

CHAPTER TWO

2. THE USE OF THE ATTRACTION-AGGREGATION-ATTACHMENT-PHEROMONE/CARBON DIOXIDE TRAP UNDER DIFFERENT FIELD CONDITIONS IN SOUTH AFRICA

The AAP/CO₂ trap was used at six different areas in South Africa (Fig.6, 1 - 6). A two-year survey was carried out at the Rietgat CGA (1). Further collections were made at the campus of the Medical University of Southern Africa (MEDUNSA) (2), and a well-managed bushveld cattle farm, Dr L van der Merwe's farm "Ludlow" near Warmbaths (3). The SABS Farm in East London (4) was an experimental farm where tick seeding had occurred for a number of years, and was typical of the Eastern Cape coastal vegetation. The AAAP/CO₂ trap was also used at two game reserves. At the Kruger National Park (5) the trap was used in the vicinity of the Skukuza main camp. The trap was also tested at the Songimvelo Game Reserve (6).

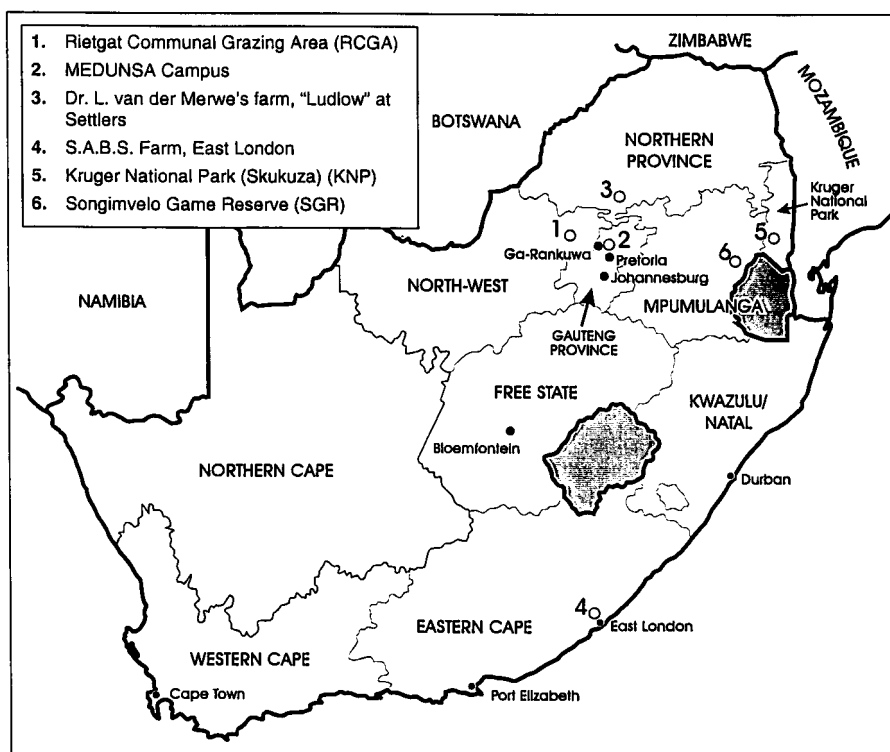


Fig. 6 Map of South Africa, illustrating the six different areas (1 - 6) where the AAP/CO₂ trap was used to collect ticks

2.1 THE COMMUNAL GRAZING AREA AT RIETGAT, NORTH WEST PROVINCE (POOR TICK MANAGEMENT)

2.1.1 INTRODUCTION

The Rietgat CGA belongs to a rural community 50 km north-west of the Medical University of South Africa (MEDUNSA). Rietgat is one of a number of similar communities in the region which include Maboloka, Wintersveldt, Bethany, Madinyane, Lethlambile and Jericho.

The Veterinary Faculty at MEDUNSA has been involved in servicing these regions for over a decade, by outreach clinics which were run at Maboloka, Lethlambile and Winterveldt. Short-term objectives of the clinics were to give veterinary students clinical exposure, as well as providing a clinical service to the local communities. Education in veterinary primary health, extension services, community development and the accumulation of research data were the long-term objectives.

In the early 1990's, Tice, Stewart, Bryson, Du Plessis & De Waal initiated a project to investigate the population dynamics of ticks, and the serological prevalence of TBD in cattle in four communally grazed areas in South Africa. The two-year survey entailed monthly visits to the CGA. Three of these CGA namely, Rietgat, Madinyane and Bethany, all in the North West Province of South Africa, were selected because dipping management was poor and erratic. These CGA were compared with another at Geluk, which borders the KNP (Fig. 6), and where tick control was for many decades historically excellent. At all of

these CGA there had been some subsidization of dipping, which was at the time being phased out, and the cattle owners now had to pay for the cost of tick control.

A survey by Tice (1997) showed that eight species, namely *A. hebraeum*, *Rhipicephalus appendiculatus*, *R. evertsi evertsi*, *R. simus*, *Boophilus decoloratus*, *B. microplus*, *Hyalomma marginatum rufipes*, and *H. truncatum* were present on cattle and goats virtually throughout the year. At Rietgat *A. hebraeum* was present on the cattle throughout the year, and the relative abundance of this tick was greater than that recorded in other bushveld areas of South Africa (Londt *et al.* 1979). There was a distinct seasonal variation in its abundance with a summer peak from October to April, and a spring/winter low from May to September. Indications were that *A. hebraeum* completed only one generation per year at Rietgat (Tice 1997). Up to 70 male ticks were collected per animal during summer and 15 to 20 in the spring/winter. *A. hebraeum* nymphs were abundant on the feet of goats throughout the year and comprised 58% of the total tick burden. As many as 30 nymphs were recorded per goat during summer, and throughout winter at least five nymphs were present.

Young cattle in the CGA were also tested for antibodies to the common TBD and antibodies to *Babesia bigemina*, *B. bovis*, *Anaplasma marginale* and *C. ruminantium* were detected. Seroprevalence of antibodies to *C. ruminantium* was high throughout the study which, when coupled with the high tick burdens, would seem to indicate endemic stability to *C. ruminantium* and other TBD at the Rietgat CGA.

The work of Tice (1997) was extended by Kiwanuka *et al.* (1995) who used participatory research and extension methods to determine which control methods were being employed to control ticks on cattle. It became apparent that tick damage to teats of cows and not TBD was the farmer's primary concern (Kiwanuka *et al.* 1995), and that *A. hebraeum* was the main contributor to this damage. More recently Stewart (1997) conducted a "resource needs' appraisal" in the Jericho region just west of Rietgat. Ticks, and more specifically, tick damage was again shown to be the most important factor affecting cattle in this region.

As a result of these surveys, reliable data on the population dynamics of the ticks and the serology of the common TBD were now available for the CGA. Reasonable progress in defining the needs of the developing farmers in these regions had also been made. Little, however, was known about the free-living stages of any of the ticks found in the region.

Drag-sampling of the vegetation for free-living larvae by Tice (1997) on the CGA in this region had indicated that the common ticks, with the exception of the *Hyalomma* spp., were present. They were, however, collected in much lower numbers than in other bushveld areas of South Africa such as the Eastern Cape Province (Rechav 1982) and the KNP (Spickett *et al.* 1991). At Rietgat Tice (1997) collected a total of only 40 *A. hebraeum* larvae after 16 flannel drags, one could deduce that either the sampling method did not reflect the true prevalence of *A. hebraeum* in the area, or the population was very small. *A. hebraeum* adults and nymphs are seldom collected by flannel dragging techniques and with this in mind, it was decided to test the efficacy of the AAAP/CO₂ trap described by Norval *et al.* (1989b) in attracting adult and nymphal *A. hebraeum* at the Rietgat CGA.

On 20 October 1992 preliminary trials were conducted with Dr Yunker of the Veterinary Research Institute, Onderstepoort, using a diethyl-ether pheromone extract obtained from feeding male *A. hebraeum* with dry ice as a CO₂ source. Thirty AAAP/CO₂ traps were placed in a variety of ecological habitats in which *A. hebraeum* adults might occur at the Rietgat CGA. Initially few of the traps appeared to be attractive although some *A. hebraeum* adults were collected, but were unfortunately frozen by the dry ice.

At only one site (Rietgat area C), which was close to a cattle kraal and had good leaf litter from surrounding *Euclea crispa* shrubs, was high tick activity seen. Consequently the traps were concentrated in this region only, and by the end of that day 78 adult ticks (35 males and 43 females) had been collected. This was the first report of the successful application of the AAAP/CO₂ trap for the sustained collection of *A. hebraeum*.

In 1996 it was decided to use the AAAP/CO₂ trap to collect adult, and possibly nymphal, *A. hebraeum* at the Rietgat CGA, and to determine the *C. ruminantium* prevalence in these ticks. The following two visits to the same site in February and March 1996 yielded negative results. As the identical pheromone and CO₂ sources were used, it was thought that other factors, possibly climatological, were involved. It was then decided to visit the Rietgat CGA on a regular basis over a two-year period, to investigate the factors affecting the attraction of free-living, unfed adult and nymphal *A. hebraeum* in this region.

2.1.2 MATERIALS AND METHODS

2.1.2.1 Geographical location in South Africa

Rietgat (25° 24S - 27° 49E), Madinyane (25° 22S - 27° 52E), Maboloa (25° 26S - 27° 51E) and Jericho (25° 20S - 27° 49E) are all rural villages (Fig. 6) between 30 and 35 km north-west of the commercial centre of Brits (25° 36S, 27° 49E). All these villages and their adjacent CGA are in the North West Province of South Africa.

2.1.2.2 Study area: Different ecological areas sampled

Rietgat is 65 km north-west of MEDUNSA, which is adjacent to the Ga-Rankuwa Hospital, and situated 31 km north-west of Pretoria, the administrative capital of South Africa. A detailed map showing the Rietgat study area (Fig. 7) was compiled from the 1987 1:50 000 map and an aerial photographic survey map (1991) obtained from the Chief Directorate of Surveys and Land Information in Pretoria.

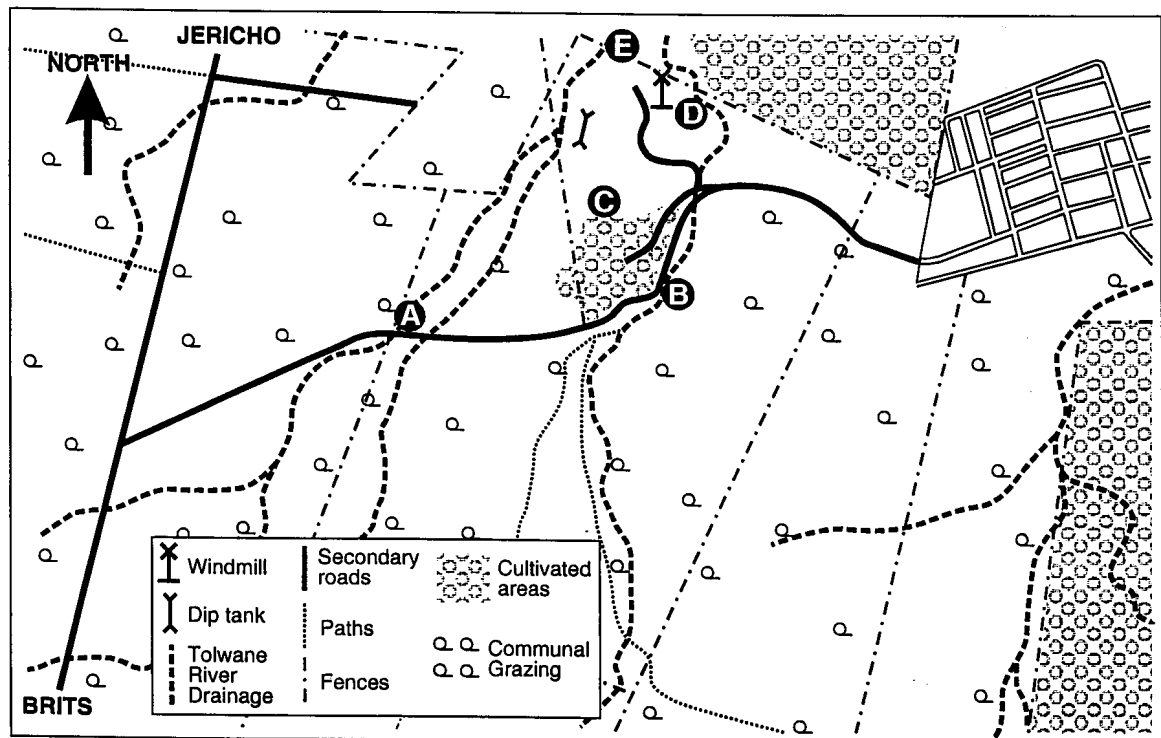


Fig. 7 Map of the Rietgat CGA, North West Province, South Africa, showing the five different ecological areas (A - E), where ticks were collected with the AAAP/CO₂ trap during the two-year field survey

The Rietgat CGA is adjacent to the small village of Lethakaneng (25° 23S - 27° 49E), and access to this area was normally obtained by using the Brits/Jericho road, although it can also be reached from the Maboloka/Jericho road. The altitude in the CGA varies from 1 074 to 1 111 metres above sea level, with the 1 100 metre contour transecting the area. Tributaries of the Tolwane river drain the GCA, and although the river was dry for long periods during the year, there were times when it flowed continually.

Historically this area had been farmed by “white” commercial farmers, but these farms were taken over by the “Apartheid Government” and given to “black” homeland farmers in the former Bophuthatswana. Evidence of previous residences, camps, fences, diptanks, windmills, reservoirs and water points was still visible and many of these structures were still in use. The whole Rietgat area was still fenced and the two access points had gates, which were permanently closed.

Most of the area was set aside for traditional communal grazing for the numerous cattle, goats and donkeys owned by the farmers, whilst other areas were used for cultivated lands, which were left fallow in winter and only used when the summer rains came. A variety of crops were grown in the region and included cotton (*Gossypium* spp.), maize (*Zea mays*), and various vegetables.

Five sampling sites, A to E, were chosen to represent the various ecological areas found at the Rietgat CGA (Fig. 7 and 8). These sampling sites were visited on a regular basis during the two-year survey, and pheromone/CO₂ traps were used to locate ticks.

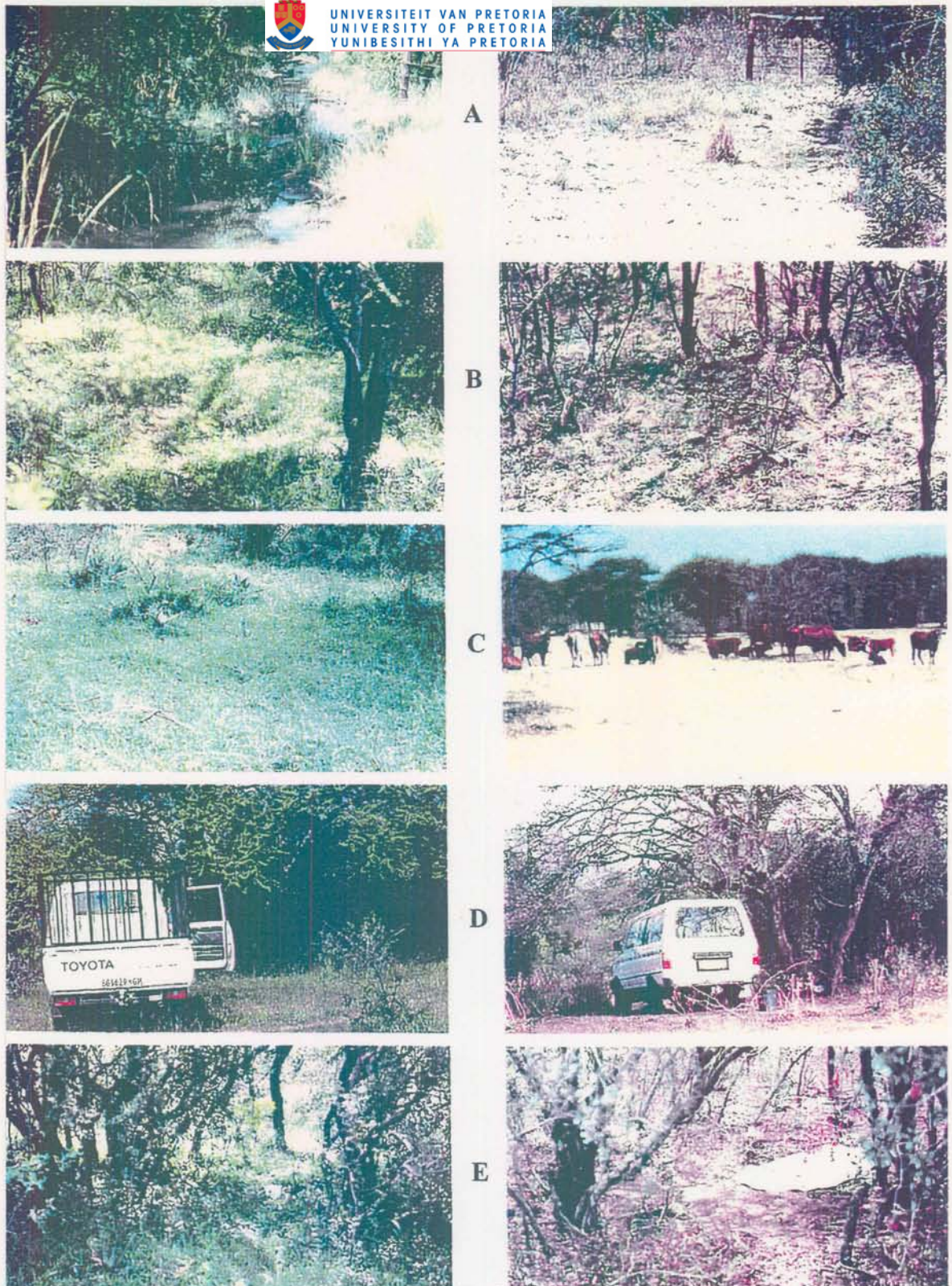


Fig. 8 Typical views of the vegetation types in midsummer (left) and mid-winter (right) at the 5 ecological sites (A - E) at the Rietgat CGA, where the AAAP/CO₂ trap was used: A) Site A, B) Site B, C) Site C, D) Site D, E) Site E

- Site A (Fig. 7 and 8): This was the first site sampled, and was located in the vicinity of the westerly entrance gate in the area where the Tolwane River crosses the road. Four micro-habitats (A/1, A/2, A/3 and A/4) were sampled on a regular basis. A/1 was mainly *Acacia karoo* and *Combretum apiculatum* veld, and was close to a watering point where cattle accumulated. It was very dry in winter, but in summer a number of annual plants were present. A/2, A/3 and A/4 were three sampling sites at the entrance gate A. The Tolwane River crosses the road at this point where there are clumps of tamboti trees (*Spirostachys africana*), with a semi-shaded, deep leaf litter. A windmill was present amongst the trees and there was evidence of cattle and game tracks throughout the area.

- Site B (Fig. 7 and 8): This site also lies close to a branch of the Tolwane river, and six micro-habitats - B/1, B/2, B/3, B/4, B/5 and B/6 - were sampled on a regular basis.

B/1: On the banks of the Tolwane river under a clump of *Acacia tortillis* and *Acacia nilotica* trees, semi-shaded and with deep leaf litter.

B/2: In the riverbed in the shade of *Combretum apiculatum* and *Rhus lancea* trees and with deep leaf litter.

B/3: Open savanna-type grass in the shade of a *Rhus lancea* (karee) tree.

B/4 and B/5: A thick overgrowth of *A. karoo* and *A. tortillis*; the traps were placed on or near a path used by grazing cattle.

B/6: A clump of *E. crispa* trees with a thick leaf litter which was moist almost throughout the year.

- Site C (Fig. 7 and 8): This was the area where the original 1992 collection of ticks was made. At least 10 collection points were tried and eventually three sites (C/1, C/2 and C/3) were found to be productive for the collection of ticks.
 - C/1: Open site next to *E. crispera* shrubs; very dry in winter, but with a meadow-like appearance in summer.
 - C/2: The original site at which Dr Tice and I collected ticks in 1992. It was close to a kraal and was in mixed *E. crispera* and *Acacia* veld, and in winter there was a deep leaf litter.
 - C/3: Site surrounding C/2, shaded and with a good leaf cover.

- Site D (Fig. 7 and 8): A clump of *E. crispera* in the shade of large *A. tortillis* trees. Deep leaf litter throughout the year.
 - D/1: Deep inside the clump of *E. crispera* with almost permanent shade and deep leaf litter.
 - D/2, D/3 and D/4: Sites surrounding D/1, much more open with numerous cacti and no leaf litter, but a sandy surface.

- Site E (Fig. 7 and 8): Situated near the diptank and characterised by a large variety of trees and shrubs.
 - E/1: Dense clump of *E. crispera* with a very deep leaf litter, which stayed moist almost throughout the year.
 - E/2: In the shade of a large tamboti tree with deep leaf litter, especially just after the tree had lost its leaves.

E/3: Small and large clumps of tamboti trees, mixed with weeping wattles (*Peltophorum africanum*) but more open than E/2.

E/4: *E. crispa* shrubs with good leaf litter.

E/5: Mixed *A. karoo* and *A. robusta* with little ground cover present.

E/6: One large tamboti tree with some leaf litter.

E/7: In the shade of a *Rhus lancea* tree.

2.1.2.3 Climate at the Rietgat CGA

2.1.2.3.1 Recording of the climatic data

Climatic data was obtained in two ways. Firstly, locally where the temperature and the relative humidity were recorded at soil level in the shade. This was done with a thermohygrometer (Solvay Animal Health), which is illustrated in Fig. 9a. The temperature and relative humidity were monitored throughout the day. Secondly, climatological data were obtained from the Brits Agro Meteorological Station which is 20 km south-east of Rietgat, and was considered to be representative for the region. The various devices used to measure climate at Brits are illustrated in Fig. 9b. These included rainfall, temperature (minimum and maximum) and relative humidity (minimum and maximum). Wind speed and evaporation data were also recorded, but were not used in this study.



a



b1



b2

- Fig. 9a) Thermohygrometer used to record the ground temperature and relative humidity at the Rietgat CGA
- b) Climatological data were collected at the Brits Agro Meteorological Station: Temperature (maximum and minimum) and relative humidity were measured on the thermohygrograph illustrated (b/1). The rainfall was recorded with a rain gauge (b/2).

At each visit to the Rietgat GCA all the micro-habitats were photographed (Nikon FE2) to illustrate ecological changes which may have occurred since the last visit. It was thus possible to monitor the climatic, vegetational and animal parameters which may have altered. At each visit the strength of the wind was recorded using a “wind index” scale, zero represented no wind and five heavy gale force winds. The occurrence of recent rains was also noted, particularly when this resulted in standing surface water. A “day length index” was also calculated with the minimum day length being the midwinter solstice (21 June), and equivalent to zero; intermediate day length (autumn and spring) as 2.5 and the midsummer solstice (21 December) as a maximum of five.

2.1.2.3.2 Seasonal variation in rainfall

The seasonal variation in rainfall measured at the meteorological station at Brits during the survey is illustrated in Fig. 10a. There were two distinct seasons, a rainy and a dry season. The rainy season starts in September, has a peak in November/December and another in February/March, and then declines in April/May. The dry season spans June/July/August.

Seasonal rainfall was closely followed by a substantial growth of vegetation at the CGA. The wettest month was March, which was followed with some more rain in April and May. In June/July surface water was still present and the grazing was in good condition. In August, September and October there was very little rain and the surface water had dried up completely. The vegetation and animals on the CGA suffered enormously as the temperature increased and grazing and surface water decreased.

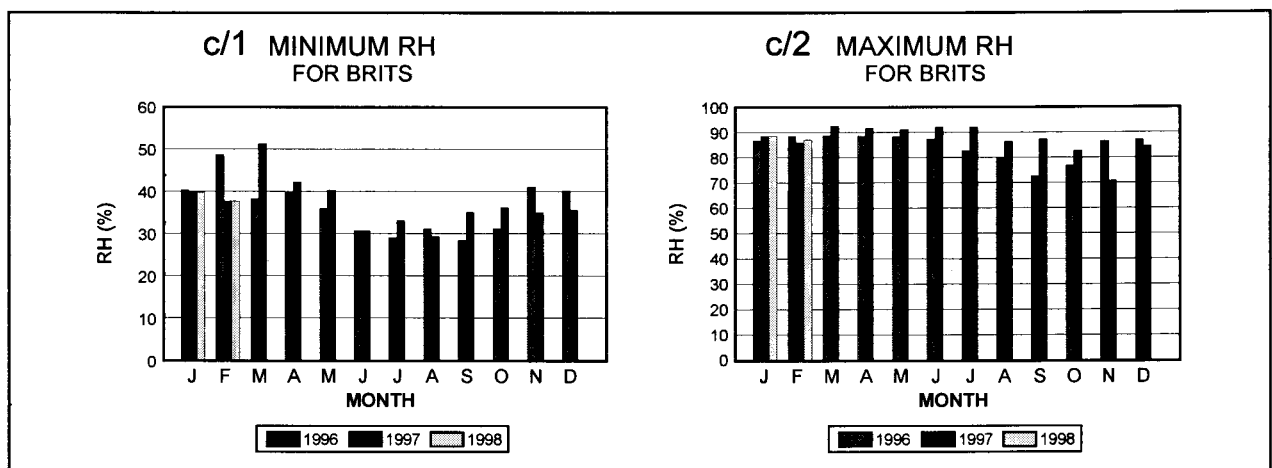
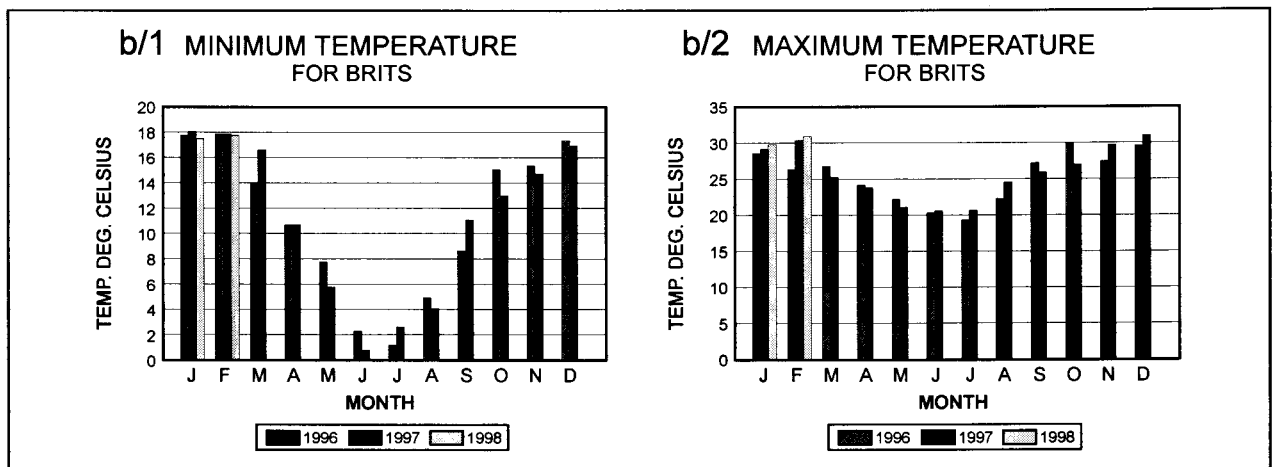
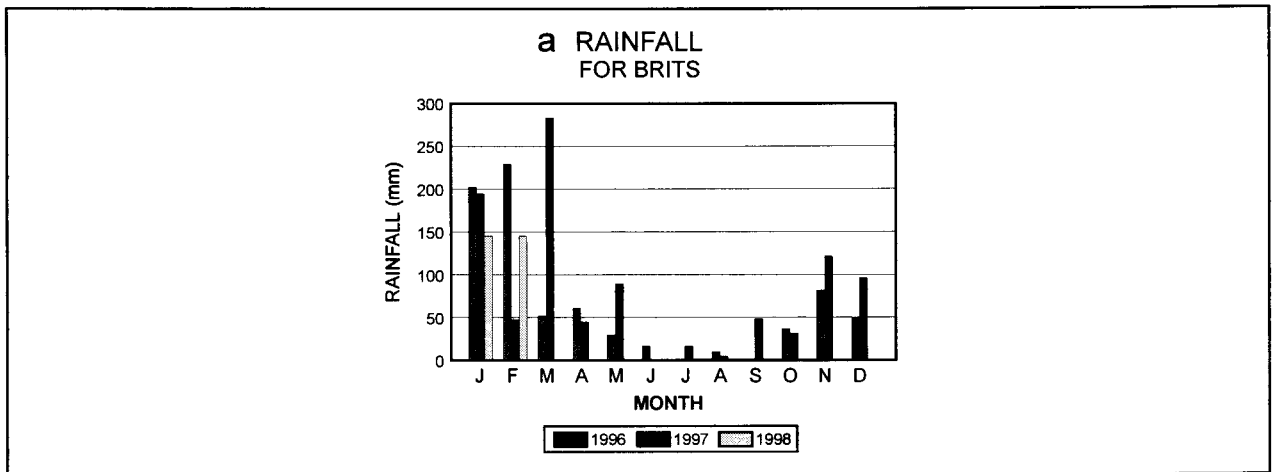


Fig. 10 Climatological data recorded at the Brits Agro Meteorological Station, 20 km south-east of the Rietgat CGA: a) Rainfall, b) Temperature (minimum b/1 and maximum b/2), c) Relative humidity (RH) (minimum c/1 and maximum c/2)

2.1.2.3.3 Seasonal variation in temperature

The minimum (b/1) and maximum (b/2) temperatures recorded at the Brits meteorological station are illustrated in Fig. 10b. The maximum temperatures at Brits (Fig. 10b/2) during the years 1996 to 1998 did not vary greatly and were normally in the range of 20°C to 30°C. Peak maximum temperatures were measured from October to February, and started to decline in March, April and May. The lowest maximum temperatures were recorded in June and July and started to rise again in the spring of August/September. The minimum temperatures at Brits showed greater variation with the lowest minima in June and July and the highest in January/February.

2.1.2.3.4 Seasonal variation in relative humidity

The seasonal variation in the RH measured at Brits is illustrated in Fig. 10c. The maximum RH (Fig. 10c/2) varied little throughout the year (74% to 90%), and was mostly in the range of 80 to 90%. The minimum RH (Fig. 10c/1) ranged from 52% to 28% and for most of the year varied between 30% and 40%.

2.1.2.3.5 Wind and day length

These were initially monitored on a regular basis, but because of difficulties in recording these parameters, a wind and day length index was eventually calculated on the day of the field visit. On the highveld of South Africa certain months of the year are more windy and this was taken into consideration. The photoperiod, i.e. the number of hours of sunshine per day, can affect the behaviour of ticks, and consequently it was decided to calculate a day length index on each visit.

2.1.2.4. Vegetation

2.1.2.4.1 Main vegetation types

The main vegetation types found in the five ecological sites at Rietgat are illustrated in Fig. 8 and 11. Tice (1997) described the vegetation in this site as sourish, mixed bushveld (Acocks 1988), but this site does not fall into any definite bushveld type, but is rather a combination of different types from mixed bushveld (Acocks type 18) through to sour bushveld (Acocks type 20). It is evident that there has been severe overgrazing and bush encroachment by both *A. karoo* and *Dichrostachys cinera*.

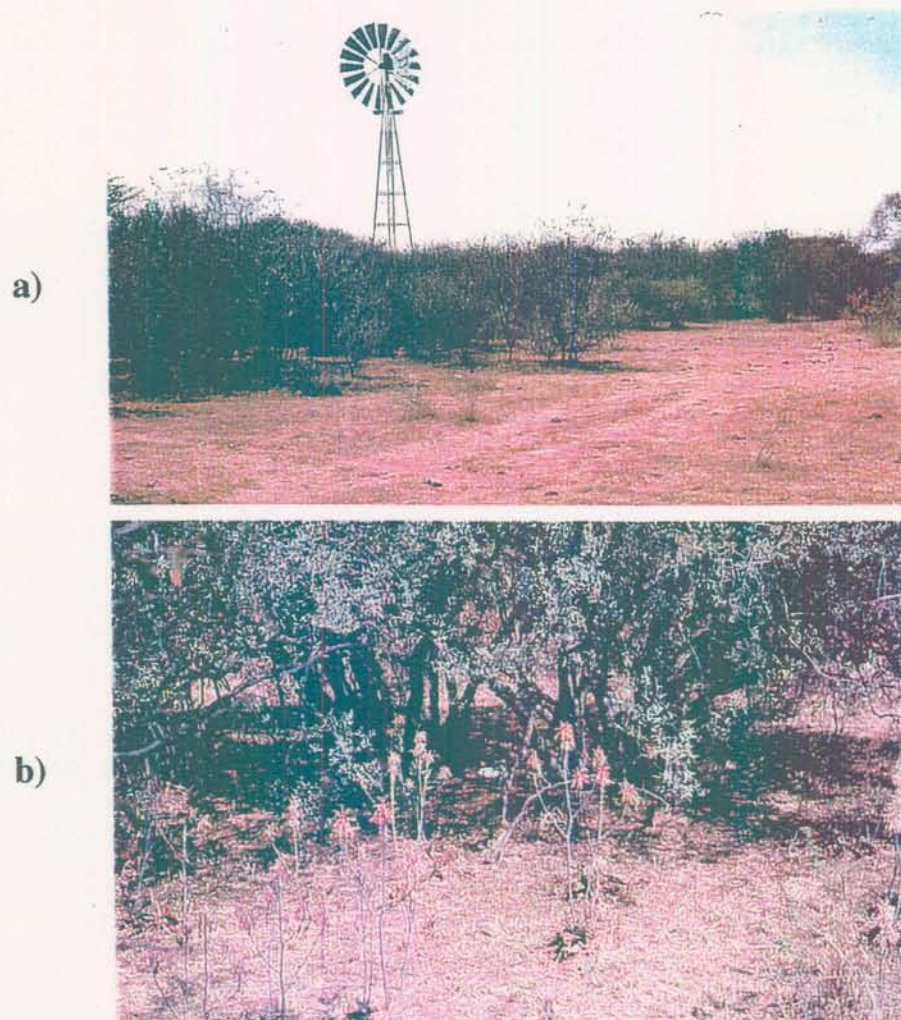


Fig. 11 The CGA at Rietgat comprised mainly *Acacia* sour veld (a) and *Euclea crispa* shrub veld (b).



Rietgat CGA:

1. *Acacia karoo* (sweet thorn)
2. *Acacia robusta* (splendid thorn)
3. *Acacia caffra* (common hook thorn)
4. *Acacia erubescens* (blue thorn)
5. *Acacia mellifera* (black thorn)
6. *Acacia tortillis* (umbrella thorn)
7. *Acacia nilotica* (scented thorn)
8. *Acacia burkei* (black monkey thorn)
9. *Boscia albitrunca* (shepherd's tree)
10. *Dichrostachys cinera* (sickle bush)
11. *Peltophorum africanum* (weeping wattle)
12. *Spirostachys africana* (tamboti)
13. *Sclerocarya caffra* (marula)
14. *Ozoroa paniculosacum* (common resin)
15. *Rhus lancea* (karee)
16. *Rhus leptodictya* (mountain karee)
17. *Rhus pyroides* (common wild currant)
18. *Maytenus heterophylla* (common spike thorn)
19. *Pappea capensis* (jacket plum)
20. *Dombeya rotundifolia* (wild pear)
21. *Combretum apiculatum* (red bush willow)
22. *Combretum zeyheri* (large fruited bush willow)
23. *Combretum hereroense* (russet bush willow)
24. *Euclea crispa* (blue guarri)
25. *Euclea undulata* (small-leaved guarri)
26. *Diospyros lycioides* (Karoo blue bush)
27. *Olea europaea* (wild olive)
28. *Carissa bispinosa* (num num)
29. *Ehretia rigida* (puzzle bush)
30. *Tarchonanthus camphoratus* (camphor bush).

The following grass species were also common at the Rietgat CGA: *Themeda triandra*, *Heteropogon contortus*, *Eragrostis rigidior*, *Aristida congesta* and *Panicum maximum*.

The grassland grazing sites appeared to be of three main types. Firstly the open savanna type grasses which had a predominance of sourveld types. These soon became unpalatable and were frequently burned, so that the cattle could graze the young leaves in spring.

Secondly the open sites where cultivated land had previously existed and which were now taken over by various invader grasses. Thirdly the grasses associated with the trees, which were more sweet veld types, and which were preferred by the cattle.

Within the CGA there were regions where one or more of the tree types predominated and these included:

- Dense *Acacia* veld, usually *A. karoo*, but also mixtures of other *Acacia* species.
- Tamboti (*S. africana*) was found close to the river, often clumped and in dense thickets.
- *Euclea* spp. - the two *Euclea* spp. occurred as shrubs or small clumps of trees often with a dense leaf litter. These areas remained shaded even when most of the other trees had lost their leaves in the very hot, dry season in early summer. The leaf litter remained reasonably moist here throughout the year.
- Mixed *Combretum* veld - the various *Combretum* species were found in forests and were associated with the river.
- *D. cinera* - dense, impenetrable thickets of sickle bush were particularly common where the land had been disturbed for agricultural purposes.
- *Peltophorum africanum* - the weeping wattle was common and also occurred in fairly dense clumps of trees.

2.1.2.4.2 Seasonal variation in vegetation cover

There were enormous changes in the vegetation on the CGA and these are illustrated in Fig. 12. These changes would have direct effects on the collector's ability to locate free-living, unfed ticks. Each ecological site was also photographed and a data bank of slides

representing the ecological changes occurring began to emerge. During the two-year cycle each year was divided into six seasonal cycles which were deemed to be representative of the seasonal changes in vegetation on the CGA.



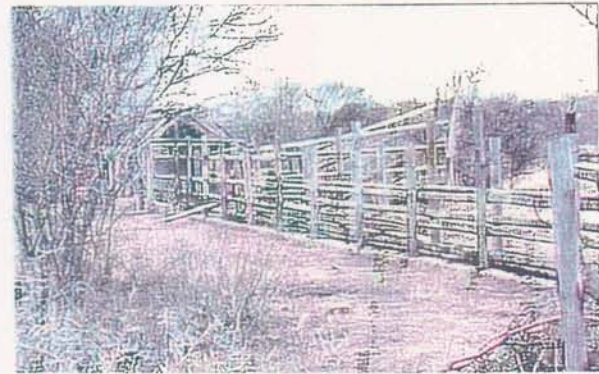
a/1



a/2



b/1



b/2



c/1



c/2

Fig. 12 Seasonal changes in the vegetational cover on the CGA at Rietgat were dramatic:

- ▶ The entrance gate in summer (a/1) and winter (a/2)
- ▶ The diptank site in summer (b/1) and winter (b/2)
- ▶ The grasslands site in summer (c/1) and winter (c/2).

- Midsummer vegetation cover (January/February) (Fig. 12 a/1). The effects of the early rains in October and November were evident on the CGA. Most of the trees had flowered and were in full leaf. The grasses were at their peak and many were seeding. This was a hot, wet period but little of the rain remained as surface water and the river ran intermittently. The increase in vegetation was starting to make tick collection difficult.
- End of summer vegetation cover (March/April) (Fig. 12 b/1). The real effects of the summer rain was evident on the CGA during this period with maximum growth in shrubs, trees and grasses. The dense undergrowth, particularly under the larger clumps of trees, made the detection of ticks almost impossible. The main communal grasslands were at their peak and were seeding. There was a lot of surface water and the Tolwane river was in full flow across the road. The cattle were in peak condition during this period.
- Early winter vegetation cover (May/June) (Fig. 12 c/2). Much of the surface water from the late rains was still present on the roads and the Tolwane river was flowing slowly. Although the CGA was beginning to dry out, as the rains had stopped, much of the grasslands and the cattle were still in good condition. Many of the grasses under the cover of the trees were still green but the open grasslands were now dry and yellow-brown. The asparagus and cotyledonous shrubs were in flower.
- Midwinter vegetation cover (July/August) (Fig. 12 a/2). Four or five months without substantial rains were now beginning to show their effects on the CGA, which were very dry and dusty. The Tolwane river flowed only intermittently, but pools of water were still present. The grassland was a yellow-brown colour, and the veld burning process had started in certain sites. Many of the trees had lost their leaves and this led to a substantial decrease in shade cover. The aloes were in full flower as were the wild pears (*Dombeya rotundifolia*), and the cattle were beginning to lose condition.

- Spring vegetation cover (September/October) (Fig. 12 b/2). The weather during this period was hot and dry although the first rains had come. The surface water was absorbed into the dry dusty CGA. The tamboti trees had also lost their leaves during this period. There was very little surface water and the animals collected at the water points. The Tolwane river was dry and there was little natural shade during this period, and only those evergreen trees and shrubs, such as *E. crispera* and *Rhus lancea*, were able to maintain the moist climate necessary for survival of free-living ticks. The open grasslands were “semi-desert” and the cattle were in very poor condition. The aloes and the cotyledons were in seed. Some of the *Acacias* were beginning to regain their leaf cover and the early *A. caffra* and *A. robusta* were in flower.
- Early summer vegetation cover (November/December) (Fig. 12 c/1). During early summer the first substantial rains fell and the Tolwane river started to flow again. The very hot conditions during this period quickly counteracted the effects of the rain. The early annual plants and grasses started emerging, and the CGA started to recover from the long, dry season. Although there was vegetation growth it was still possible to see adult and nymphal free-living ticks when they were attracted to the trap.

In conclusion the CGA at Rietgat experienced large seasonal variations in vegetation cover which were monitored photographically. This showed that at certain times of the year the vegetation on the CGA was so dense that it was not possible to see the free-living ticks. At other times of the year only a few clumps of shaded vegetation remained and these times were optimal for the collection of free-living ticks which had accumulated there.

2.1.2.4.3 Leaf litter

A. hebraeum is a tick which requires high environmental temperatures and humidity to survive (Norval 1977), and is not found in those regions of South Africa which are too dry

and cold. It is also known to prefer shaded, moist habitats (Rechav, 1982), and with the exception of the larvae, is thought to spend the majority of its non-parasitic life beneath leaf litter (Norval *et al.* 1989b).

During the warm summer rainy season the relative humidity and temperature of most of the ecological micro-habitats at the Rietgat CGA would probably be suitable for the survival of free-living *A. hebraeum*. In the dry, cold season, however, only those sites which remain shaded and moist would be suitable micro-habitats for the free-living ticks. As the majority of the trees on the CGA lose their leaves in winter the surface habitat around their stems would not be an ideal place for the ticks to survive. At Rietgat the *E. crispa*, *Rhus lancea* and *S. africana* clumps have a deep leaf litter which remains moist even in the depth of the dry season. These sites would be ideal for the survival of free-living *A. hebraeum* adults and nymphs.



Fig. 13 Leaf litter, which was especially abundant under the shade of *Euclea crispa* shrubs, remained moist throughout the year. An adult *A. hebraeum* can be seen (arrow) emerging from the leaf litter.

2.1.2.5 Livestock/wildlife on the Rietgat CGA

Cattle on the CGA were in good condition at the beginning of the dry season (Fig. 14), but their condition declined rapidly towards the end of winter.

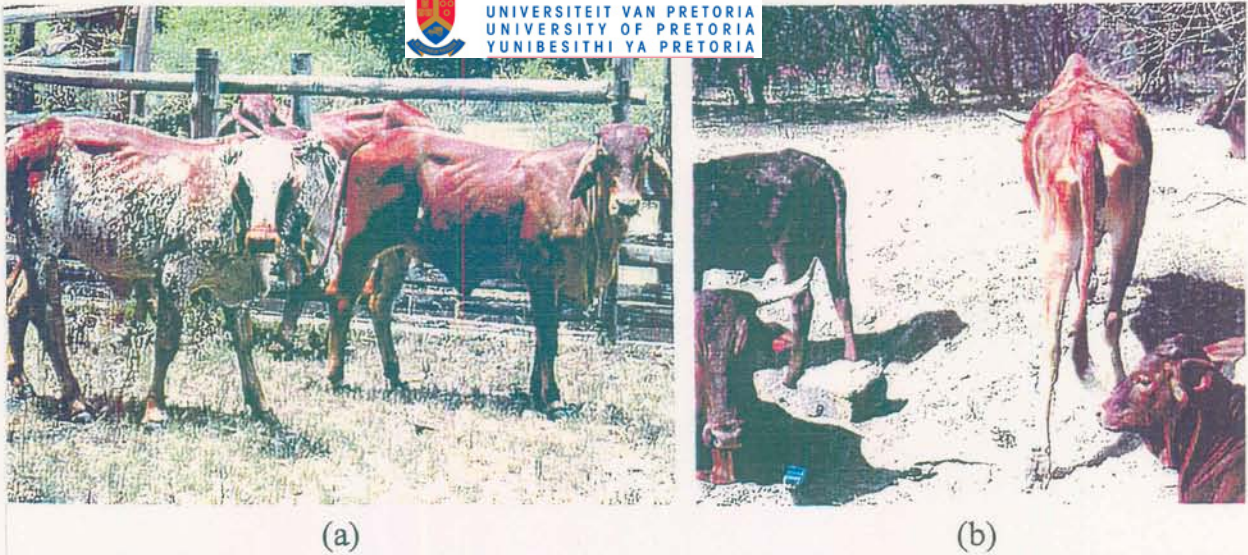


Fig. 14 Mixed type cattle breeds on the Rietgat CGA were in good condition at the beginning of winter (a), but their condition declined rapidly towards the end of winter (b).

In 1991/2/3 there were 153 cattle, 30 sheep and 60 goats belonging to 17 owners (Tice 1995). Most of the cattle were Brahmans and Afrikaners, as well as Brahman-Afrikaner crosses. Some dairy breeds (Simmentaler, Drakensberger, Friesland and Jersey) were also evident in the cross-bred cattle. The goats were all indigenous types and the sheep, Dorper cross-breeds. Pigs (large white and indigenous types) were also present at a small piggery. Donkeys, chickens, geese, turkeys, pigeons and dogs were also common at the communal village. A diptank which was not functioning during the 1991/2/3 project (Tice 1997) was still not functional during this project and the owners appeared to use either no form of tick control, or hand spray or pour-ons. It was difficult to assess the numbers of wild animals present on the CGA. Following discussions with local farmers it was clear that some wild animals were still present, as they were hunted with packs of dogs.

2.1.2.6 Sample size determination

2.1.2.6.1 Factors affecting sample size

Elbers, Stegman, de Jong, Lambers, Koning & Hunneman (1995) stated that during a survey

five essential parameters need to be considered. These are population size, expected prevalence of the disease, permissible error, desired level of confidence and the variance in the disease prevalence.

- Population size

The study population in this survey was the total free-living, unfed adult and nymphal *A. hebraeum* population at the Rietgat CGA. It was presumed that the populations of *A. hebraeum* in the surrounding CGA had similar characteristics to the study population. It was not possible to extrapolate the findings to other populations in South Africa, and the size of the tick population at the Rietgat CGA was assumed to be infinite. The sampling method was not random, as the AAAP/CO₂ trap attracted localised populations (clusters) of ticks (Gummow pers. comm. 1996). To overcome this it was decided to double the size of the required sample. Another sampling bias with this type of survey was that ticks tend to be collected more frequently from one or more high density sites, in order to overcome this problem, up to 25 ecological sites were sampled to get a more representative sample.

- Expected prevalence of disease

There have been no *C. ruminantium* prevalence surveys done on the specific tick population of the Rietgat CGA. Research in other parts of South Africa indicated a prevalence of 5% to 10% (Du Plessis 1985; Du Plessis *et al.* 1992), and in Zimbabwe a much higher prevalence (P) of 0% to 45% in free-living *A. hebraeum* (Norval *et al.* 1990). Because of this it was decided to do a pilot study in which 150 adult ticks were collected and an

estimate of disease prevalence was done on this sub-sample. Once the results of this estimate were available a more accurate sample size could be calculated.

- Permissible error (Desired absolute precision)

The desired absolute precision of a sample is normally the choice of the investigators. In this project, because of financial constraints, it was decided that a 5% level of precision would give a cost-effective sample.

- Desired level of confidence

In general a 95% confidence level gives accurate results.

- Variance

Because our survey population was sampled in clusters, it was decided to double the required sample and to avoid sampling only specific clusters.

2.1.2.6.2 Calculation of the required sample size

It was decided that 2%, 5% and 10% prevalence levels would be used in the initial sample size estimation. These would be adjusted according to the pilot survey results ($n = 150$), and the final estimate would be made when that information became available. The P used for all calculations (Canon & Roe 1982) involved a 95% desired level of confidence and a 5% desired absolute precision.

It was calculated that the prevalence levels would require the following sample sizes, i.e. number of ticks collected:

PREVALENCE	REQUIRED SAMPLE SIZE
● 2% (P = 0.02)	31
● 5% (P = 0.05)	73
● 10% (P = 0.10)	139
● 20% (P = 0.20)	246
● 50% (P = 0.50)	385

As the prevalence increases, the larger the sample size required. The pilot survey using the PCR assay indicated that 4.7% ($\pm 5\%$) of the ticks ($n = 150$) were infected with *C. ruminantium*, and that a field sample of 73 ticks ($P = 0.05$) would probably be sufficient. However, in order to counteract the effects of cluster sampling and other inherent problems with the sampling method, it was decided to substantially increase the required sample size, and eventually 434 adult ticks were collected from the Rietgat CGA for PCR analysis to detect *C. ruminantium*.

2.1.2.7 Field collection of ticks using the AAAP/CO₂ trap

2.1.2.7.1 The apparatus used to collect the ticks in the field

The apparatus used to collect the free-living, unfed *A. hebraeum* is illustrated in Fig. 15.

This included the following:

- Dry ice blocks (5½ kg)
- Pheromone in a small brown glass container
- Metal pegs and foam rubber pieces for pheromone
- 1 pair of large canvas garden gloves to handle the dry ice
- 1 large plastic bucket (black) to carry the dry ice
- 1 smaller plastic bucket (white) to mix pheromone onto the foam rubber pieces
- “Cooler box” to store the pheromone in the field
- Thermohygrometer
- Plastic storage containers for collection of ticks

- Record book, camera, pencil and forceps
- Small mat to lie on
- garden rake to clean site.



Fig. 15 The apparatus used to collect the ticks in the field.

2.1.2.7.2 Pheromone source

The pheromone used in the field collection of free-living, unfed *A. hebraeum* adult and nymphs was obtained from a mixture of feeding male *A. hebraeum* ticks stored in 99% diethyl-ether (Unilab-Saarchem (Pty) Ltd). The adult feeding males were collected from a number of undipped cattle. These cattle were owned by farmers in the area, and were either

restrained on the ground with ropes, or placed in a crush whilst the ticks were collected. Approximately 1 ml of diethyl-ether per tick was used. The main storage bottle was an empty, used 2,5 l brown diethyl-ether bottle with a good screw-on cap. The bottle was kept in the dark in a cool place. Batches of feeding male *A. hebraeum* ticks were added when they became available. The bottle was shaken periodically to mix the volatile pheromone with the diethyl-ether.

For field trips small quantities (5 - 10 ml) of diethyl-ether-pheromone were transferred to 50 ml brown glass bottles which were stored in a cooler box. When field work involved travelling by plane, such as to the East London site, it was necessary to send the pheromone ahead via courier to avoid any dangers involved in the transport of such a volatile and flammable substance.

2.1.2.7.3 CO₂ source

The CO₂ source used throughout the survey was dry ice. Initially small pieces of broken dry ice were used, but it was soon realised that large solid blocks (5½ kg) (Fig. 16) were more efficient. They disseminated CO₂ over a much wider area, were easier to move and lasted longer in the field. Two blocks of dry ice, which were supplied in a box, were collected early in the morning from the Onderstepoort Research Institute and taken to the field. A block of dry ice gave a good, strong source of CO₂ all day.



Fig. 16 A large 5½ kg block of dry ice was used as a source of CO₂. Pheromone-impregnated foam rubber pieces were attached to metal pegs (n = 1 to 4), close to the dry ice.

2.1.2.7.4 Using the AAAP/CO₂ trap in the field

Initial visits were at 07:00 in the morning, but after field experiments at the KNP (Bryson *et al.* 1999) indicated that these ticks only become active after 09:00, then field work commenced at 09:00. On each visit to the Rietgat CGA a certain procedure which was found to give the best results, was followed with the AAAP/CO₂ trap. On arriving at a site the climatological parameters were first recorded. Soil temperature and relative humidity were measured in the shade and recorded throughout the day. Recent rains were noted and the wind index calculated. Any ecological changes were noted and the site was photographed with a Nikon FE camera to record these changes.

(a)



(b)



Fig. 17a) + b) The AAAP/CO₂ traps were easy to use in the field, and were often moved around. Six or more traps were used at a time (a), and the traps could be concentrated in sites where tick activity was highest (b).

A number of dry ice blocks were positioned in close proximity (6 - 8 at a time) around a clump of trees or down a path in order to maximise the effect of the CO₂/pheromone (Fig. 17a). Small (4 cm diameter) pieces of circular foam rubber which were collected from used medicine containers, were fitted onto metal pegs (Fig. 16) and these were placed in a plastic bucket. Ten ml of diethyl-ether/pheromone mixture was then poured onto the foam

rubbers/pegs in the small white plastic bucket. Care was taken to ensure that all the foam rubbers were impregnated with the pheromone ether. The pegs (4 at a time) were then placed around the CO₂ source. It was particularly important that at least one of these pheromone sources was downwind of the CO₂. As the CO₂ gave off a white visible trail it was easy to monitor the direction of the wind. By using six, or even eight blocks of dry ice/pheromone, it was easy to surround a large site with CO₂/pheromone and to maximise the effects of the trap (Fig. 17a).

2.1.2.7.5 Collecting adult ticks

When surveying a new area it was important to locate a site or sites with high tick density. This was not always easy and required patience, time and a good understanding of the ecology of the tick. Certain areas such as the farms at Warmbaths and MEDUNSA were not ideal places to use the trap for reasons which will be discussed later. Rietgat and the KNP were, however, ideal areas because of the high density of ticks in these sites.

When using the traps in an exploratory way it is important to exclude sites where ticks cannot survive, and to rather concentrate on those sites in which ticks are most likely to accumulate. The latter include shade, moisture, leaf litter, cattle paths and kraals, bushes, game tracks and water points. At these sites the traps can be moved quickly, i.e. every 5 - 10 minutes until tick activity is observed (Fig. 17a), then all the traps can be moved into this site and to concentrate the collecting effort here.

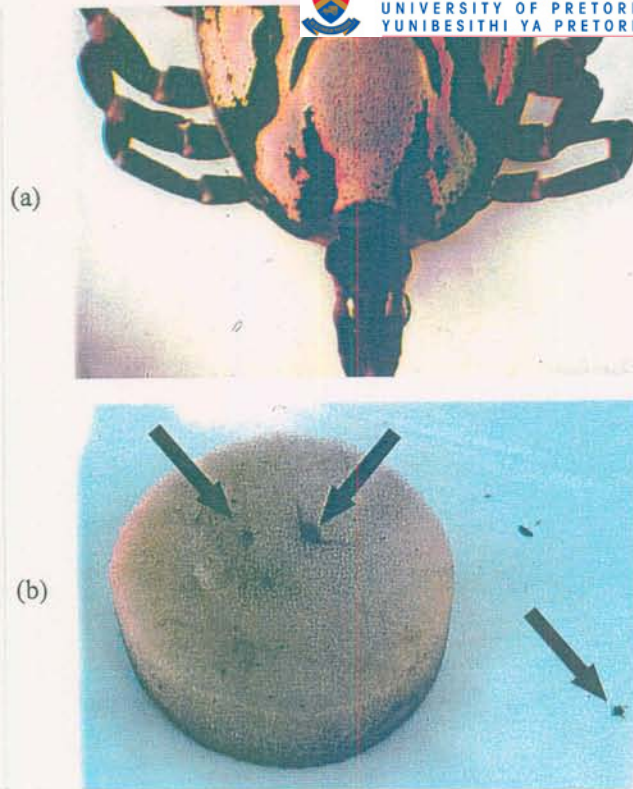


Fig. 18a Adult *A. hebraeum* (male).

Fig. 18b Nymphal *A. hebraeum* (arrows) were also attracted to the AAAP/CO₂ trap, and often attached to the pheromone-impregnated foam rubber pieces.

The collection of adult *A. hebraeum* (Fig. 18a), was comparatively easy as the ticks rapidly move towards the trap and are clearly visible because of their movement. In ecological sites with deep leaf litter, it was imperative to clear the litter with a small rake to make the ticks more visible. Once the ticks had been located they could be collected either by hand or with forceps. The traps could also be placed in an open, sandy space (Fig. 17b), and the ticks could then be enticed out of the dense vegetation and into the open area where they were more easily visible. The same principle was employed when using a cattle path (Fig. 17a), and ticks from the surrounding bushes would be attracted onto the path.

The traps had to be visited on a regular basis in order to stop the ticks from getting too close

to the dry ice, which froze and killed them. Once the adult ticks detected the CO₂/pheromone, they scuttled around in an erratic manner with their front legs waving excitedly. In an area of high tick density, three or more ticks could be caught at a time, all of them making their way to the trap. It was also important to walk around the area where the traps were located, as adult ticks were attracted from several metres and were often collected some distance from the trap. Once the ticks had been caught they were transferred to small plastic containers with an air hole and stored. Care was taken not to leave these containers in the direct sunlight and to store them in the shade. Adult ticks collected during the field surveys were transferred to the containers and stored in a humidifier.

2.1.2.7.6 Collecting nymphal ticks

At Rietgat it was noticed that numbers of small ticks were also attracted to the traps. A sample of these ticks was collected and later identified as *A. hebraeum* nymphs. Certain ecological sites were far better for the collection of *A. hebraeum* nymphs than others. These sites, A/3, B/1, B/6 and E/6 (Fig. 22), all had good shade and a deep leaf litter. The most successful method of collecting these ticks was to put four or more traps in one site. After 30 minutes one would lie down between the traps and carefully view the leaf litter from a distance of 30 cm. The nymphal ticks could be seen moving around in an excited manner, but the collection of the nymphs was difficult as they are small. One could either use fine forceps to collect the nymphs or alternatively one's fingers and take a pinch of soil and leaf litter where the nymph was and place it on white paper and grasp the nymph with fine forceps when it moves.

One or more nymphs were frequently attracted to the pheromone impregnated foam rubbers (Fig. 18b) placed in the vicinity of the CO₂ block. The nymphs collected were also placed in plastic vials and later stored in a humidifier. Both adult and nymphal *A. hebraeum* were transported to Zimbabwe in makeshift humidifiers. An important aspect with free-living, unfed ticks is to keep them moist (cotton wool soaked in water) and to clean the humidifier regularly. These free-living, unfed ticks can survive for several months using this method. This is better than using ticks collected off cattle as these die within a few days or at best weeks. Ticks collected off cattle are also susceptible to fungal and other infections, probably as a result of infected and damaged mouthparts. Field collected ticks are usually clean, flat and easier to keep alive.

2.1.3 RESULTS

2.1.3.1 Total number of ticks collected during the survey at the Rietgat CGA

The total numbers of adult and nymphal *A. hebraeum* collected at the Rietgat CGA are summarized in Tables 4, 5, 6, 7 and 8, and Fig. 19 and 20. There were a total of 23 visits to the Rietgat CGA survey area and 22 of them took place during the 1996/7/8 period. A total of 1 196 adult ticks were collected with the AAAP trap (Fig. 19), with an average of 52 ticks per day (Fig. 20). Fifty-one percent of these ticks were females (n = 604) and 49% were males (n = 592). Sixty-two percent of the total number of ticks (n = 1 934) caught with the AAAP trap were collected at the Rietgat CGA (Table 8, Fig. 19). Ninety-five percent of the total nymphs collected during the survey (n = 309) were from the Rietgat region (n = 292) (Table 8, Fig. 19).

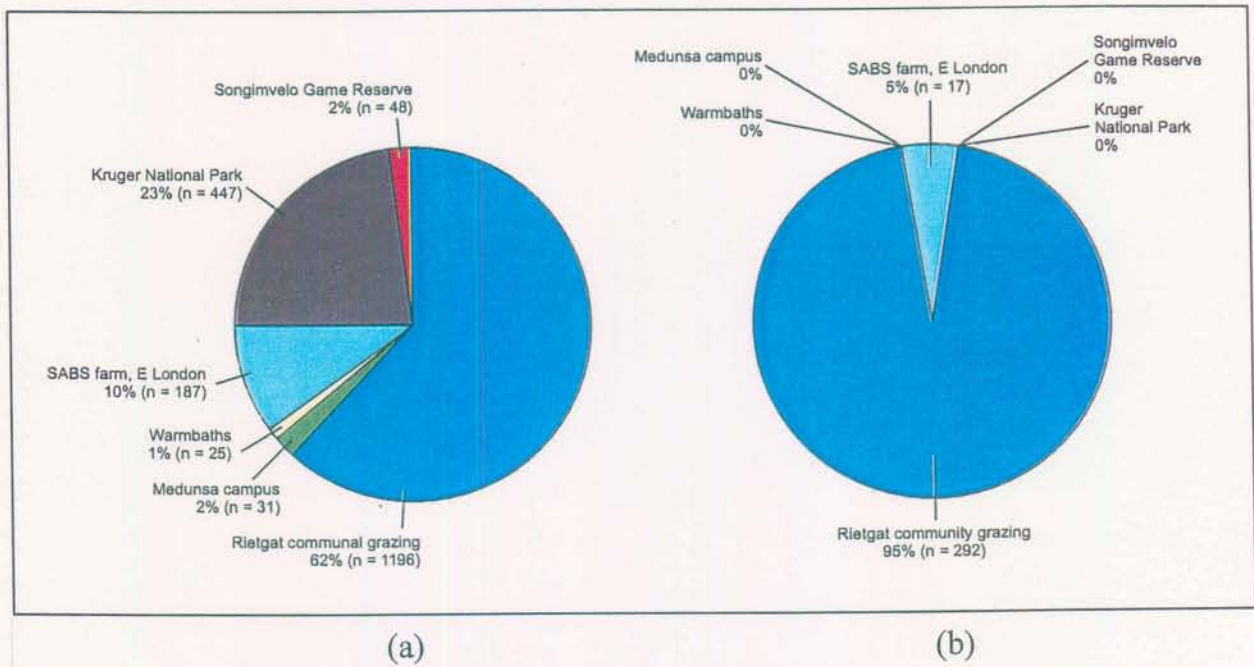


Fig. 19 Total number of adult (n = 1 934) (a) and nymphal (n = 309) (b) *A. hebraeum* collected with the AAAP/CO₂ trap at 6 areas of South Africa.

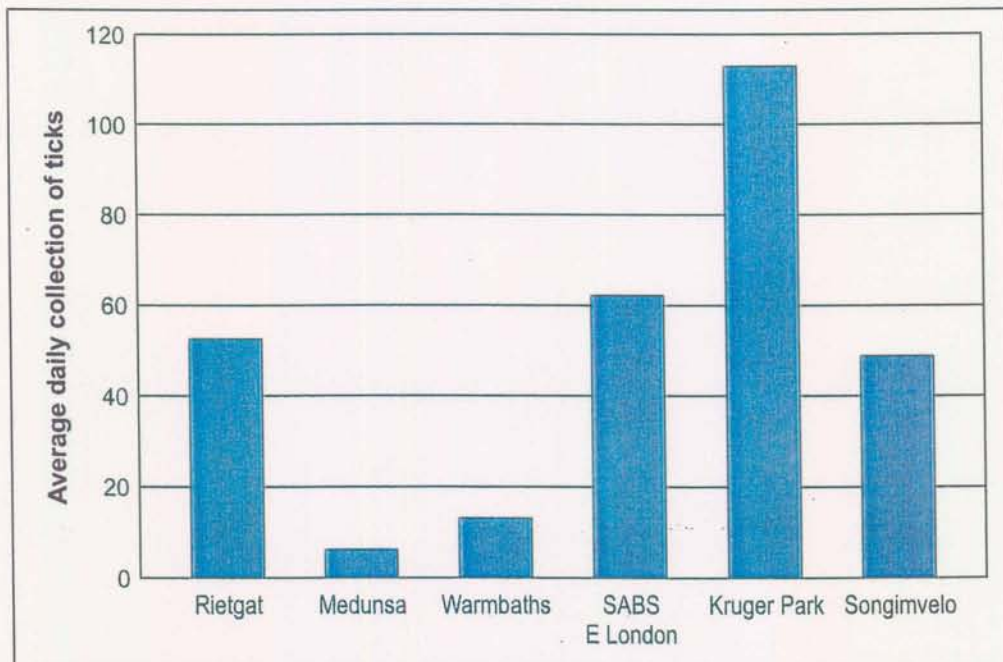


Fig. 20 Average daily collection of adult *A. hebraeum* using the AAAP/CO₂ trap at the different field sites.

TABLE 4 Total numbers of adult and nymphal *Amblyomma hebraeum* collected using the AAAP/CO₂ trap, at the Rietgat CGA, North West Province, South Africa

VISIT NO.	DATE	ADULT TICKS			NYMPHAL TICKS	NUMBER OF VISITS THAT MONTH	AVERAGE DAILY COLLECTION OF ADULT TICKS DURING THAT MONTH	AVERAGE DAILY COLLECTION OF NYMPHAL TICKS DURING THAT MONTH
		T	M	F				
1	1992-10-20	78	35	43	0	1	78	0
2	1996-02-06	0	0	0	1	1	0	1
3	1996-03-07	0	0	0	0	1	0	0
4	1996-07-02	2	1	1	0	1	2	0
5	1996-09-18	96	48	48	0	4	47.25	0
6	1996-09-19	28	13	15	0	1	1	0
7	1996-09-26	55	27	28	0	1	1	0
8	1996-09-27	10	5	5	0	1	1	0
9	1996-10-29	258	130	128	0	2	202	0
10	1996-10-30	146	60	86	0	1	1	0
11	1996-11-19	139	66	73	0	2	111	0
12	1996-11-30	83	49	34	0	1	1	0
13	1997-01-21	18	12	6	0	2	9.5	.0
14	1997-01-30	1	0	1	0	1	1	.0
15	1997-02-13	2	1	1	0	1	2	.0
16	1997-04-11	7	4	3	0	1	7	.0
17	1997-06-25	3	1	2	0	1	3	.0
18	1997-08-26	3	2	1	10	1	3	10
19	1997-09-04	6	3	3	57	2	9.5	50
20	1997-09-27	13	6	7	43	1	1	1
21	1997-11-20	81	41	40	80	2	111	77.5
22	1997-11-25	141	72	69	75	1	1	1
23	1998-01-30	26	16	10	26	1	26	26
TOTAL		1 196	592	604	292	23		

RESULTS

1. Total adults ticks collected = **1 196** 2. Total nymphal ticks collected = **292** 3. Total collection days = **23**
 4. Average adult ticks per day = **52** 5. Average nymphal ticks per day = **13**

TABLE 5 Nymphal *A. hebraeum* collected from various ecological micro-habitats during the survey at Rietgat, North West Province, South Africa (1996 - 1998)

AREA	DATE OF COLLECTION							TOTAL
	1996-02-06	1997-08-26	1997-09-04	1997-09-27	1997-11-20	1997-11-25	1998-01-30	
A/1								
A/2								
A/3						30	21	51
A/4								
B/1		10	29		20			59
B/2								
B/3								
B/4								
B/5								
B/6			28	22	10			60
C/1	1							1
C/2								
C/3								
D/1								
D/2								
D/3						15		15
D/4								
D/5								
E/1				10	20	10	0	40
E/2				11	30	20	5	66
E/3								
E/4								
E/5								
E/6								
E/7								
TOTAL	1	10	57	43	80	75	26	292

1. Total nymphs collected = 292

2. BLANK SQUARES = Not sampled

TABLE 6 Adult *A. hebraeum* collected from 25 ecological micro-habitats sampled on a regular basis at Rietgat, North West Province, South Africa

A R E A	1992	1996 DATES OF COLLECTION											1997 DATES OF COLLECTION										1998	TOTAL	
	10-20	02-06	03-07	07-02	09-18	09-19	09-26	09-27	10-29	10-30	11-19	11-30	01-21	01-30	02-13	04-11	06-25	08-26	09-04	09-27	11-20	11-25	01-30	TICKS	
A/1						0		0		0					0	0	0	0		0	0	0	0	0	0
A/2						0		0		0						0	0		0	0	95		0	95	
A/3						13		0		0					0	0	2		2	70			8	95	
A/4						0		3							0	0	0		1	5	0		0	9	
B/1										64			0			0	0	0	4	1	2	0	1	72	
B/2-5										0			0		0	0	0	0	0	0	0	0	0	0	
B/6										0			0		0	0	0	2	1	4	0		0	7	
C/1		0	0	2						0			0		0	0	0	0	0	0	0	0	0	2	
C/2	78	0	0	0	50		55			58			0		2	0	0	0	0	0	0	0	0	243	
C/3		0	0	0						24		45	0	1	0	0	1	0	0	0	0	0	0	0	71
D/1									5	0	0	23	0	0	0	0	0	0	0	0	0	0	8	36	
D/2									35	0	18	15	1	0	0	0	2	0	0	1	0	38	0	110	
D/3									68		26		0			0	0	0	0	4	0	0	0	98	
D/4									19		10		0			0	0	0				0	0	29	
D/5									0		0		0			0	0	0	0	0	0	0	0	0	
E.1					46	15		7	96		23		0		0	5	0	0	0	0	0	8	9	209	
E.2					0	0		0			62		11		0	0	0	0	0	3	0	0	0	76	
E.3					0	0		0			0		6		0	2	0	1	0	0		0		9	
E.4					0	0		0	15		0		0		0	0	0	0	0	0	0	0	0	15	
E.5					0	0		0	10		0		0		0	0	0	0	0	0	0	0	0	10	
E/6					0	0		0	10		0		0											10	
E/7					0	0		0			0		0			0	0	0	0	0			0	0	
TOTAL	78	0	0	2	96	28	55	10	258	146	139	83	18	1	2	7	3	3	6	13	81	141	26	1196	

Total number of ticks collected = 1 196 Adults (592 Males and 604 Females)

0 = No ticks collected

BLANK SQUARES = Not sampled

TABLE 7 Ticks other than *Amblyomma hebraeum*, which were attracted to the AAAP/CO₂ trap

DATE OF COLLECTION	SPECIES	SEX		AREA
		MALE	FEMALE	
1996-11-08	1 x <i>Rhipicephalus appendiculatus</i>	1	0	Rietgat CGA
1996-11-11	8 x <i>R. simus</i>	5	3	MEDUNSA
1996-11-15	2 x <i>R. evertsi</i>	2	0	MEDUNSA
1997-09-09	3 x <i>R. evertsi</i>	2	1	Warmbaths
1998-01-31	8 x <i>R. simus</i>	5	3	Rietgat CGA
	4 x <i>R. appendiculatus</i>	3	1	
	4 x <i>R. evertsi</i>	3	1	
	1 x <i>Hyalomma marginatum rufipes</i>	1	0	

TABLE 8 Total *A. hebraeum* adults and nymphs collected with the AAAP/CO₂ trap at the various areas in South Africa

AREA	ADULT TICKS	% OF THE TOTAL COLLECTION OF ADULTS	NYMPHAL TICKS	% OF THE TOTAL COLLECTION OF NYMPHS	DAYS SPENT COLLECTING TICKS	AVERAGE ADULT TICKS COLLECTED PER DAY ON EACH VISIT
Rietgat CGA	1 196	62	292	95	23	52
MEDUNSA	31	1.6	0	0	5	6.2
Warmbaths	25	1.3	0	0	2	12.5
SABS (East London)	187	9.6	17	5	3	62.3
Kruger National Park	447	23	0	0	4	111.75
Songimvelo Game Reserve	48	2.5	0	0	1	48
TOTAL COLLECTION	1 934	100	309	100%	38	

2.1.3.2 Seasonal abundance of the ticks collected

■ Adults

Adult *A. hebraeum* showed a definite seasonal pattern of abundance in both 1997 and 1998 (Fig. 21). The first adult ticks collected with the trap after winter were in August and September. This was followed with peak numbers in October/November. There was a steady decline in January/February and from March through to August very few adult *A. hebraeum* were collected. Seasonal peaks thus occurred during the dry, hot period of September/ October/November. During this period the first rains fell but had not had time to affect the vegetational cover on the CGA.

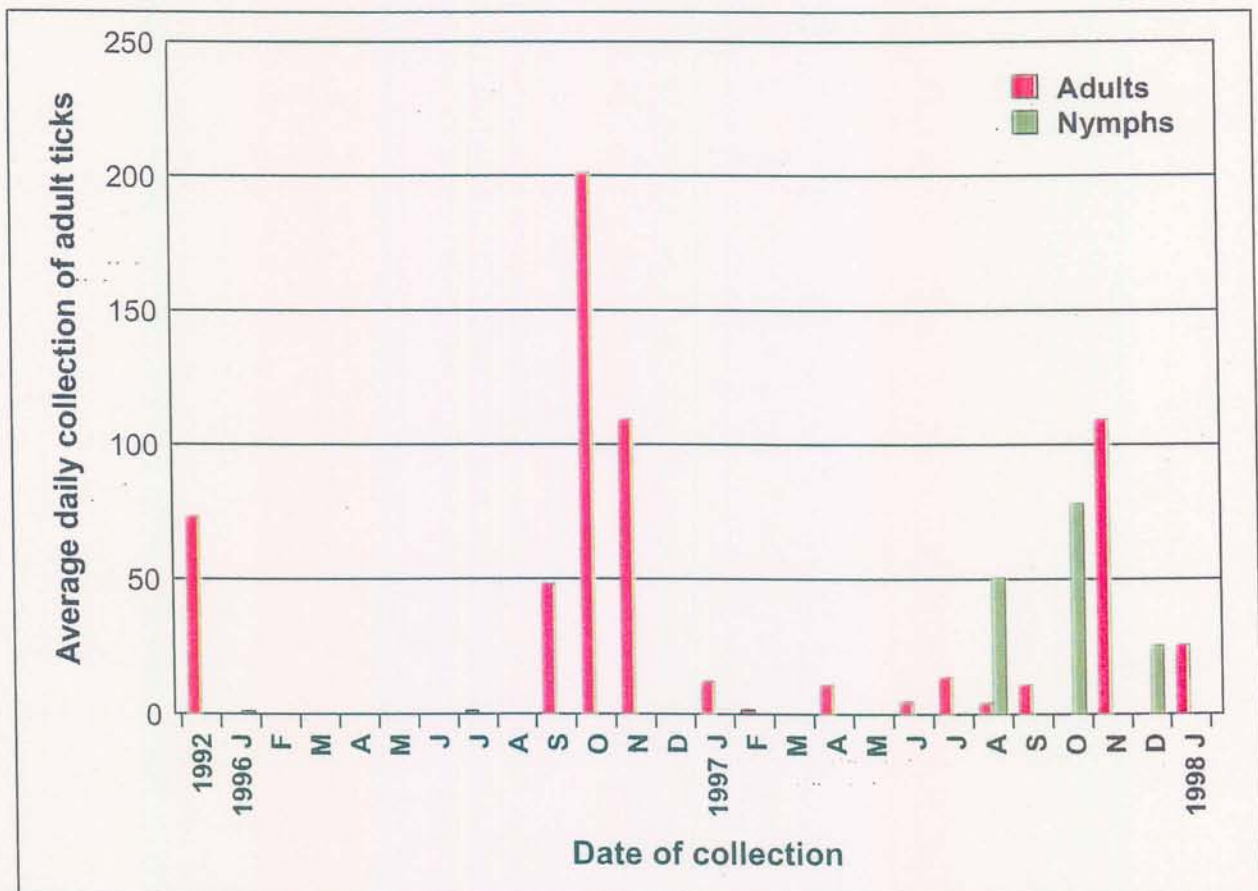


Fig. 21 Seasonal variation in the numbers of adult and nymphal *A. hebraeum* collected with the AAAP/CO₂ trap at the Rietgat CGA.

In October 1992 when the CGA at Rietgat was first visited, ticks were collected successfully with the AAAP/CO₂ trap. Follow-up visits during the late summer (February/March) resulted in no ticks being collected at exactly the same site. This change in tick activity can now be explained by changes in climate and vegetation, and will be discussed later in more detail.

■ Nymphs

Nymphs were only collected during a short period towards the end of the survey. This was mainly because at the commencement of the project, it was not realised that the AAAP/CO₂ trap could attract nymphs. As a consequence no data were collected for the first one and a half years. During the six-month period when nymphs were sampled, successful collections in certain ecological areas were made on a regular basis (Fig. 22). Although they did not appear to have the same seasonal periodicity as the adults, a more in-depth investigation would be necessary to confirm this.

2.1.3.3 Variation in tick numbers collected at the various ecological areas

■ Overall results

Twenty-five ecological micro-habitats were sampled on a regular basis during the survey. It was clear for both the adults and the nymphs that certain ecological areas were preferred, whilst others had no ticks at all (Tables 5 and 6, and Fig. 22).

■ Individual sites sampled

- Areas A/2 and A/3 (Fig. 8A), which were associated with a clump of tamboti trees and were close to the Tolwane river, were good areas for collecting both adult and nymphal ticks.

- Area B (Fig. 7 and 8B) was not a highly productive area with the exceptions of areas B/1 and B/6, both of which were heavily shaded with a good, deep leaf litter and were associated with a high nymphal population.
- Area C (Fig. 7 and 8C) was the original site at which the AAAP/CO₂ trap was tested in 1992. C/2 and C/3 were both good areas for collecting adult ticks but no nymphs were found.

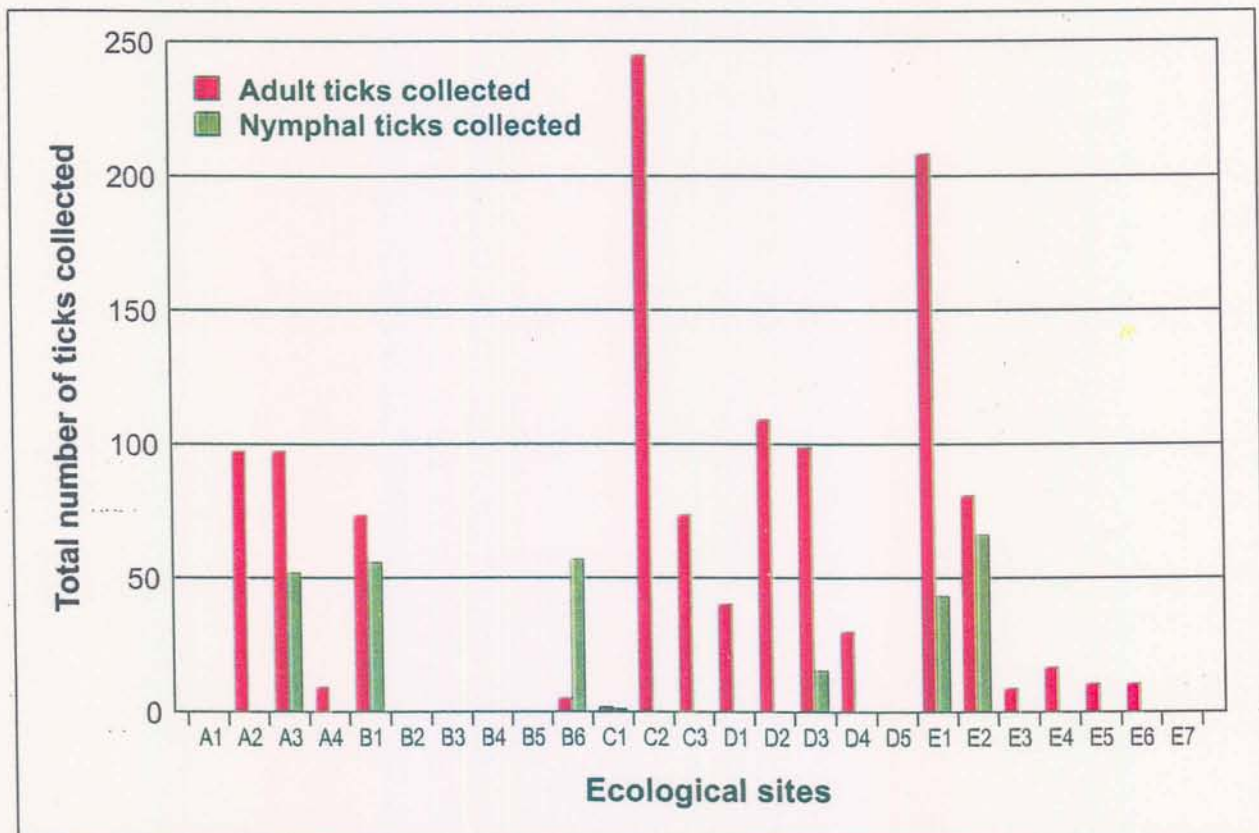


Fig. 22 Numbers of adult and nymphal *A. hebraeum* collected at the various ecological sites at the Rietgat CGA.

- Area D (Fig. 7 and 8D) and the surrounding area was a reasonably good habitat at which to collect adult ticks. D/2 and D/3, which were more open areas, were found to be better than the deeply shaded D/1.

- Area E (Fig. 7 and 8E), which was close to the diptank, encompassed very good sites E/1, E/2, as well as mediocre ones E/3, E/4, E/5, E/6 and E/7, where very few ticks were collected. E/1 was mixed *E. crispera* and *Rhus lancea* trees with a dense leaf litter which was moist throughout the year even during the severe, hot, dry period of the year, when most of the trees and shrubs had lost their leaves. The evergreen *E. crispera* and *Rhus lancea* appeared to lose their leaves throughout the year and this resulted in year round, moist leaf litter and shade for the ticks. The other area E/2, was a typical tamboti clump with a dense leaf litter in late winter/spring and this resulted in a good, moist, shaded environment for the ticks.

2.1.3.4 Sex ratio of the population of *A. hebraeum*

The sex ration of female to male ticks collected with the AAAP/CO₂ trap at the Rietgat CGA was 51% females to 49% males.

2.1.3.5 Other tick species attracted to the AAAP/CO₂ trap

Various other tick species were also attracted to the traps. The ticks collected and identified are listed in Table 7. *R. appendiculatus*, *R. simus*, *R. evertsi evertsi*, and *H. marginatum rufipes* were collected and identified at Rietgat.

2.1.4 DISCUSSION

2.1.4.1 Factors affecting the efficacy of the AAAP/CO₂ trap

2.1.4.1.1 CO₂ Source

Initially small chopped pieces of dry ice were used after breaking up a large 5½ kg block. This method was successful, but the dry ice was difficult to move around, dissipated quite quickly and did not last the entire day. The “lunch box” tick trap of Norval, Butler & Yunker (1989b), using fed males of *A. hebraeum* over which a stream of CO₂ from a CO₂ cylinder was passed, was also tried in the field. A very poor response to this trap resulted probably because of the very low levels of CO₂ emitted from the cylinder. Various other combinations of CO₂ cylinders and fed male ticks were tested at the Rietgat CGA, all with poor results. The CO₂ cylinders were expensive to purchase and maintain, and certainly could not be left unattended because of the possibility of theft. The cylinders were heavy and the CO₂ did not last long under field conditions, and had to be frequently refilled. In certain areas facilities to refill these cylinders were unavailable. The most important disadvantage was the inability of the CO₂ cylinder to disperse the CO₂ over a large area and it was also impractical to use six or eight cylinders at a time.

For the above-mentioned reasons it was deemed necessary to use a more flexible, movable and inexpensive source which dispersed CO₂ over a wide area and mimicked the breath of

a grazing bovine. Large blocks of dry ice were found to be ideal for this purpose and they were easy to move around, either with one's shoes or with gloves. It was possible to concentrate the CO₂ by surrounding the area with 6 - 8 blocks of dry ice. Ticks in the area would then be affected by one or other of the CO₂ blocks in the region. These blocks were easy to move along the paths onto other sites, so that one had a better chance of discovering new habitats. The blocks lasted for at least a day and could be left unattended without fear of theft, and when the day's sampling was finished they could be left in the veld and did not cause any environmental damage. Periodically cattle would investigate the blocks of dry ice, but were careful not to get too close due to the very cold CO₂. The cattle probably made the traps more efficient because of the extra CO₂ they exhaled, but caused concern when they tried to eat the pheromone impregnated foam rubber/metal pegs. The most important advantage of these large CO₂ blocks was their ability to disperse high concentrations of CO₂ over a wide area and deep into the leaf litter.

There are few reports on the sustained capture of *A. hebraeum* adults in the wild. The most important reason for the failure to collect these ticks was probably a poor CO₂ source. It is imperative to have a strong source of CO₂, and to select sites carefully. If an area is sampled during the peak season of tick abundance (September to November), and no ticks are collected, then one can be reasonably sure there are no ticks in the area.

2.1.4.1.2 Pheromone source

When problems were experienced with the AAAP/CO₂ trap and no ticks were collected, it was necessary to examine various factors to determine where the problem was. The same CO₂ source was always used so this was ruled out. However, the pheromone used did vary from batch to batch, and this change brought about variability which needed to be tested under field conditions.

This new diethyl-ether pheromone extract was tested under field conditions at Rietgat and found to be effective, and subsequently was the only pheromone used for the remainder of the project at all six areas in South Africa. Initially in 1992 a few drops of pheromone extract were applied to small pieces of filter paper which were then placed on a twig or a small piece of a branch close to the CO₂. Later it was found that the pheromone on the paper did not last as the paper was unable to absorb sufficient pheromone, and could also easily be blown away by wind. For this reason the small foam rubber pieces collected from pill containers were preferred. These were attached to metal pegs and were more durable. They could also be dipped into diethyl-ether pheromone extract which had been poured into a plastic bucket. This dipping process made it easier to make up multiple pheromone sources at the same time, and also ensured that all the volatile diethyl-ether was absorbed by the foam rubber pieces.

2.1.4.1.3 Day length/Time of collection

The effect of day length and time of collection on tick emergence was difficult to monitor, and should be done under controlled laboratory conditions. The seasonal effect of increased or decreased day length must, however, play an important role in the emergence of adult *A. hebraeum*. This would be in association with temperature, rainfall, RH and other factors. When day length was at a minimum in South Africa (midwinter solstice in June) then no or very few ticks were collected. When day length was at its maximum (midsummer solstice in December) the ticks were far more active. When day length starts to increase in September/October there was a substantial increase in tick activity.

2.1.4.1.4 Temperature

The minimum and maximum temperatures recorded at Brits have already been discussed (section 2.1.2.3.3). It is difficult to assess the direct effect of temperature in isolation from other factors, especially day length and RH. However, when soil temperature and RH were monitored over a 72 hour collection period in the KNP, a relationship between the daytime temperature and the number of ticks collected per hour was evident (Fig. 34). Although trapping ticks in the KNP started at 06:00, few were collected till 08:00. Low temperatures and day length would also contribute to this effect. The rapid decline of temperature, followed by the almost total absence of *A. hebraeum* adults collected at Rietgat during winter could also be attributed to the decline in temperature and the frost associated with this season.

2.1.4.1.5 Rainfall

The seasonal variation in rainfall at the Brits meteorological station has already been discussed (section 2.1.2.3.2). It is apparent that there was an indirect effect on adult tick activity at Rietgat. During the cool, dry winter when no rain fell, there was virtually no adult tick activity. In September/October the first rains occurred and these coincided with an increase in adult tick activity making it the optimal time to collect ticks. These early rains resulted in a flush of vegetation which started to show its effect in January/February/March, resulting in poor visibility of the ticks. This was compounded by further good rains in January, February and March resulting in maximum vegetation cover in April.

2.1.4.1.6 Relative humidity

The seasonal variation in RH at Brits has already been discussed (section 2.1.2.3.4). Data in the KNP (Fig. 34) showed that the RH was initially high at 06:00 (70%). It then declined steadily throughout the day to a minimum of 10% at 14:00, and then began to rise again. No clear relationship between RH and the total number of ticks collected with the trap could be established. The higher RH's measured in summer (Fig. 10c) are probably more conducive to the survival of *A. hebraeum* than the much lower RH's in winter. The very low RH measured at the end of the dry season (August/September) made tick survival difficult, and as a consequence, they congregated in shaded, moist areas with deep leaf litter. During this period it was much easier to collect adult ticks as the traps could be concentrated in these areas.

2.1.4.1.7 Wind

Although wind speed was recorded at Brits, this data was not extrapolated to Rietgat. The wind index at the Rietgat CGA was recorded on each visit, but this area is not very windy and the wind had little effect on the use of the AAAP/CO₂ trap. When strong winds were present such as in East London, they would affect the functioning of the trap. The breezes which were common at the Rietgat CGA assisted the use of the trap, and by placing the trap upwind, the breeze would distribute the CO₂/pheromones over a much wider area and would reach downwind ticks.

2.1.4.1.8 Vegetation cover/leaf litter/shade

The seasonal changes in vegetation on the Rietgat CGA were dramatic, and a major effect was to substantially decrease the visibility of adult ticks. During the summer when the RH was high in most ecological areas on the CGA, adult ticks were able to migrate into the thick bush, and as a consequence, they were no longer visible or easy to trap. As the dry winter progressed, more and more of the CGA dried up.

2.1.4.1.9 Animal presence/kraals

Within its distribution range *A. hebraeum* will always be found in and around areas associated with domestic and wild animals. Kraals are particularly favoured and engorged female ticks detach and accumulate here. Other areas of high tick activity are water points and cattle/game tracks used on a regular basis. The presence of cattle or goat faeces can be

a good indicator of ticks, especially in the case of goats which rest under trees and deposit ticks and faeces in the area (Fig. 27).

2.1.5 CONCLUSIONS

The survey at the Rietgat CGA was the first to employ the AAAP/CO₂ trap on a sustained basis to collect adult *A. hebraeum*. A total of 1 196 adult ticks were collected with the trap at an average of 50 ticks per day on each visit. These results were most satisfactory as workers in Zimbabwe (Peter 1995), and in other parts of Africa (Hassan, pers. comm. 1997), have experienced difficulties in using the trap on a sustained basis, and collected only laboratory-reared ticks. The present survey has demonstrated that if specific parameters are met, the AAAP/CO₂ trap can be used successfully over a sustained period to monitor and collect adult and nymphal *A. hebraeum*.

The ecological factors which affect the efficacy of the trap make it clear that one cannot just go to an area and start collecting *A. hebraeum* adults. Visits should be undertaken on a regular basis throughout the year, and on each visit probable sites selected according to clearly defined parameters. It is imperative that a strong source of CO₂ be used and preferably in the form of large blocks of dry ice.

The pheromone must be extracted from male ticks from undipped cattle, and at least 200 feeding males should be used for the original stock. One of the most difficult and frustrating

tasks was to locate possible new free-living clusters of adult *A. hebraeum*. This requires a good field knowledge of the African bushveld, and a reasonable knowledge of the ecological requirements of the tick. Above all, it requires persistence, as frequently no adult ticks will be collected. It was important then to return on a monthly basis and to spend the entire day testing possible sites. At Rietgat, it is obviously preferable to go in September/October as there would be a much better chance of success. It is also advisable to periodically test the efficacy of the AAAP/CO₂ trap with laboratory-reared ticks.

It is difficult to conclude that there are no *A. hebraeum* ticks in an area, as was the case at Rietgat during the winter, where the ticks were present but were not attracted to the traps. Population dynamics data had, however, indicated that *A. hebraeum* were present on the goats and cattle throughout the year (Tice 1997), but that they were only attracted to the traps for a short period in late spring.

An important new use for the trap at Rietgat was the collection of nymphal *A. hebraeum*. Previous workers (Norval, Peter & Meltzer 1992) had suggested that *A. hebraeum* nymphs were attracted to the AAAP/CO₂ trap. This is the first time that large numbers of *A. hebraeum* nymphs have been collected in the field. These free-living, unfed ticks are going to be assayed by PCR to determine the prevalence of *C. ruminantium* organism in the nymphs.

2.2 THE PRODUCTION ANIMAL UNIT, MEDICAL UNIVERSITY OF SOUTHERN AFRICA (MEDUNSA) (SEMI-INTENSIVE GRAZING; MEDIUM TICK CONTROL)

2.2.1 INTRODUCTION

The campus of MEDUNSA was selected as one of the sites to test the AAAP/CO₂ trap because domestic animals grazing the camps had low numbers of *A. hebraeum*. In addition heartwater had been diagnosed in the indigenous goats in this region. Camps in which animals had a high heartwater mortality (Donkin pers. comm. 1998) were selected. Heartwater mortality in the Saanen goats kept on zero grazing at the Animal Production Unit also occurred periodically (Donkin pers. comm. 1998).

2.2.2 MATERIALS AND METHODS

2.2.2.1 Geographic location

MEDUNSA is 31 km north-west of Pretoria in the Gauteng Province of South Africa (Fig. 6).

2.2.2.2 Study area

The location of the study area is illustrated in Fig. 23. The three main sampling areas, camps A, B and C, were adjacent to the student residences (H), the sewage works (K) and the main entrance (A). The sampling sites are labelled 1 to 10.

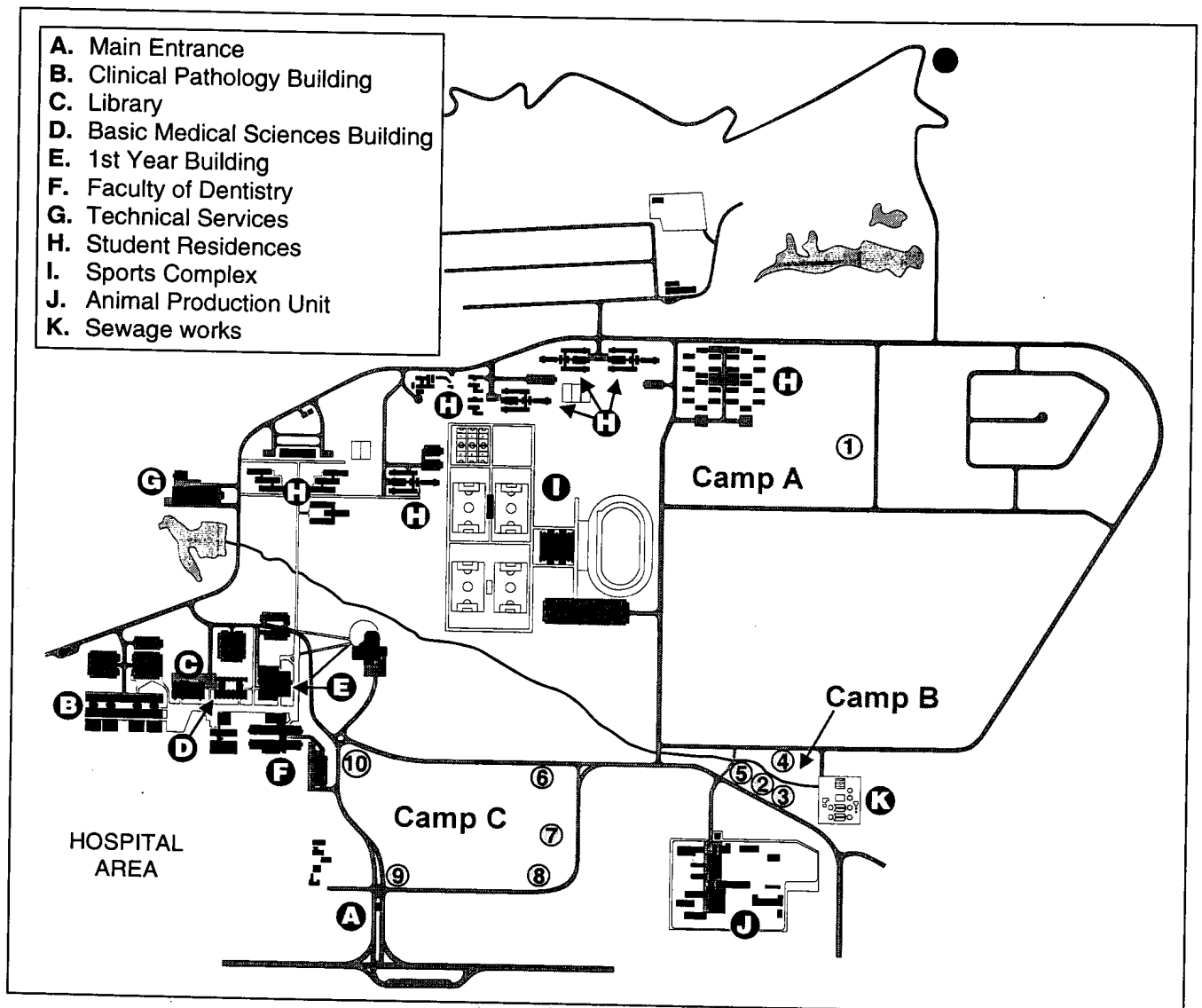


Fig. 23 The different camps at MEDUNSA (A - C), and sites (1 - 10) where the AAAP/CO₂ trap was used to collect ticks.

- Site 1 (Camp A): This site was adjacent to the student residences (H), and was chosen because Nguni cattle were grazing the camp. There was a dense invasion of *A. karoo*, making access to the camp difficult. The soil was the characteristic black loam (turf) (Fig. 24).



Fig. 24 Ticks were not common on the black loam (turf) found in certain camps at MEDUNSA.

- Sites 2 - 5: (Camp B): These sites were situated close to the sewage works (K) and the Animal Production Unit (J). A stream originating from the sewage works (K) crossed the camps to the dam. Invasion by *A. karoo* was evident, and indigenous goats were kept in this camp for most of the year.

- Sites 6 - 10: (Camp C): A large camp next to the main gate (A) which was inhabited by indigenous goats throughout the year and periodically grazed by cattle. Invasion by *A. karoo* was evident and *A. nilotica* and *A. robusta* were also present.

2.2.2.3 Climate

The climate at MEDUNSA was similar to that described for Brits. Soil temperature, the RH, vegetation index, wind index and day length index were all recorded. Recent rain fall was also noted (see Table 9).

2.2.2.4 Vegetation and fauna

All the camps were invaded by *A. karoo* and to a lesser extent *D. cinera*, *Acacia nilotica*, *A. caffra* and *A. robusta*. *Peltophorum africanum* and *Rhus lancea* were also present.



Fig. 25 Typical mixed *Acacia* spp. camps at the campus of MEDUNSA.

2.2.2.5 Livestock

The Nguni beef cattle of the Animal Production Unit grazed the various camps at different times of the year (Fig. 26 a/1). The indigenous goats also grazed camp C, and occasionally camp B (Fig. 26 a/2).

TABLE 9 *Amblyomma hebraeum* collected using the AAAP/CO₂ trap at the Production Unit, MEDUNSA, South Africa

VISIT NO.	COLLECTION DATE	AREA SAMPLED (MAP)	ADULT TICKS			NYMPHAL TICKS	VISITS PER MONTH	NO. OF TRAPS USED	AVERAGE TICKS PER DAY		**1 RAINFALL	**2 RH	**3 TICK ACTIVITY INDEX (0 - 5)	*4 VEGETATION COVER INDEX (0 - 5)	**5 TEMPERATURE	**6 WIND INDEX (0 - 5)	**7 DAY LENGTH INDEX (0 - 5)	TIME OF DAY	
			T	M	F				A	N									
1	1996-07-23	6, 7, 8, 9, 10	0	0	0	0	1	20	0	0	None	N/R	0	1	N/R	1	0	11:00 - 14:00	
2	1996-11-06	2	3	2	1	0	3	5	8	0	Yes	43	1	3	31°C	3	3		
		3, 4, 5	0	0	0	0		5	0	45		31°C							
TOTAL			3	2	1														
3	1996-11-14	6 & 7	0	0	0	0		60			Yes	65	1	3	26°C	2	3	09:00 - 13:35	
		8	10	6	4														0
		9	0	0	0														0
		10	1	0	1														0
		TOTAL			11														6
4	1996-11-15 NOVEMBER 1996	8	9	4	5	0		20		Ground wet - overcast	66	1	3	26°C	2	3	09:00 - 15:00		
		TOTAL	23															67	23°C
5	1997-11-21 NOVEMBER 1997	1	0	0	0	0	1	48	8	0	Yes	43	1	3	25°C	2	3	09:00 - 13:24	
		2	0	0	0							30			30°C				
		3	0	0	0							15			33°C				
		4	0	0	0														
		5	0	0	0														
		6	0	0	0														
		7	0	0	0														
		8	6	2	4														
		9	1	1	0														
		TOTAL			8							3			5				

RESULTS: 1. Total adult ticks collected = 31 2. Total days collecting = 5 3. Average adult ticks collected per day = 6

**1 RAINFALL	**2 RH (Relative Humidity)	**3 TICK ACTIVITY INDEX (0 - 5)	**4 VEGETATION COVER INDEX (0 - 5)	**5 TEMPERATURE	**6 WIND INDEX	**7 DAY LENGTH INDEX (0 - 5)
Recent rains Average monthly rainfall recorded at the nearest meteorological station	Measured on the ground in full shade	0 = Zero tick activity 1 = A few ticks present 2 = Some activity - low numbers 3 = Medium tick activity 4 = Ticks active 5 = Ticks very active	0 = None 1 = Little 2 = Some 3 = Medium 4 = Good 5 = Dense	Measured on the ground in full shade. Minimum (min.) and maximum (max.) also recorded if possible	0 = Zero winds 1 = Slight breezes 2 = Medium breezes 3 = Windy day 4 = Strong winds 5 = Heavy, gale force	0 = Minimum 2.5 = Intermediate 5 = Maximum

T = Total M = Male F = Female A = Adult N = Nymphal 0 = No ticks collected NR = Not recorded

BLANK SQUARES = Negative



a/1

a/2

Fig. 26 Nguni cattle (a/1) and indigenous goats (a/2) were grazing on the campus at MEDUNSA.



Fig. 27 *A. hebraeum* was more common in areas where goat droppings accumulated.

2.2.2.6 Sample size determination

The same statistical approach as that used at Rietgat was followed at MEDUNSA to calculate the sample size.

2.2.2.7 Collection of ticks with the AAAP/CO₂ trap

In order to collect *A. hebraeum* adults, five visits were made to the camps at MEDUNSA. During this period 158 traps were used at ten sample sites. The same sampling procedure as that at Rietgat was followed, using multiple blocks of dry ice surrounded by pheromone impregnated foam rubber pegs, and moved around until tick activity was observed. Sampling started at 09:00 and usually ended by mid-afternoon.

2.2.3 RESULTS

Only 31 ticks in total were collected during five sampling days giving an average of six adult ticks per day. This was the lowest figure for all of the sites sampled (Table 9, Fig. 20) and represented only 2% of all the *A. hebraeum* collected with the traps (Fig. 19a). No nymphal ticks were collected (Fig. 19b), but eight *R. simus* adults and two *R. evertsi evertsi* males were attracted to the traps (Table 7).

2.2.4 DISCUSSION

At the MEDUNSA collection site, *A. hebraeum* adults were not common either on domestic animals, (Donkin pers. comm. 1998) or in the veld, as indicated by the poor response to the AAAP/CO₂ trap. No nymphal *A. hebraeum* were found in any of the camps, although this tick occurred on goats in these camps (Donkin pers. comm. 1998). There were considerable differences between tick activity here and at Rietgat or the KNP. Tick activity is difficult to compare, especially if the comparisons are between different areas, different times of the year and different times of the day. In an attempt to assess tick activity subjectively, a tick activity index was produced (see Table 9). Tick activity ranged from no activity (0) to very

active (5). At Rietgat and the KNP the index measured five on several occasions, but at MEDUNSA activity ranged between 0 and 1. It was a difficult place to work, as the veld had been invaded by numerous *Acacias*, and after six or seven hours of collection in the midsummer sun, no or very few ticks had been collected. It was hoped to calculate the prevalence of *C. ruminantium* in the ticks but this was not possible.

An average of only six adult ticks were collected per visit (Fig. 20). Most of these were from a single sampling site in the shade of a large *A. nilotica* tree, and where goats were common, as indicated by piles of their faeces (Fig. 27). Most of the other areas sampled had a characteristic black loam (turf) (Fig. 24), which is a clay-like soil. In summer the turf got wet and it was difficult to walk on. The black colouration of the soil made the visibility of the ticks much more difficult. These areas were also closely associated with *A. karoo* and *A. nilotica*.

2.2.5 CONCLUSIONS

When there was a high concentration of free-living, unfed adult *A. hebraeum* in the field such as at Rietgat, then the response was good. In a low activity area at MEDUNSA, however, the tick response was always poor and despite many hours of trapping very few ticks were collected. As the trap was concurrently being used successfully at Rietgat, there were obviously no inherent problems with the trap design, but rather a very low population of adult ticks at MEDUNSA. The tick control management at MEDUNSA, which included regular acaricide application to cattle and goats, must also have reduced the population. The Nguni cattle were also seldom infested with large numbers of ticks.

2.3 DR L VAN DER MERWE'S FARM (LUDLOW) NEAR WARMBATHS,
NORTHERN PROVINCE, SOUTH AFRICA - COMMERCIAL
BUSHVELD FARM (GOOD TICK MANAGEMENT)

2.3.1 INTRODUCTION

This farm was chosen as a representative of a typical, well-managed bushveld cattle farm with a heartwater problem. A preliminary visit indicated that *A. hebraeum* was present on the farm and that pour-on acaricides were regularly used to control ticks. The farm was owned and managed by Dr Lente van der Merwe, a veterinarian with a particular interest in heartwater. Dr van der Merwe has published on the clinical symptoms of heartwater and done extensive vaccination trial work. Her farm was also selected by the U.F./USAID/SADC Heartwater Project in Zimbabwe to test a new, inactivated heartwater vaccine (Mahan 1997). It was therefore important that live, adult ticks be caught, so that the strain of *C. ruminantium* causing heartwater in this area could be characterised.

2.3.2 MATERIALS AND METHODS

2.3.2.1 Geographical location

The location of this farm is illustrated in Fig. 6. The farm comprises 675 hectares of land and is essentially a cattle/grain farm. It lies 18 km south-east of the town of Warmbaths (25°10' South; 28°20' East) and close to the village of Settlers.

2.3.2.2 Study area

A detailed map, supplied by Dr van der Merwe, is reproduced in Fig. 28.

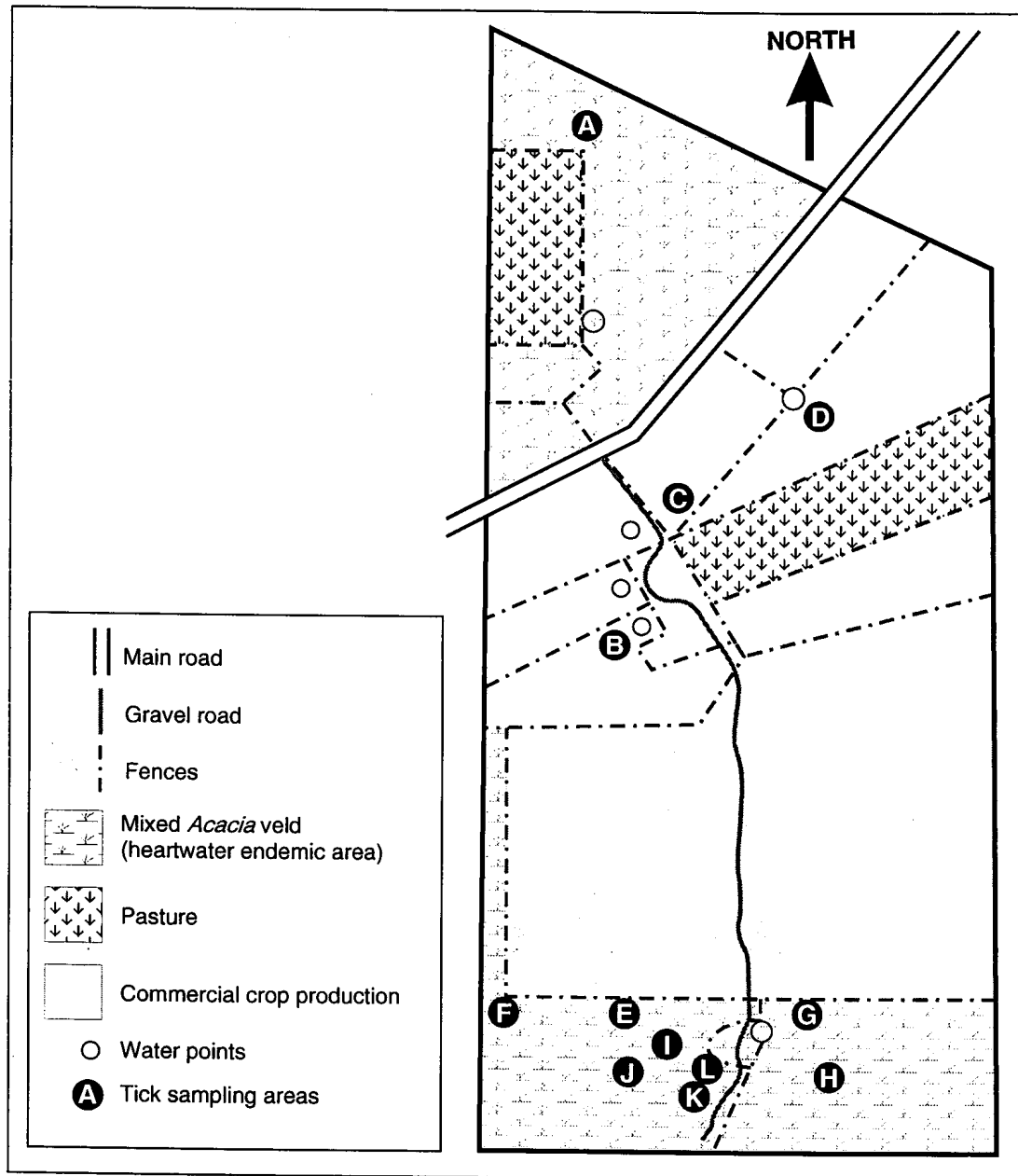


Fig. 28 Diagrammatic layout of the farm "Ludlow", near Warmbaths, Northern Province, South Africa.

■ Tick sampling sites

The farm is transected by the Warmbaths-Settlers road and the first sampling site (A) was on the northern border of the farm, in the mixed *Acacia* veld and close to a well-used cattle kraal. Site B was located in a camp adjacent to the main homestead. Sites C and D were in camps just north-east of the homestead and in dense, mixed *Acacia* veld. Bonsmara bulls were also kept in these paddocks. Sites E to K were in the mixed *Acacia* veld (Fig. 28) in the southern most camp on the farm. Most of the Bonsmara stud cattle grazed in this vicinity, and a well-used cattle kraal, crush and water point were operating at the time of sampling. Sites E to K were all similar type sites, usually in the shade of *A. karoo*, *A. nilotica* and *A. tortillis* which were abundant in these camps. Many cattle tracks were visible in the grass veld and the black turf soil was similar to that found at MEDUNSA.

2.3.2.3 Climate

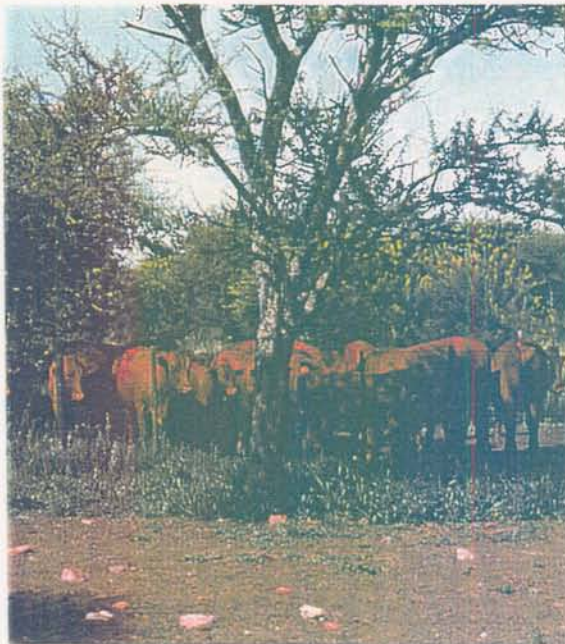
The climate at Warmbaths was similar to that at Brits, and detailed climatic data were not recorded as only five visits were made. The average annual rainfall recorded at Ludlow was 562 mm and this was mainly in summer. On each visit to the farm the RH and soil temperature were measured. The occurrence of recent rain was also noted and wind and day length indices estimated.

2.3.2.4 Vegetation

The vegetation consisted of mixed bushveld and cultivated lands. The mixed bushveld comprised *Acacia* species typical for this area, and was in many ways similar to that at MEDUNSA with *A. karoo* invasion on black turf (Fig. 29a, b and c).



(a)



(b)



(c)

Fig. 29a) Typical black turfveld at “Ludlow”, with *A. nilotica*, *A. tortillis* and *A. karoo* the most dominant.

b) Bonsmara stud cattle bred on the farm were fairly resistant to the common TBD.

c) Collection of *A. hebraeum* at the kraal region of “Ludlow” farm, the black loam is clearly visible

2.3.2.5 **Livestock**

The farm is a registered Bonsmara stud farm and the bulls were kept close to the homestead, and the rest of the herd grazed on the mixed *Acacia* bushveld and old cultivated lands. The cattle were treated with a pour-on acaricide when tick levels were considered excessive.

2.3.2.6 **Sample size determination**

The same statistical approach to the sample size determination as that at Rietgat was used at Warmbaths.

2.3.2.7 **Collection of ticks**

2.3.2.7.1 Use of the AAAP/CO₂ trap

The AAAP/CO₂ trap was used on two separate visits to Warmbaths in September and November 1997. On the first visit sites (E - L), all in the southern section of the farm, were sampled. All these sites were close to the main cattle kraal and watering point in this region (Fig. 29b), and where the black turf veld had been invaded by *A. nilotica*, *A. tortillis* and *A. karoo* the dominant tree species (Fig. 29a). Leaf litter on the turf and *Acacia* veld was limited.

On the second visit to this farm, the same sites (E - L) as previously were sampled, but other sites were also sampled including some near the homestead (B, C and D) and in the northern areas (A) of the farm.

TABLE 10 Ticks collected on Dr L van der Merwe's farm "Ludlow", near Settlers, South Africa

VISIT NO.	COLLECTION DATE	AREA SAMPLED (MAP)	ADULT TICKS			NYMPHAL TICKS	VISITS PER MONTH	NO. OF TRAPS USED	AVERAGE TICKS PER DAY		**1	**2	**3	**4	**5	**6	**7	TIME OF DAY
			T	M	F				A	N	RAIN-FALL	RH	TICK ACTIVITY INDEX (0 - 5)	VEGETATION COVER INDEX (0 - 5)	TEMPERATURE	WIND INDEX (0 - 5)	DAY LENGTH INDEX (0 - 5)	
1	1997-06-17										NO	N/R						
2	1997-09-09	E F G H I J K L TOTAL	0 0 3 0 0 0 19 0 22	0 0 1 0 0 0 13 0 14	0 0 2 0 0 0 6 0 8	0 0 0 0 0 0 0 0 0	1	8 8 8 8 8	2 2	0	No	80 80	2	1	20°C 31°C	2	2	09:40 - 14:00
3	1997-11-12	G H I J K L B C D A TOTAL	0 0 0 0 2 0 0 0 0 1 3	0 0 0 0 0 0 0 0 0 1 1	0 0 0 0 2 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0 0	1	4 4 4 4 4 4 4 4 4 4	3	0	Over-cast Over-cast	63 62 62	0 0 0 0 1 0 0 0 1	4	26°C 20°C 31°C	1 1	3	09:00 11:00 12:30
4	1997-12-11	*242 adult ticks (mostly male) collected from 30 cattle *Sent to Dr Mahan, Zimbabwe																
5	1998-01-21	Visited Dr Van der Merwe's farm with Dr Mahan, to discuss details of the Heartwater trial																
RESULTS:																		
1. Total adult ticks collected = 25 2. Total days collecting = 2 3. Average adult ticks collected per day = 12,5																		

**1 RAINFALL	**2 RH (Relative Humidity)	**3 TICK ACTIVITY INDEX (0-5)	**4 VEGETATION COVER INDEX (0-5)	**5 TEMPERATURE	**6 WIND INDEX	**7 DAY LENGTH INDEX (0-5)
Presence of recent rains noted. Average monthly rainfall recorded at the nearest meteorological station	Measured on the ground in full shade	0 = Zero tick activity 1 = A few ticks present 2 = Some activity - low numbers 3 = Medium tick activity 4 = Ticks active 5 = Ticks very active	0 = None 1 = Little 2 = Some 3 = Medium 4 = Good 5 = Dense	Measured on the ground in full shade. Minimum (min.) and maximum (max.) also recorded if possible	0 = Zero winds 1 = Slight breezes 2 = Medium breezes 3 = Windy day 4 = Strong winds 5 = Heavy, gale force	0 = Minimum 2,5 = Intermediate 5 = Maximum

T = Total M = Male F = Female A = Adult N = Nymphal 0 = No ticks collected N/R = Not recorded BLANK SQUARES = Negative

2.3.2.7.2 Collection of ticks off cattle

Because of the lack of success in collecting adult ticks at Ludlow farm using the trap, adult *A. hebraeum* were eventually collected from cattle on the fourth visit (Table 10). These ticks were sent to Zimbabwe for characterisation of the local *C. ruminantium* strain.

2.3.3 RESULTS

The results of the visits to Ludlow are summarized in Table 10. A total of 25 adult ticks were collected during two separate visits. An average of 12.5 ticks per visit and no nymphal ticks were collected.

2.3.4 DISCUSSION

It was important for the characterisation of the heartwater strains that a statistically relevant field sample of free-living, unfed ticks be caught with the AAAP/CO₂ trap. However, when two days of trapping involving 12 trapping sites, resulted in only 25 ticks, of which 21 (84%) were from one site (K), it was decided that the trap was not successful in attracting *A. hebraeum* adults and nymphs on this farm. In order to obtain a sample of adult ticks, the cattle were put into a crush (Fig. 29c), and ticks were removed manually. From discussions with Dr van der Merwe and observation of the cattle, it was obvious that medium to heavy burdens of *A. hebraeum* were in fact, present on the farm.

If the different areas where the AAAP/CO₂ trap was used are compared (Fig. 19a & 20), then only 1% of the total ticks were collected at Warmbaths and no nymphs (Fig. 19b). The average daily collection of adult *A. hebraeum* (Fig. 20) at Warmbaths was the second lowest of the six areas sampled.

A number of reasons probably resulted in the poor tick catch at Ludlow farm. Firstly the good management here, coupled with the ongoing pour-on tick control, undoubtedly resulted in decreased ticks in the veld. The landscape type, *A. karoo*/black turf, was similar to that at MEDUNSA and in both areas tick collecting was disappointing.

2.3.5 CONCLUSIONS

The trap can only work optimally if there are medium to high densities of ticks in the area. If one is working on a well-managed farm with good tick control, then there are going to be fewer free-living, unfed adult and nymphal ticks in the veld. Thus it will be more difficult to collect ticks in any significant numbers.

2.4 THE SOUTH AFRICAN BUREAU OF STANDARD'S (SABS) EXPERIMENTAL FARM AT EAST LONDON, EASTERN CAPE PROVINCE, SOUTH AFRICA (MEDIUM TO GOOD TICK CONTROL; TICKS SEEDED)

2.4.1 INTRODUCTION

The Eastern Cape Province of South Africa's coastal zone has a very high population of *A. hebraeum*. The SABS farm at East London was at first chosen to test the AAAP/CO₂ trap primarily to trap ticks to test the new inactivated UF/USAID/SADC heartwater vaccine, and was also ideally suited due to the ongoing tick experiments on the property. Heartwater is an enormous problem for Angora goat farmers in the region.

2.4.2 MATERIALS AND METHODS

2.4.2.1 Geographical location

East London is situated on the south-eastern coastline of South Africa and is 1 100 km from Pretoria (Fig. 6). The exact geographical position is 25°50' - 33°10'. The farm is set amongst coastal vegetation and within view of the Indian Ocean.

2.4.2.2 Study area

The layout of “Little Go” farm is illustrated in Fig. 30. Most of the farm was typical Eastern Cape coastal vegetation but large areas were under pastures. The main gate was at the north-western end of the farm, and the main buildings of the farm where the crush and all the cattle handling facilities were located, were at the southern end of the farm (homestead).

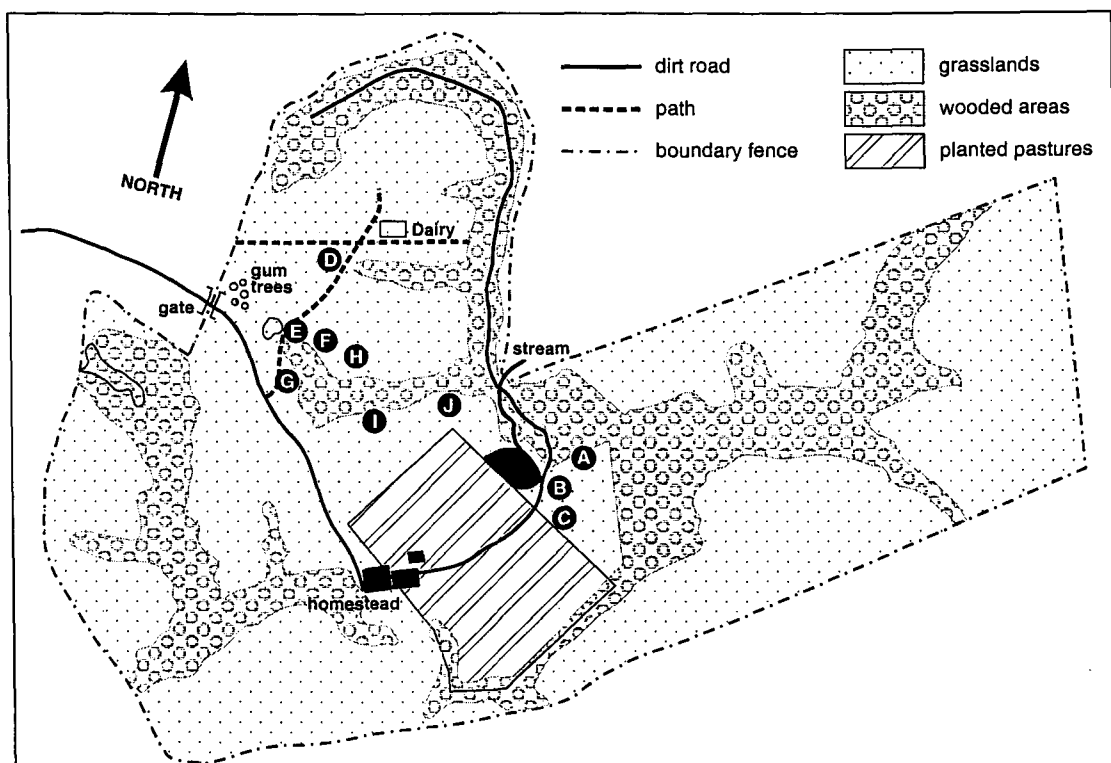


Fig. 30 Diagrammatic representation of the SABS farm, “Little Go”, near East London, Eastern Cape Province, South Africa. The AAAP/CO₂ trap was used at sites A to J.

2.4.2.3 **Climate**

As the East London farm was only visited once for a period of four days during December 1997, no detailed climatic data were collected. However, East London is midway between the humid, tropical East Coast and the temperate Mediterranean type climate of the West Coast of South Africa. Rainfall is mainly during summer and the climate in summer is hot and humid. Strong coastal winds may occur. A heavy, gale force wind (5, wind index, Table 11) was blowing during two of the three days' field collection trips. These winds reduce the summer temperatures and a maximum of 29°C was measured in December 1997.

2.4.2.4 **Vegetation and fauna**

The SABS farm lies within a few kilometres of the Indian Ocean and obviously this has dramatic effects on the vegetation. The vegetation is described by Acocks (1988) as coastal forest and thorn veld. Although most of the coastal forest (Fig. 31a) had been removed, gullies and valleys containing original coastal forest still remained. *A. karoo* and other *Acacias* are invading this region and parts of the farm now correspond to Acocks type 7 vegetation (Fig. 31b) (Eastern Cape Province thornveld). The grasses were dense and sourish mixed species (Fig. 31) (Acocks 1988).

(a)



(b)



Fig. 31a) Typical coastal vegetation where ticks were collected at the SABS farm near East London.

b) The AAAP/CO₂ traps were placed on a path next to a clump of coastal vegetation along which ticks were successfully collected at the SABS farm, East London.

2.4.2.5 Livestock

The species of domestic animals on the experimental farm varied according to the needs of the trials in operation. Beef and dairy cattle, Angora goats and dogs (Beagles) were present during this trial period.

2.4.2.6 Sample size determination

The same statistical approach to sample size determination was carried out as at Rietgat CGA.

2.4.2.7 Collection of ticks

2.4.2.7.1 With the AAAP/CO₂ trap

The collection method used at East London had to be adapted to the facilities available. No dry ice blocks were available, so small quantities of chipped dry ice were used (Afrox, East London) and placed in muslin bags (± 1 kg in total), otherwise the same method and pheromone extract was used as at Rietgat.

Day 1: Tick collecting took place only during the afternoon as it was really only an exploratory exercise. Twenty-four traps were placed in various areas along the road adjacent to the dam (Fig. 30, sites A, B and C). The vegetation was dense coastal forest invaded by *A. karoo* and associated with a stream. The tick activity index in this area was only one, and only two *A. hebraeum* adults were collected. As this field work was designed to assist the UF/USAID/SADC Heartwater project to determine the field strain of heartwater present, it was imperative that more ticks be collected during the four-day visit.

Day 2: After discussions with the SABS personnel and a neighbouring farmer, it was decided to try another area where the cattle apparently pick up many ticks. This area was near the main gate (Fig. 30), and lay between the road and the dairy. This site contained two gullies of deep, coastal forests (Fig. 31b), surrounded by grasslands and two paths ran through it, one to the dairy and another to the dam.

Initial collection (Fig. 31b) started deep in the shade of the coastal forest and soon ticks were being collected by all three collectors. As the tick numbers started to decline it was necessary to move to the periphery of the coastal forest, where once again good tick activity was found (Fig. 31b, Fig. 30 sites F and H). After this the traps were moved onto the paths and into the open (Fig. 30 sites E and G), and eventually when tick activity started to decline here the traps were moved into the open grasslands and again good tick activity was found (Fig. 30 sites I and J).

Day 3: Similar sites to that on Day 2 were sampled. The wind on both days 2 and 3 was described as gale force but appeared not to affect the efficiency of the trap. Trapping occurred throughout the day from 08:00 until 18:00 on both days (Table 11).

2.4.2.7.2 Ticks collected off cattle

A. hebraeum adults (n = 268) were collected off cattle on day 4 (1997-12-18) and these ticks were sent by courier to Zimbabwe for characterisation of the strain of *C. ruminantium*. A sample of these ticks was later fed on heartwater-susceptible sheep at the laboratory in Harare.

2.4.3 RESULTS

A total of 187 adult ticks were collected using the AAAP/CO₂ trap during the three days collecting at the SABS farm at East London (average of 62 adult ticks per day). Seventeen nymphs were also collected during the visit. Certain ecological sites (Fig. 30 D, E, F, G and H) yielded far more ticks than others (A, B, C, I and J).

2.4.4 DISCUSSION

This project was a good example of how the AAAP/CO₂ trap had to be adapted to local conditions. Initially it was thought that there were very few adult *A. hebraeum* on the farm. However, because of the flexibility of the AAAP/CO₂, which could be moved over large areas of the farm, good tick collection areas were eventually located. The traps were then concentrated in these areas. The pockets of coastal forest in the grasslands, were found to be the best tick collecting sites, and by moving the traps quickly, and by having three operators, a wide variety of habitats could be covered.

The 187 adult ticks collected by the trap at the SABS farm at East London represented 9.6% of the total number of ticks collected during the whole study (Table 8, Fig. 19a). The 17 nymphs represented 5% of the total nymphs caught (Fig. 19b). The average, daily collection of adult ticks at the SABS farm, East London (Fig. 20) (n = 62) was considerably more than at MEDUNSA (n = 6) or Warmbaths (n = 12).

2.4.5 CONCLUSIONS

The sample of nearly 200 ticks collected on a well-managed, mixed farm, demonstrated that the AAAP/CO₂ trap can be used to locate and collect an adequate sample of free-living, unfed *A. hebraeum* if the ticks are present.

2.5 SKUKUZA REST CAMP, KRUGER NATIONAL PARK MPUMALANGA PROVINCE, SOUTH AFRICAN GAME RESERVE (NO TICK CONTROL)

2.5.1 INTRODUCTION

The KNP was chosen as one of the six sites to test the AAAP/CO₂ 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 the foot-and-mouth fencing had been established. The KNP was also one of those areas where the influence of man and domestic animals was minimal. This area was possibly the closest to the original Africa, before modern man, cattle and goats arrived. It was presumed that if *C. ruminantium* was present, it circulated only in the wild animals and the ticks. The main objective of this study was to collect free-living, unfed *A. hebraeum* which had not fed on domestic animals and determine the prevalence of *C. ruminantium* in these ticks.

2.5.2 MATERIALS AND METHODS

2.5.2.1 Geographical location

The KNP lies along the easterly border of South Africa with Mocambique, and in the north with Zimbabwe (Fig. 6). The Skukuza Rest Camp (25°00' - 31° 35'), is 482 km north-east of Pretoria and all the tick collecting was done around this camp.

2.5.2.2 Study area

The trapping area around the Skukuza Rest Camp is illustrated in Fig. 32. This camp is located between the Sabie and the Nwashitsaka rivers, and much of the field work was conducted around the Nwashitsaka Research Guest Camp.

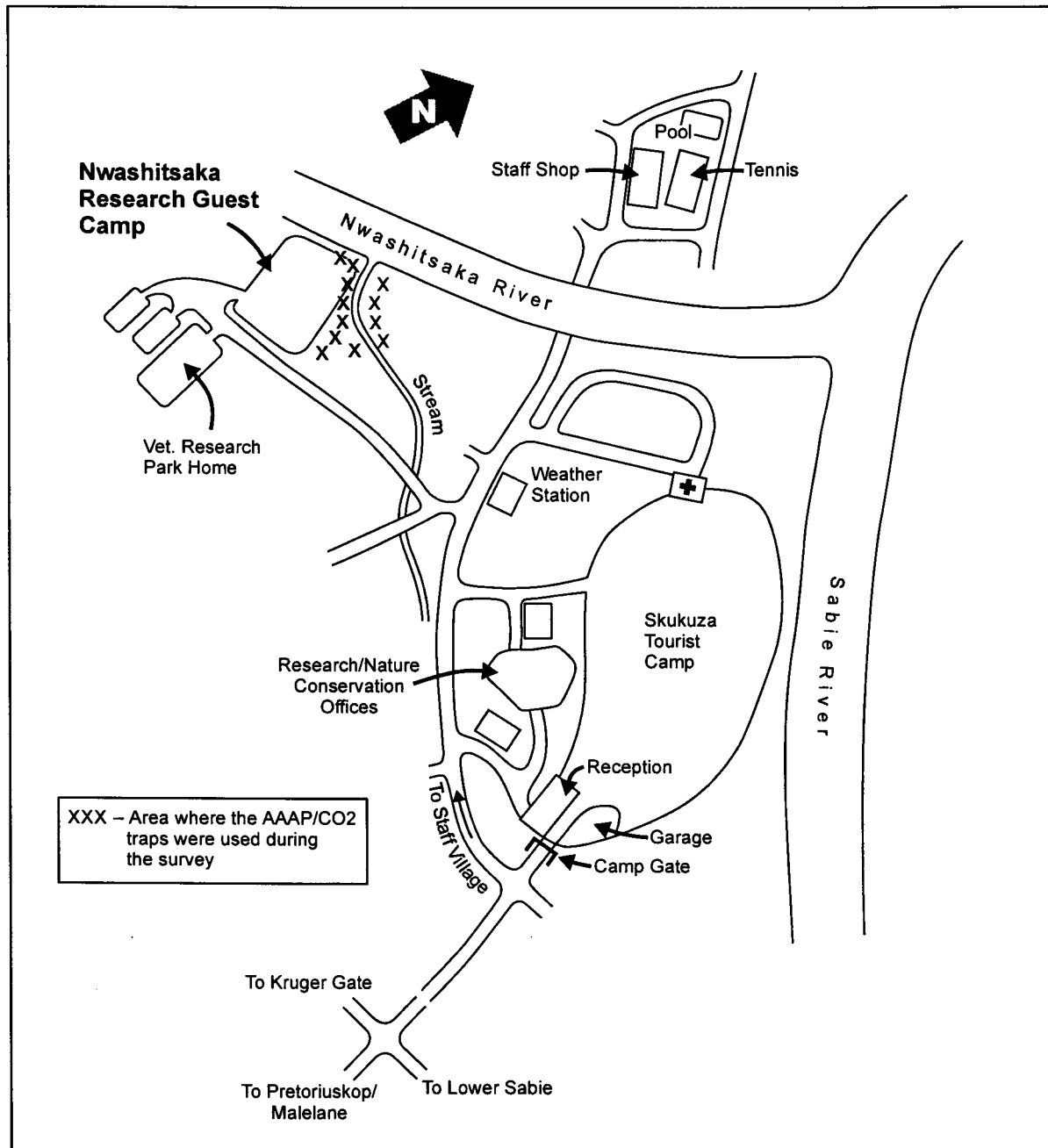


Fig. 32 Map of the Skukuza region in the Kruger National Park illustrating the Nwashitsaka Guest Camp where most of the AAAP/CO₂ traps were used.

2.5.2.3 Climate

The climate was dry and tropical rains normally occur from November to March (average total 561 mm per annum) with the cool period from April to September. The maximum temperatures in midsummer were in the lower thirties and the minimum temperatures in winter between 5°C and 8°C with no frost. The maximum RH in summer was in the upper 90's and the minimum in winter in the lower 20's. The main climatic problem in the KNP is the unpredictability of the rains and this often results in serious droughts, such as the 1998 drought in the northern part of the KNP. During these drought periods many of the rivers dry up and the wildlife is forced to use borehole water.

2.5.2.4 Vegetation and fauna

Gertenbach (1983) described the specific landscape type in the Skukuza area of the KNP as mixed Combretum and Terminalia woodland (type 4). He also indicated that detailed studies on the climate, soils, vegetation and animal life in the KNP have recently been published. This data base has made it possible to zonate the KNP into landscapes.

2.5.2.5 Livestock

There are no domestic livestock in the KNP, and this has been the situation for many decades. This is the result of establishing game-proof fencing to stop the movement of wild animals into surrounding cattle ranches and thus the spread of foot-and-mouth disease. The buffalo (*Syncerus caffer*) herds in the KNP are infected with the foot-and-mouth disease virus.

2.5.2.6 The use of the AAAP/CO₂ trap

2.5.2.6.1 Collection of ticks

The AAAP/CO₂ trap was used on two separate occasions at the Skukuza Rest Camp in the KNP. The first visit in December 1992 was a preliminary trip to test the trap, using Dr Tice's original pheromone solution and small pieces of dry ice, and the trap was used in a gully adjacent to the Nwashitsaka Research Guest Camp (Fig. 32 and 33).



Fig. 33 The gully adjacent to the Nwashitsaka Research Guest Camp at Skukuza, KNP, South Africa, where the ticks were collected.

(Acknowledgement Prof. I G Horak).

The test site was surrounded by various bushveld trees including *Dichrostachys cinera*, *Grewia* spp. and many tamboti (*Spirostachys africana*). There was a good leaf litter under the shade of these trees and various wild animals were seen in the vicinity and game tracks and faeces were common.

On the second visit to the KNP in September 1996, a number of 5½ kg blocks of dry ice were taken along and stored in the -70°C freezer of the State Veterinary office, and were used when required. The same gully, adjacent to the Research Camp (Fig. 32 and 33), near the Nwashitsaka river was again sampled, but this time with a new pheromone solution and with 5½ kg blocks of dry ice. Other possible areas in and around the Skukuza Rest Camp were also sampled with the assistance of a Pretoria Technikon student, Mr Duncan MacFadyen. All the ecological vegetation types tested were within easy reach of the Skukuza Rest Camp.

2.5.2.6.2 Soil temperature and RH measurement

On 16 September 1997 the RH and soil temperature were monitored in the gully adjacent to the Nwashitsaka Research Guest Camp. The objective of this experiment was to determine whether a relationship existed between the soil temperature/RH and the collection of free-living, unfed adult *A. hebraeum*. These parameters were measured with the thermohygrometer used at Rietgat (Fig. 9a), and recorded in full shade and sometimes semi-shade.

2.5.3 RESULTS

2.5.3.1 Collection of ticks

Four hundred and forty-seven ticks were collected in the KNP over a four-day period, with an average daily collection of 112 ticks per day. *A. hebraeum* nymphs were not collected at the KNP during the survey.

TABLE 12 *Amblyomma hebraeum* collected at Skukuza, Kruger National Park, South Africa, using the AAAP/CO₂ trap

VISIT NO.	COLLECTION DATE	AREA SAMPLED (MAP)	ADULT TICKS			NYMPHAL TICKS	NO. OF VISITS PER MONTH	NO. OF TRAPS USED	AVERAGE TICKS PER DAY		**1 RAIN-FALL	**2 RH	**3 TICK ACTIVITY INDEX (0 - 5)	**4 VEGETATION COVER INDEX (0 - 5)	**5 TEMPERATURE (FULL SHADE)	*6 WIND INDEX (0 - 5)	**7 DAY LENGTH INDEX	TIME OF DAY
			T	M	F				A	N								
1	1992-12-06	Skukuza Scientific Camp Site (SSCS)	88	45	43	0	1	10	88	0	Yes	N/R	5	4	N/R	1	5	09:00 - 16:00
2	1997-09-15	SSCS	110	42	68	0	3	30	110	0	No	N/R	5	3	N/R	2	2	15:00 - 17:00
3	1997-09-16	SSCS	112	51	61	0	3	30	112	0	No	Max. 75% Min. + 10%	5	3	Min. 13°C Max. 51°C	1	2	
4	1997-09-17	Tamboi Riverbed	42	22	20	0	3	30	137	0	No	N/R	5	3	N/R	1	2	09:00 - 15:00
5	1997-09-17	Fishing hole	95	38	57	0	3	30	0	0	No	N/R	5	3	N/R	1	2	N/R
	SEPTEMBER	TOTAL	447	198	249	0												

RESULTS: 1. Total adult ticks collected = **447** 2. Total days of collecting = **4** 3. Average ticks collected per day = **112**

**1 RAINFALL	**2 RH (Relative Humidity)	**3 TICK ACTIVITY INDEX (0 - 5)	**4 VEGETATION COVER INDEX (0 - 5)	**5 TEMPERATURE	**6 WIND INDEX	**7 DAY LENGTH INDEX (0 - 5)
Presence of recent rains noted. Average monthly rainfall recorded at the nearest meteorological station	Measured on the ground in full shade	0 = Zero tick activity 1 = A few ticks present 2 = Some activity - low numbers 3 = Medium tick activity 4 = Ticks active 5 = Ticks very active	0 = None 1 = Little 2 = Some 3 = Medium 4 = Good 5 = Dense	Measured on the ground in full shade. Minimum (min.) and maximum (max.) also recorded if possible	0 = Zero winds 1 = Slight breezes 2 = Medium breezes 3 = Windy day 4 = Strong winds 5 = Heavy, gale force	0 = Minimum 2.5 = Intermediate 5 = Maximum

T = Total M = Male F = Female A = Adult N = Nymphal 0 = No ticks collected N/R = Not recorded

2.5.3.2 Temperature and relative humidity, and its possible effect on tick collection with the AAAP/CO₂ trap

Trapping of ticks on the second day (1997-09-17) started at 06:00 and the soil temperature (T°C) and the RH were monitored throughout the day until 17:00 (Fig. 34). At 06:00 the T°C was relatively low (15°C) and no ticks were collected. As the day progressed and the T°C increased to over 20°C (07:00), the ticks then began to respond to the trap. From 09:00 the ticks really started to become active and this continued throughout the day with a peak in the early to late afternoon (14:00 - 16:00). The RH started at a high of 75% (06:00), but declined steadily throughout the day to reach a minimum of 10% at 14:00 and then slowly increased to 40% at 17:00.

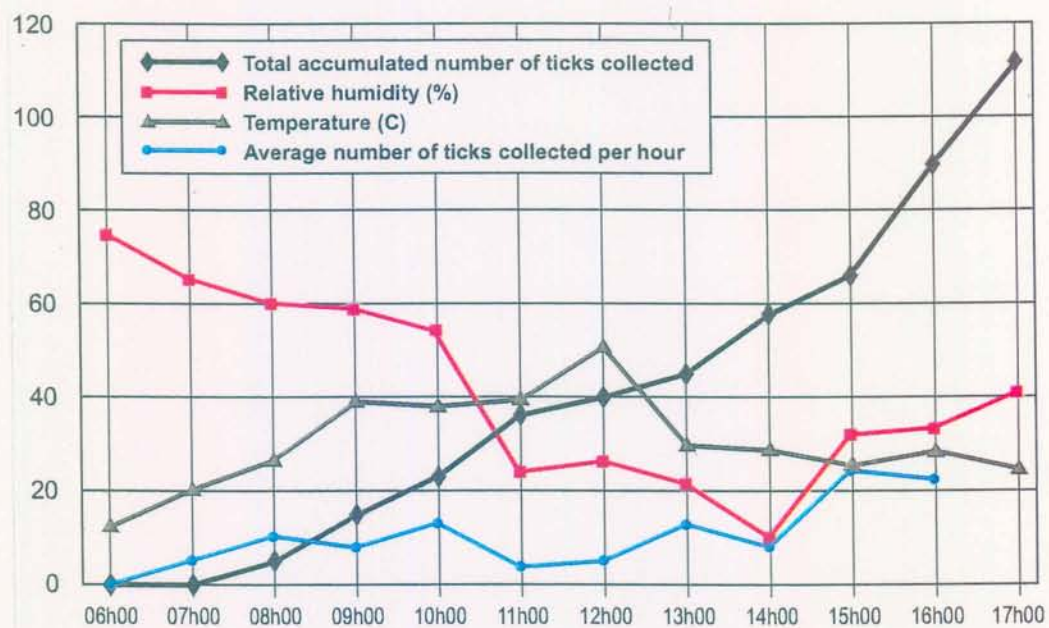


Fig. 34 Total accumulated adult *A. hebraeum* (—◆—) collected with the AAAP/CO₂ trap during one day of collection (1997-09-17) at the Nwashitsaka Research Guest Camp, Skukuza, KNP, South Africa. The average ticks collected per hour (—●—), relative humidity (—■—) and temperature (—▲—) were also recorded.

2.5.4 DISCUSSION

2.5.4.1 Ticks collected

The 447 ticks collected in the KNP comprised 23% of the total number of ticks collected in the study (Table 8, Fig. 19a). No nymphal ticks were collected (Fig. 19b), and the average, daily collection of 112 adult ticks was almost twice that of any other area sampled (Fig. 20). The KNP had the highest density of *A. hebraeum* adults of the six localities sampled (Fig. 20). The AAAP/CO₂ was successfully used in a number of different ecological areas in the KNP, having high tick activity. The tick activity index (Table 12) in all these areas and on each of the collecting days was a maximum of five. This indicated a high population of free-living, unfed *A. hebraeum* in the KNP. Certainly this would confirm the findings of Horak (1995), who found the *A. hebraeum* to be a common tick on many of the KNP wild animals. Spickett *et al.* (1991) also found that free-living *A. hebraeum* larvae were the most prevalent larval species in the KNP. However, a more intensive study needs to be done to locate *A. hebraeum* nymphs in the KNP, which were not detected by drag-sampling or AAAP/CO₂ trapping.

2.5.4.2 Temperature and relative humidity measurements

There appeared to be a direct relationship between the soil T°C and the activity of the ticks. Certainly early in the morning (06:00) there was no tick activity, and this only improved after 09:00 when the tick activity increased substantially. Certainly one can conclude that field work with these ticks should not start until after 09:00, as little tick activity was present before this. The effect of RH on tick collection was more difficult to ascertain, but there appears to be no direct relationship.

2.5.5 CONCLUSIONS

The KNP was an easy place to collect *A. hebraeum* adults with the AAAP/CO₂ trap probably as a result of a high population of ticks due to ideal climatic conditions, no acaricidal control and an abundance of wild animal hosts. All these factors would have led to a sustained high population of free-living, unfed adult *A. hebraeum*. Future research should investigate seasonal changes in the population of adult *A. hebraeum*, and whether it is possible to collect adult ticks in midwinter, as well as where the nymphal ticks can be found.

2.6 THE SONGIMVELO GAME RESERVE, MPUMALANGA PROVINCE, SOUTH AFRICAN GAME RESERVE (NO TICK CONTROL)

2.6.1 INTRODUCTION

The Songimvelo Game Reserve (SGR) was originally chosen as an area to test the AAAP/CO₂ trap because of its proximity to "Geluk". "Geluk" was one of Tice's (1995) CGA used in his project as an example of an intensively dipped CGA (Tice 1995).

2.6.2 MATERIALS AND METHODS

2.6.2.1 Geographical location

The SGR (30° 52' - 31° 00' East; 28° 57' - 26° 05' South) is close to Badplaas, Mpumalanga Province, and also close to Swaziland. It was established in 1986 by the amalgamation of sparsely populated lands which had been communally grazed.

2.6.2.2 Study area

No map of the area was available.

2.6.2.3 Climate

As the SGR is close to the KNP it can be assumed that their overall climatic conditions are reasonably similar, i.e. lowveld, hot summer and no frost (rainfall 700 mm per annum).

2.6.2.4 Vegetation and fauna

Lowveld and sour bushveld. A large variety of wild animals have been introduced into the SGR and these include elephants.

2.6.2.5 Livestock

None.

2.6.2.6 Collection of ticks

Ticks were collected by three operators using the original pheromone and chips of dry ice (Tice 1997).

2.6.3 RESULTS

The results of the one visit to the SGR are summarized in Table 8 and 13, and Fig. 19a & 20. Forty-eight adult (24 male and 24 female) *A. hebraeum* were collected during this visit (Fig. 20) (average of 48 ticks per day).

TABLE 13 *Amblyomma hebraeum* adults collected using the AAAP/CO₂ trap at Songimvelo Game Reserve

VISIT NO.	DATE	ADULT TICKS			NYMPHAL TICKS	NUMBER OF VISITS THAT MONTH	AVERAGE ADULT TICKS COLLECTED PER VISIT
		T	M	F			
1	1992-12-02	48	24	24	0	1	48
TOTAL		48	24	24		1	48

T = Total M = Male F = Female

2.6.4 DISCUSSION

The AAAP/CO₂ trap worked well in both game reserves where there was a high density of free-living adult *A. hebraeum* as indicated by the high tick activity index. The 48 ticks collected in the SGR comprised only 2% of the total number of adult ticks collected (Fig. 19a), and no nymphal ticks were collected (Fig. 19b). The number of ticks collected during a single day (n = 48) was good (Fig. 20), and on the same level as at Rietgat.

2.6.5 CONCLUSION

The game reserves proved to be the best localities for the optimum use of the AAAP/CO₂ trap, probably as a result of the high density of the population of *A. hebraeum* in these controlled environments. The high population of undipped hosts in both reserves would have contributed to the artificially high populations of *A. hebraeum*. Ideal climatic conditions, coupled with the lack of frost would also have had a positive effect on the free-living population of *A. hebraeum* in these game reserves.

2.7 OVERALL DISCUSSION AND CONCLUSIONS ON THE USE OF THE AAAP/CO₂ TRAP

A total of just under 2 000 (1 934) adult *A. hebraeum* and 309 nymphs were collected with the AAAP/CO₂ trap at the geographically different sites. There were certain areas where the trap worked well (KNP, SGR, Rietgat and East London), and others (Warmbaths and MEDUNSA), where the sustained use of the trap could not be justified.

The various factors affecting the use of the trap have been discussed in detail, and the time of year was far the most important. There was a definite seasonal variation in response to the trap, and in winter very few ticks were collected at any of the sites, and it would be enlightening to test the trap in the KNP in midwinter. The effect of temperature on tick collection has important implications because much of the field work, especially in summer, is done early in the morning (04:00 to 06:00). Winter and early morning collections must be avoided and rather concentrate on the early to late afternoon in spring and early summer.

An important observation made during this study was the effect of vegetational cover. In South Africa, as in other parts of the world, many of the trees lose their leaves, especially towards the end of summer and during autumn. Some trees only lose their leaves in spring (tamboti) and some continuously (*E. crispa*). The trees which lose their leaves continuously give rise to deep litter, creating excellent habitats for adult and nymphal ticks during the dry, cold winter. They also constitute excellent sites to collect ticks which concentrate there.

Dense, living vegetation cover had negative effects on the visibility of the ticks to the collector, and especially in late summer when the vegetation was so dense it was almost impossible to see adult ticks.

The effects of RH and rainfall are more difficult to assess. However, it would appear that as the RH declines in winter, especially after all the surface water has evaporated (late spring), conditions become unfavourable for free-living, unfed adult ticks. Some of these ticks probably survive by moving to habitats where RH is maintained. At Rietgat at the end of winter the only areas where these moist conditions are maintained are shaded, moist leaf litter. As the summer progresses and the rains fall, then larger portions of the CGA develop suitable RH conditions. Consequently collections made during the late winter/early summer period (September, October and November) would have the greatest chance of success.

The biggest advantage of the AAAP/CO₂ trap is its simplicity and flexibility. It did not require very specific conditions to work, the CO₂ source is usually easy to obtain and once the pheromone solution has been made it lasts for years if stored correctly. All items used in the trap are inexpensive consumables which are easily replaceable.