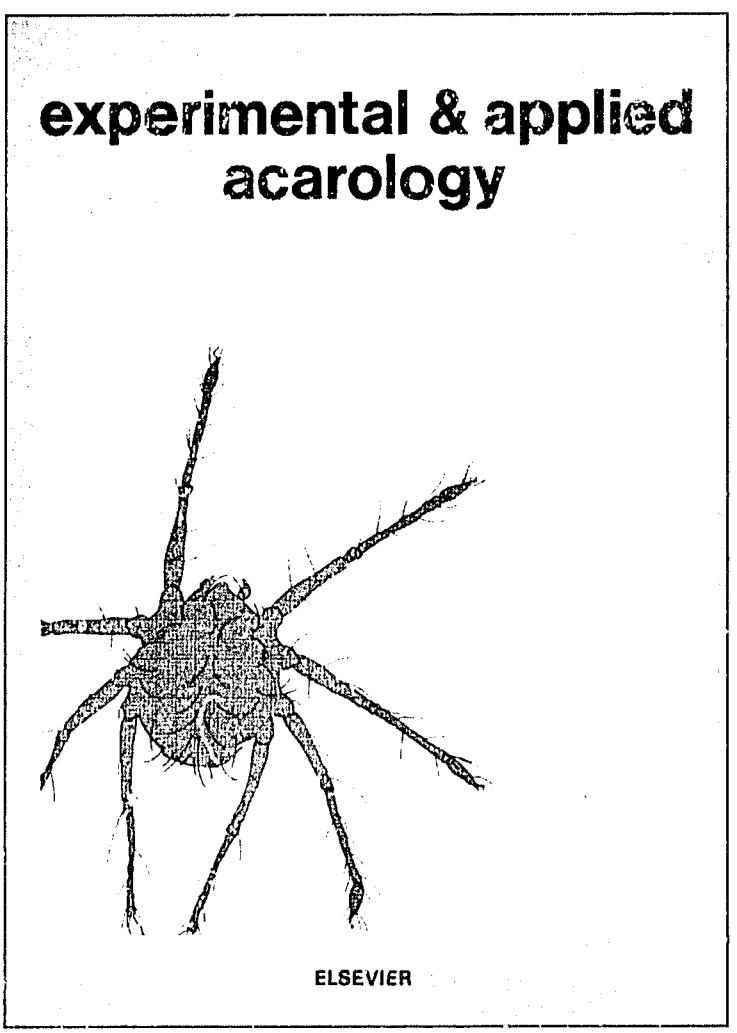

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Field Sampling of Unfed Adults of *Amblyomma hebraeum* Koch

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ABSTRACT

Norval, R.A.I., Yunker, C.E. and Butler, J.F., 1987. Field sampling of unfed adults of *Amblyomma hebraeum* Koch. *Exp. Appl. Acarol.*, 3: 213-217.

No methods have previously been available for the field sampling of unfed adults of *Amblyomma hebraeum*. We released 2000 unfed adults into a 5-m^2 area in mopane woodland in southeastern Zimbabwe. The ticks sought shelter beneath the debris on the soil surface but emerged and became active in host seeking when stimulated. Effective stimuli were the odours of cattle and sheep and high concentrations of carbon dioxide from dry ice. The ticks did not respond to low concentrations of carbon dioxide, vibrations generated by stamping the ground with poles, or two volatile components of ox breath known to attract tsetse flies. The presence of humans in the release area also had little or no effect in stimulating the ticks. As adults of *A. hebraeum* are large and conspicuous they can easily be collected from the soil surface, either manually or using a vacuum device, after stimulation by cattle or the release of high concentrations of carbon dioxide.

INTRODUCTION

Amlyomma hebraeum Koch, 1844 is the most commonly occurring vector of heartwater (caused by *Cowdria ruminantium*) in cattle-producing areas of Zimbabwe (Norval, 1983). To gain a better understanding of the epidemiology of this important disease, it will be necessary to determine infection rates in field populations of *A. hebraeum*. However, the exact whereabouts in the environment of unfed adults of this species is not known and no methods of sampling them have been described. It is known that these ticks are not collected by blanket-dragging techniques or by conventional static carbon dioxide traps (Norval, 1974). Adults of *A.hebraeum*, unlike those of many other Ixodidae,

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do not ascend vegetation and assume a questing posture while awaiting contact with a host.

In this paper we describe the responses of unfed adults of *A.hebraeum* to the odours of cattle, sheep and humans, vibrations, high and low concentrations of carbon dioxide, and acetone and octenol. The last two compounds occur in ox breath and are highly attractive to tsetse flies (Vale and Hall, 1985). A simple method of sampling the ticks is described.

MATERIALS AND METHODS

The study was carried out at the Mbizi Quarantine Area (31°05'E, 21°25'S) in the southeastern lowveld of Zimbabwe, where both *A.hebraeum* and heartwater are known to be endemic. Two thousand laboratory-reared adults of *A.hebraeum* were released into a 5-m^2 area in a paddock in mopani (*Colophospermum mopane*) woodland. The release area was shaded by mopani trees and ground cover consisted of tufts of short grass, dry leaves, twigs, bark and dry cow dung. The area was visited 2 h after the release of the ticks and then 14–19 days later. The first visit was to determine the whereabouts of the ticks in the release area and subsequents visits were to test their responses to various stimuli.

The stimuli tested were:

- one person standing motionless in the release area for 10 minutes;
- three persons walking about the release area for 10 minutes;
- a weak source of carbon dioxide, obtained by mixing 100 g sodium bicarbonate with 300 ml 5% acetic acid, placed immediately outside the release area on the upwind side for 20 min;
- a strong source of carbon dioxide, obtained by placing a block of dry ice (±5 kg) immediately outside the release area on the upwind side for 10 min;
- one calf with legs tied to prevent movement, placed immediately outside the release area on the upwind side for 10 min;
- one adult sheep with legs tied to prevent movement, placed immediately outside the release area on the upwind side for 10 min;
- 40 cows stationary 5–10 m outside the release area on the upwind side for 10 min;
- 20 cows walked once through the release area (1–2 min);
- six poles used by three persons to stamp the ground in the release area for 10 min to simulate vibrations caused by walking cattle;
- one open vial containing acetone placed immediately outside the release area on the upwind side for 10 min;
- one open vial containing octenol placed immediately outside the release area on the upwind side for 10 min.

All tests were carried out between 0800 and 1700 h from 1 to 5 May 1986,

TABLE 1

Numbers of adults of *Amblyomma hebraeum* that became active on the soil surface of the 5-m^2 release area in relation to various stimuli

Stimuli	No. active ticks
One person, motionless (10 min)	0
Three persons, walking (10 min)	3
Weak source carbon dioxide (20 min)	0
Strong source carbon dioxide (10 min)	>200
One calf, immobilized (10 min)	>100
One sheep, immobilized (10 min)	> 50
Forty cows, 5-10 m outside release area (10 min)	>100
Twenty cows walked through release area (1-2 min)	>300
Vibration caused by stamping of six poles (10 min)	0
Acetone (10 min)	0
Octenol (10 min)	0

when the days were warm (20–27°C), the skies cloudless and a weak northwesterly wind prevailed. The tests involving the weak and strong artificial source of carbon dioxide, 20 cows walking through the release area and vibrations caused by the stamping of poles were repeated between 0800 and 1000 h and 1500 and 1700 h to see if the results differed between the mornings and afternoons. Between tests sufficient time was allowed for all ticks to conceal theselves beneath the debris on the soil surface. The tick response at the end of each test was determined by counting the numbers of active ticks on the soil surface.

Two methods of collecting active ticks from the soil surface were tested. The ticks were either collected manually and placed in vials or were collected using the vacuum device described by Butler et al. (1984, 1985) and then sorted from the debris and placed in vials. The ticks were re-released after counting.

RESULTS

The presence of humans in the release area elicited little or no response, as was the case with the weak source of carbon dioxide, the release of acetone or octenol and the vibrations caused by the stamping of the ground (Table 1). The odour of a single motionless sheep did cause a response, as did that of a single motionless calf. The greatest responses were caused by high doses of carbon dioxide from dry ice and 20 cows walking over the release area. The odour of 40 cows standing 5–10 m outside the release area also elicited a clear response. The maximum distances at which ticks responded to the sheep or

calf were 2–3 m and to the dry ice 3–4 m. The tests repeated under morning and afternoon conditions gave identical results.

Manual collection of the active ticks from the soil surface proved to be considerably more rapid than collection using the vacuum device. This was due to the time required to sort the ticks from the debris after vacuuming.

DISCUSSION

This study was initiated in an attempt to devise a method for sampling unattached adult *A.hebraeum* in order to facilitate epidemiological studies of heartwater. A preliminary search for these ticks in a number of potential microhabitats of the study areas during February 1986 had previously yielded negative results. Here, samples of ground litter, animal burrows, and crevices in and under tree bark were made by means of the vacuum device, and by employing CO_2 traps that emitted synthetic pheromone vapors (Butler et al., 1984). The pheromone mixture used approximated the assembly–aggregation pheromone of *Amblyomma variegatum* (Schoni et al., 1984), which is known to strongly attract both sexes of *A. hebraeum* on the host (Rechav et al., 1982).

Several authors (Garcia, 1962; Wilson at al., 1972; Eads et al., 1982; Gray, 1985) have shown that ixodid ticks can be sampled using carbon dioxide traps, and carbon dioxide and other ox odours have been found to attract biting flies (Vale and Hall, 1985). In the present study we have shown that adults of *A.hebraeum* do respond to carbon dioxide, but only in the high volumes that can be obtained from sublimating dry ice or in the breath of large mammals. A weak source of carbon dioxide, obtained by mixing sodium bicarbonate and acetic acid, was used to attract argasid ticks parasitic on reptiles (Butler et al., 1984), but this did not stimulate adults of *A.hebraeum* in the present study. In the latter species, the threshold for the carbon dioxide response is obviously high, and for this reason the ticks are stimulated only when their large mammal hosts (Norval, 1983) are in the immediate vicinity. Humans cause little or no stimulation of the ticks, possibly because their breath is emitted high above the ground.

Once active on the soil surface the ticks did not show a marked degree of aggregation round the source of carbon dioxide but rather appeared to move in a random pattern until a host was encountered. It is probably because of the high carbon dioxide threshold and the behaviour of the ticks once stimulated that previous attempts to sample adults of *A.hebraeum* with static carbon dioxide traps (Norval, 1974) were unsuccessful.

It is not known if adults of *A.hebraeum* respond to any host odours other than carbon dioxide, but they were not stimulated by amounts of the volatile substances acetone and octenol, known to attract tsetse flies.

Because of the rapid response of adults of *A.hebraeum* to high doses of carbon dioxide it may be possible to sample the ticks without the use of traps. Cattle or dry ice can be used to stimulate adults to become active on the soil

surface within a few minutes and these can then be collected. This might provide a means of collecting unfed adults from the field. If incorporated with mark-release-recapture techniques (Sonenshine et al., 1976), it could be used to estimate vector-population densities.

Although the vacuum device of Butler et al. (1984) may be used to collect *A.hebraeum* adults after stimulation, manual collection is preferable in areas where loose debris occurs.

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