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Collection of free-living nymphs and adults of *Amblyomma hebraeum* (Acari: Ixodidae) with pheromone/carbon dioxide traps at 5 different ecological sites in heartwater endemic regions of South Africa

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Abstract. The capture of free-living adults and nymphs of *Amblyomma hebraeum*, the main vector of heartwater in domestic and wild ruminants in South Africa, by means of attraction–aggregation–attachment–pheromone/carbon dioxide traps at five endemic localities in South Africa is described. Although the traps were used successfully at each of the localities, no determination of their efficiency in relation to the actual abundance of ticks at a particular site was made. This study confirmed that the traps could be used in a variety of ecological areas to locate populations of free-living adult *A. hebraeum*.

Key words: *Amblyomma hebraeum*, ticks, heartwater, field collection, pheromone traps

Introduction

Amblyomma hebraeum (Koch) commonly occurs on a wide range of hosts in South Africa (Horak *et al*., 1987). It is also the most important vector of *Cowdria ruminantium*, the causative organism of heartwater in domestic and wild ruminants in South Africa. Although the tick's distribution is well-described (Walker and Olwage, 1987), little is known about its localised micro-habitat in the field (Yunker and Norval, 1991).

Unlike several ixodid tick species, free-living, unfed *A. hebraeum* nymphs and adults do not ascend vegetation to await passing hosts, but seek shelter beneath debris on the soil surface. They become active in response to the attraction–aggregation–attachment–pheromone (AAAP) secreted by feeding male ticks and the carbon dioxide (CO_2) exhaled by their hosts (Norval *et al.*,

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1987). Host location in male and female *A. hebraeum* involves two main responses. Firstly, a response to CO_2 which initiates active searching that is non-directional, and secondly, a directional response to AAAP (Norval *et al.*, 1989b). Two traps were previously designed to collect laboratory-reared *A. hebraeum* that had been released into the field (Norval *et al.*, 1989b). Both involved passing CO_2 over *A. hebraeum* males that had fed and were producing AAAP. Norval *et al.* (1988, 1992) concluded that *A. hebraeum* nymphs were attracted to CO_2 alone, as well as to a combination of CO_2 and AAAP. Adult *A. hebraeum* were, however, not attracted to CO_2 alone, but responded when pheromone was added (Norval *et al.*, 1987, 1992).

The use of AAAP/CO_2 traps has been-described by Butler *et al.* (1984), Norval *et al.* (1989b) and Yunker *et al.* (1990). In their studies the ticks captured were mostly laboratory-reared and had been released under clearly defined experimental conditions. However, Rechav and Whitehead (1978), Norval *et al.* (1989a) and Yunker *et al.* (1990), noted that wild *A. hebraeum* adults were attracted to the pheromone source together with experimentally released ticks. Subsequently there have been two reports on the collection over a sustained period of time of free-living, unfed nymphs and adult *A. hebraeum* with AAAP/CO_2 traps. Peter *et al.* (1999) collected 292 adult *A. hebraeum* with the traps in the Kruger National Park (KNP), and Bryson *et al.* (2000) collected 292 nymphs and 1196 adult *A. hebraeum* over a period of 2 years at a communal grazing area (CGA) in South Africa. Their findings indicated that, provided certain climatic, seasonal and vegetational factors were taken into consideration, AAAP/CO_2 traps could be used successfully at those specific sites for the capture of free-living nymphs and adults of *A. hebraeum* (Bryson *et al.*, 2000).

During this project, five very different sites, from a geographical, ecological and tick management viewpoint, were chosen to test the attractiveness of the AAAP/CO_2 traps. All of these sites were located in heartwater endemic areas of South Africa, and the ticks were collected as part of a wider project to test for heartwater prevalence in localised populations of free-living, unfed adult *A. hebraeum* (Bryson, 2000).

Materials and Methods

Trap construction

Details of the trap design have been provided by Bryson (2000) and Bryson *et al.* (2000), and are briefly repeated here.

The trap comprised a carbon dioxide source and pheromone source. Initially small pieces of broken dry ice were used, as the CO_2 source, but later large solid blocks (5.5 kg, 15 cm × 15 cm) of dry ice were preferred. Pheromone was obtained by immersing feeding male *A. hebraeum*, collected from undipped cattle, in 99% diethyl-ether at a rate of approximately one tick per millilitre of ether. The ticks in ether were stored in a 2.5 l brown glass bottle with a tight-fitting screw-on lid and kept in a cool dark place. Batches of feeding male *A. hebraeum* were added when they became available, and the bottle was shaken periodically to mix the volatile pheromone with the diethyl-ether. Small quantities (5–10 ml) of ether–pheromone extract were transferred to a 50 ml brown glass bottle for field trips and this was stored in an insulated box to keep it cool.

Operation of the traps in the field

On each visit to a field site, climatological parameters were recorded, and soil temperature and relative humidity were measured in the shade and recorded throughout the day with a thermo-hygrometer, and the occurrence of recent rain was also noted. The climatological parameters were recorded as described in the previous paper (Bryson *et al.*, 2000).

Circular pieces of foam rubber (4 cm diameter) were placed in a small plastic bucket and the ether/pheromone extract was poured on to them. The pheromone-soaked foam rubber pieces were then fitted on to four metal pegs (25 cm in length), which were hammered into the ground and surrounded the CO_2 source. The foam rubber pieces were positioned between 10 cm and 15 cm above the soil surface, and radiated around the CO_2 source at a range of 10–30 cm. As the dry ice gave off a white trail it was easy to monitor the direction of the wind and place the traps upwind of potential collection sites. The maximum period of trap efficacy was one day (24 h), unless the dry ice could be stored in a −70°C freezer, as was the case at the KNP.

When the traps were used in an exploratory fashion it was important to exclude localities in which ticks could not survive and to concentrate on those sites where they were most likely to accumulate. The latter include shady bushes, leaf litter, cattle paths, kraals, game tracks and water points. At these sites the traps could be moved every 5–10 min until tick activity was observed, and then all the traps could be moved to the active site.

To enhance the ticks' visibility the traps could be placed in an open, sandy space close to clumps of trees or on a cattle path. The ticks enticed out of the surrounding vegetation and into the open were then readily seen. It was important to inspect the area surrounding the traps, because adult ticks were attracted from several metres away, and were often collected some distance from the traps (maximum 6 m).

By using six or more blocks of dry ice, each with pegs and their foam rubber attachments, it was possible to surround a large site and thus maximise the effects of the traps. The optimum distance between the traps was approximately 1–2 m, and six traps could attract adult ticks within a maximum area of 36 m^2 (6 m × 6 m).

Collection of nymphs

Amblyomma hebraeum nymphs were attracted to the traps, but were difficult to collect because of their small size. The most successful method of collecting them was to place four or more traps at a single site. After 30 min the operator would lie down between the traps and from a distance of approximately 30 cm above soil level carefully inspect the surrounding leaf litter in which the nymphs could be seen moving in an excited fashion.

Individual nymphs could be caught with fine-tipped forceps, or a pinch of soil and leaf litter containing the nymph could be taken, placed on white paper and the nymph grasped with forceps when it moved. Nymphs were frequently attracted to the pheromone-impregnated foam rubber pieces in which they would attach, and as a consequence, could readily be collected from the rubber pieces.

Collection of adult A. hebraeum

The collection of adult *A. hebraeum* was straightforward as the ticks were clearly visible because of their large size as they scuttled towards the traps. Once the ticks had been caught they were transferred to small plastic containers with perforated stoppers and stored in a humidifier.

Field sites

Five field sites, all in heartwater-endemic areas of South Africa, were selected to determine whether ticks would be attracted to the traps in a variety of different ecological habitats.

MEDUNSA

The campus of the Medical University of Southern Africa (MEDUNSA) (25°40′S–27°56′E) is 30 km north-west of Pretoria, in the Gauteng Province of South Africa. This site was selected to test the traps because the domestic stock using the camps were known to carry low numbers of *A. hebraeum*. Camps which were tested were selected because of a history of heartwater mortality in the goats using these camps (Donkin, pers. comm., 1998).

The vegetation on the campus is sourveld with mixed acacia invaders (*Acacia karoo*, *A. nilotica* and *A. robusta*) (Acocks, 1988). The soil is a black loam (turf), and cattle, horses and goats grazed in the camps during the collection period. Seven species of ixodid ticks are known to occur on the goats in this region (Bryson *et al.*, 2001), and *R. evertsi evertsi*, *A. hebraeum* and *R. appendiculatus* are the most prevalent species.

Tick control was mainly aimed at preventing foot abscessation in the goats by controlling *A. hebraeum* with foot baths and hand spraying. Ten sites within the three main camps were sampled during 1996/7. Five separate visits were made to the camps at MEDUNSA (09:00–15:00 h), and 158 settings of the AAAP/CO_2 traps were used to locate ticks.

Ludlow farm, Warmbaths, Northern Province

'Ludlow' (25°10′S–28°20′E) is a well-managed bushveld cattle farm where heartwater is problematic, and consequently young calves are vaccinated at an early age. The farm has been used to test prospective heartwater vaccines (Van der Merwe, pers. comm., 1998). Ludlow is a registered Bonsmara stud farm and there were few ticks on the cattle. The herd is grazed on mixed *Acacia* bushveld and old cultivated lands. For tick control pour-on acaricides are used on a regular basis. The vegetation is a black turfveld (Acocks, 1988), with invasion of *A. karoo*, *A. nilotica* and *A. tortillis*, and 12 sites were sampled on two visits during 1997 using 88 settings of the traps. Trapping started at 09:00 h on both days and continued until the early afternoon (14:00 h). Temperature and relative humidity were monitored throughout the day.

'Little Go' farm, East London, Eastern Cape Province

'Little Go' farm (33°4′S–27°47′E) was chosen because of the abundance of *A. hebraeum* on the farm, and the high prevalence of heartwater in cattle, sheep and goats in the region. East London is situated on the south-eastern coast line and is 1100 km from Pretoria. The vegetation is described by Acocks (1988) as coastal forest and thorn veld, and although most of the indigenous coastal vegetation has been removed, gullies and valleys containing this vegetation still remained. *A. karoo* and other acacias have also invaded the area, and the grasses are sourish mixed species (Acocks, 1988).

Historically the farm has been used for acaricide efficacy trials conducted by the South African Bureau of Standards (SABS) and ticks have been seeded on to the pastures. The species of domestic animals on the experimental farm varied according to the trials in operation at that time. Beef and dairy cattle, Angora goats and dogs (Beagles) were present during the trial period.

Two and a half days were spent collecting ticks, but as blocks of dry ice could not be obtained, chipped dry ice (1 kg) wrapped in muslin bags was used. Trapping on day 1 (15 December 1997) started at 16:00 h and 24 traps were set at three sites, but there was low tick activity. The following two days (16 December 1997, 17 December 1997; 08:00–16:00 h), the traps were moved to seven other localities and the tick response improved considerably. A total of 150 traps were set during the $2\frac{1}{2}$-day collection period.

Kruger National Park, Mpumalanga Province

The Kruger National Park (KNP) was chosen as one of the five sites to test the AAAP/CO_2 trap because no form of tick control had been applied in the area for many decades. Wild and domestic animals had also been kept apart for almost a century, especially since fencing had been erected to keep game and domestic stock apart. The KNP is also one of those areas where the influence of man and domestic animals is minimal. This area is possibly the closest to the original Africa, before modern man, cattle and goats arrived. It was presumed that if *Cowdria ruminantium* was present, it circulated only in the wild animals and the ticks.

Skukuza Tourist Rest Camp (25°00′S–31°35′E) is situated in the southern central region of the park, and most of the tick collections with the traps were done in a gully next to the Nwaswitshaka Research Camp at Skukuza. Other sample sites were all close to the Skukuza Tourist Rest Camp. The vegetation in this landscape zone is described as thickets of the Sabie and Crocodile Rivers (Gertenbach, 1983), and at Skukuza the vegetation is mixed *Combretum* and *Terminalia* woodland (Gertenbach, 1983). There are no domestic livestock in the KNP, and the ticks feed exclusively on the wide range of wild animals and bird species in the park.

A total of 130 traps were set during the 4 days (6 December 1992, 15 September 1997, 16 September 1997 and 17 September 1999) of tick collection. On one of these days (17 September 1997) a subjective assessment of the relationship between the number of ticks collected and the soil temperature or relative humidity (RH) was made. Both climatic parameters were monitored with a thermo-hygrograph on an hourly basis from 06:00 h till 17:00 h.

Songimvelo Game Reserve, Mpumalanga Province

This private game reserve (from 25°57′ to 26°55′S and 30°52′ to 31°00E), is close to the KNP, and was visited only on one occasion (2 December 1992). The game reserve was chosen as a test site because it has only recently been established, after the amalgamation of communally grazed lands. The vegetation is sour bushveld (Acocks, 1988), and it has a similar climate to that of the KNP. There are no domestic stock in Songimvelo Game Reserve (SGR), but a large variety of wild animals have been introduced during the past two decades. No form of tick control was practised at the time of the tick collection, although some private game farms need to use some form of tick control. Twenty traps were set at a number of sites which represented the common ecological areas in the SGR. The same tick collection method was

Table 1. Total numbers of *A. hebraeum* nymphs and adults collected with AAAP/CO_2 traps in various heartwater-endemic areas in South Africa

Locality	Number of ticks collected		Days spent collecting
	Nymphs	Adults	
MEDUNSA	0	31	5
Warmbaths 'Ludlow' farm	0	25	2
East London 'Little Go' farm	17	187	3
Kruger National Park	0	447	4
Songimvelo Game Reserve	0	48	1
Total	17	738	15

used as described for the KNP, and trapping started at 10:00 h and continued throughout the day until 16:00 h.

Results

A total of 738 free-living, unfed adults and 17 nymphs of *A. hebraeum* were collected with the traps at the 5 field sites (Table 1).

MEDUNSA

Thirty-one adults and no nymphs were collected during 5 sampling days (Table 1). Eight *Rhipicephalus simus* adults and 2 *R. evertsi evertsi* males were also attracted to the traps (Table 2).

Table 2. Ticks, other than *Amblyomma hebraeum* that have been attracted to AAAP/CO_2 traps

Locality	Date	Tick species	Ticks collected	
			Males	Females
Rietgat CGA	1996-11-08	*Rhipicephalus appendiculatus*	1	0
Rietgat CGA	1998-01-31	*Hyalomma marginatum rufipes*	1	0
		Rhipicephalus appendiculatus	3	1
		Rhipicephalus evertsi evertsi	3	1
		Rhipicephalus simus	5	3
MEDUNSA	1996-11-11	*Rhipicephalus simus*	5	3
MEDUNSA	1996-11-15	*Rhipicephalus evertsi evertsi*	2	0
'Ludlow' Farm	1997-09-09	*Rhipicephalus evertsi evertsi*	2	1

CGA: Communal grazing area.

'Ludlow' farm, Warmbaths

Twenty-five adult *A. hebraeum* were collected on this farm, and 3 *R. evertsi evertsi* adults were also attracted to the traps (Table 2).

'Little Go' farm, East London

A total of 187 adults and 17 nymphs of *A. hebraeum* were trapped during the 3 days of collection.

Kruger National Park

Four hundred and forty-seven adult ticks were collected over a 4-day period. The soil temperature and the RH were monitored throughout 1 day from 06:00 h until 17:00 h. At 06:00 h the temperature was relatively low (15°C) and no ticks were collected. As the day progressed and the temperature increased to over 20°C (07:00 h), the ticks began to respond to the traps. From 09:00 h onwards activity increased and continued throughout the day with a peak in the early to late afternoon (14:00–16:00 h). The RH started at a high of 75% (06:00 h), but declined steadily throughout the day to reach a minimum of 10% at 14:00 h and then slowly increased to 40% at 17:00 h.

Songimvelo game reserve

Forty-eight adult *A. hebraeum* were collected during the visit to this game reserve.

Discussion

The survey at the Rietgat CGA (Bryson *et al.*, 2000) was the first to employ the AAAP/CO_2 traps on a sustained basis to collect free-living, unfed nymphs and adult *A. hebraeum*. A total of 292 nymphs and 1196 adults were collected during this two-year survey (Bryson *et al.*, 2000). These results were most encouraging as workers in Zimbabwe and in other parts of Africa have experienced difficulties in using the traps on a sustained basis, and had collected only laboratory-reared ticks (Peter, 1995; Hassan, pers. comm., 1997).

Amblyomma hebraeum adults were not common on domestic animals at the MEDUNSA collection site (Donkin, pers. comm., 1998), or in the field, as indicated by the low tick collection success of the traps. Although *A. hebraeum* nymphs occurred on goats at the collection sites (Donkin, pers. comm., 1998), none were collected with the traps. Most of the ticks at MEDUNSA were collected from a single sampling site in the shade of a large *A. nilotica* tree under which goats frequently rested. The soil at most of the sites sampled at MEDUNSA was a black loam and its dark colour masked the visibility of the ticks.

If the assumption is made that where there is a high concentration of free-living, unfed adult *A. hebraeum* in the field, such as apparently occurred at the Rietgat CGA (Bryson *et al.*, 2000), many ticks should be collected, the converse should also be true. In an area of presumed low tick density, such as MEDUNSA, very few ticks were collected despite many hours of trapping. There was obviously no inherent problem with the trap design as they elicited a high tick response at the Rietgat CGA, where they were concurrently being used (Bryson *et al.*, 2000). Tick control at MEDUNSA, which included regular acaricide application to cattle and goats, probably contributed to the reduction in the population of free-living ticks.

At Warmbaths (Ludlow farm) 2 days of trapping during which 12 trapping sites were used, resulted in only 25 ticks, of which 21 originated from a single site. Although few ticks were collected on this farm, medium to heavy burdens of *A. hebraeum* were sometimes encountered on livestock (Van der Merwe, pers. comm., 1997). A number of reasons probably accounted for the poor tick catch at Ludlow farm. Good management, coupled with ongoing pour-on acaricide tick control, undoubtedly resulted in decreased numbers of free-living ticks. The landscape type, *A. karoo*/black turf, was similar to that at MEDUNSA, and in both areas tick collection was disappointing.

The experience at Little Go farm near East London is a good example of how the use of the traps can to be adapted to local conditions. Initially, it was thought that there were few adult *A. hebraeum* on the farm. However, because the traps were portable they were moved to several places on the farm, and good tick collection sites were eventually located. The pockets of coastal forest in the grasslands were the most productive sites, and by concentrating the traps in these pockets and moving them frequently, a large area was eventually sampled. The East London farm was also the only locality at which nymphs were collected (Table 1). This demonstrated that AAAP/CO_2 traps can be used to locate and collect large numbers of free-living, unfed *A. hebraeum* even on well-managed farms on which ticks may only be present in ecologically suitable pockets.

Of the five localities sampled the greatest number of adult *A. hebraeum* was collected in the KNP, and besides the gully sites near the research camp the traps were also successfully used at a number of other sites. This indicated a high population of free-living, unfed adult *A. hebraeum* confirming the observations of Horak (1998) that *A. hebraeum* is a common tick on many of the wild animals in the KNP. Spickett *et al.* (1991) also found that *A. hebraeum* was the most abundant larval species collected by drag-sampling the vegetation in the KNP. However, a more intensive study needs to be done to locate *A. hebraeum* nymphs in the KNP as few of these were detected by drag-sampling and none by AAAP/CO_2 trapping.

There appeared to be a direct relationship between soil temperature and tick activity as measured throughout 1 day in the gully near the Nwaswitshaka Research Camp in the KNP. At 06:00 h when the soil temperature was low there was no tick activity, however, this increased substantially after 09:00 h once the temperature rose. Provided this observation can be repeated at this and at other localities, it would appear that field-work on the adults of this species should not start before 09:00 h. The effect of RH on tick collection was more difficult to ascertain, but there appeared to be an inverse relationship during the single day that observations were made.

The KNP is an excellent locality at which to collect adult *A. hebraeum* because of the tick's abundance there. This is in part due to ideal vegetation and climatic conditions with virtually no frost, no acaricidal control and an abundance of wild animal hosts. Future research in the KNP should investigate seasonal changes in the population of adult *A. hebraeum*, and whether it is possible to collect adult ticks in midwinter. The traps also worked well at the SGR implying that there were large numbers of free-living adult *A. hebraeum* at this locality.

An important observation made during an earlier study was the effect of vegetation cover on tick abundance (Bryson *et al.*, 2000). In South Africa many of the trees lose their leaves, especially towards the end of summer and during autumn (March–May). These leaves give rise to a deep litter, creating excellent habitats for adult and nymphal ticks during the dry, cold winter. They also constitute excellent sites to collect ticks. However, dense, living vegetation can have a negative effect on the visibility of ticks to the collector, especially in late summer when the cover is so dense that it is almost impossible to see adult ticks. The earlier study also revealed that there was a seasonal response to the traps, and that during winter very few ticks were collected (Bryson *et al.*, 2000).

Conclusions

Provided large numbers of free-living adult *A. hebraeum* are present and the operator is prepared to spend time finding favourable habitats, these ticks can be successfully collected in a variety of geographically isolated ecological areas using a battery of AAAP/CO_2 traps.

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