THE ROLE OF PREDATORS, PARASITOIDS AND PATHOGENS IN REGULATING NATURAL POPULATIONS OF THE NON-PARASITIC STAGES OF RHIPICEPHALUS APPENDICULATUS NEUMANN AND OTHER LIVESTOCK TICKS, AND RELATED ASPECTS OF THE TICKS' ECOLOGY

BY

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DECLARATIONS

This thesis is my original work and has not been presented for a degree in any other University.

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ABSTRACT

Natural enemies of the important ticks in Kenya were studied with a view to assessing their role in regulating natural populations, and their possible use in biological control of ticks.

Predators of engorged females of Rhipicephalus appendiculatus and Amblyomma variegatum in the field were found to be rodents, ants, spiders, birds, lizards and shrews. In the field there was about 43% predation of R. appendiculatus females, 46% of A. variegatum females and 36% of engorged R.appendiculatus nymphs. Death due to environmental factors did not exceed 7% for any group while predation contributed by small animals was 7%. Domestic chickens were found to be effective tick control agents in a cattle boma where they ate 86% of engorged ticks put out there. These results have shown that the effect of predators should not be ignored in making a computer model for R. appendiculatus.

A hymenopteran parasitoid resembled Hunterellus hookeri and Ixodiphagus texanus in some aspects, and differing in other aspects was found in A. variegatum nymphs from the Trans-Mara area, infesting 49% of 463 nymphs collected over a period of one year. This is the first record of a parasitoid of A. variegatum. The parasitoid was however not found in nymphs of A. variegatum from Rusinga Island. A 40% infestation of A. variegatum of unfed nymphs was achieved in

the laboratory when the ratio of parasitoids to nymphs was 1:3.

The bacteria Proteus mirabilis, Pseudomonas sp. and Serratia marcescens were isolated from engorged ticks which had been in the grass for 8 days, causing about 10% mortality, while laboratory colonies were infected with Enterobacter cloacae, Escherischia coli and Staphylococcus aureus. Only 1% of 484 ticks were found to be infected with fungi; Mucor sp., Fusarium sp. and Aspergillus sp.

Experimental infections of adult R. appendiculatus with Beauveria bassiana and Metarhizium anisopliae resulted in 73% and 30% mortalities respectively

Engorged females of R. appendiculatus were found to have a dropping off rhythm , with about 71% of them dropping between 0600 and 1000 hours, while 66% of the engorged nymphs dropped off between 1400 and 1800 hours. There was no definite rhythm of drop-off for larvae however. The drop-off rhythm in females and nymphs was not affected by feeding on tick-sensitised animals or by their time of application on animals. Onset of drop-off was delayed by 24 hours in both cases. These results indicate that delaying animals in the cattle boma until 1000 hours and bringing them in at around 1600 hours would allow most engorged ticks to drop in unfavourable places, and this procedure would therefore be useful in an integrated tick management package.

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CHAPTER ONE

1. INTRODUCTION

1.1. Economic importance of Rhipicephalus appendiculatus.

The ixodid tick Rhipicephalus appendiculatus Neumann 1901 is a three-host tick, and all its instars feed mainly on cattte. It is also commonly found feeding on sheep, goats and on many wild bovids especially the African buffalo, Syncerus caffer, the eland, Taurotragus oryx, and the water buck, Kobus sp.(Yeoman and Walker, 1967). R. appendiculatus is the vector of the haemoprotozoan Theileria parva Theiler, of which T. p.parva is the causative organism for the deadly East Coast fever (ECF), in cattle, and Theileria parva lawrencei causes the equally serious corridor disease. In endemic areas ECF causes up to 70% mortality in exotic breeds, i.e. Bos taurus or their crosses. However, for the pure indigenous cattle, Bos indicus, mortalities are not as great due to a higher level of acquired immunity. Non-immune adult Bos indicus cattle can be susceptible to ticks. A single infected tick can cause the death of a susceptible animal (Lewis, 1950; Young, 1981).

Other organisms transmitted by R. appendiculatus are Theileria taurotragi, which also causes a mild form of theileriosis; the virus of Nairobi sheep disease; and the rickettsia causing tick-bite fever in man. The presence of large numbers of R. appendiculatus may also cause tick

toxicosis, as well as exposing animals to secondary infection from tick-bite wounds. Although many young cattle recover from the disease and become immune to ECF, they still continue to act as ECF carriers (Young et al., 1986). Wildlife also serves as T.p. lawrencei and T. taurotragi reservoirs (Young et al., 1981; Irvin et al., 1981).

Rhipicephalus appendiculatus is widely distributed in East, Central and Southern Africa (Hoogstraal, 1956). In Kenya it is found in all provinces, except the dry northern region, wherever there is suitable habitat, with an annual rainfall of over 500 millimeters (Walker, 1974).

Amblyomma variegatum is also a three-host tick. It is responsible for transmitting Cowdria ruminantium, which causes heart-water in cattle, sheep and goats. The long Amblyomma mouthparts also cause abcesses which may lead to udder damage and to other secondary infections.

Boophilus decoloratus is a one-host tick which feeds mainly on cattle, and is a vector for the causative organisms of babesiosis and anaplasmosis in cattle.

1.2. Tick control

Current methods of controlling ticks rely heavily on the application of acaricides to the host in order to kill the parasitic stages. Plunge dips are easy to use and suitable for large numbers of animals, but

where there are few dips, this method can be time consuming as animals have to travel long distances. Dipping is also dangerous for weak animals and very young calves. On the other hand, hand-spraying is the most efficient method, but is only practical for farmers who have only a few cattle. A mechanical spray race is more rapid, and the acaricide is always freshly prepared. Spray races have to be maintained with spare parts, however, and this renders them difficult to use in developing countries where the spare parts are not available.

There are many costs involved in the use of acaricides. Together with the high cost of the chemicals themselves, there is increasing cost involved in testing, development and marketing of a new product. Skilled labour is also needed to man the plunge-dips. When a tick population is subjected to chemical acaricides only the most resistant ones survive, which live on to start a generation of resistant ticks. Wharton and Roulston (1970) reviewed the resistance of ticks to chemicals. Arsenical dips were the first to be used. Resistance appeared after 50 years of arsenic use in South Africa, while Rhodesia (Zimbabwe) reported resistance in 1963, and Malawi in 1969 (Jones-Davis, 1972). Other acaricides which have been used in many countries, and to which resistance has been reported, include lindane, malathion, BHC and DDT. In Australia,

there has been sequential development of resistance in Boophilus microplus to arsenic, DDT, BHC and to organophosphates (Wharton, 1979). Failure to use the right concentrations of the chemicals and uncontrolled movement of animals also enhance the development of acaricide resistance (Cunningham, 1981; Keating, 1983). Chemical acaricides are also toxic to livestock and man, and have toxic residues as well. Alternative methods of control are being sought which can be incorporated into an integrated control system.

The available alternatives include pasture spelling, habitat modification, sterile male technique, natural host resistance to tick infestation, artificially induced immunity to ticks, the use plants with acaricidal properties, and also natural enemies of ticks, namely; predators, parasites and pathogens. Pasture spelling involves leaving the grazing land unused for long periods until the available free-living stages of ticks die without finding a host. This method has been recommended for the control of B. microplus (Sutherst et al., 1979), because the larva is the only free-living stage (Wilkinson, 1964), and can therefore be eliminated in about 6 months. For a three-host tick, however, this method would not be practical, because adults of R. appendiculatus can survive for over 18 months. Moreover, pasture spelling has had little appeal for a number of reasons including the cost of fencing in range lands, and

lack of grazing land in high potential areas in developing countries. The unfed stages of ticks need enough moisture to survive. Modifying the habitat by reduction of vegetation is likely to reduce the number of ticks (Hair and Howell, 1970). Heavy grazing, burning pastures and drainage of some areas are methods which can be used to effect this. However, these methods are not practical for large scale use.

Genetic control measures, such as cytoplasmic incompatibility, hybrid sterility, and distorted sex ratios could be incorporated in integrated control packages (Knipling et al., 1968). The sterile male technique has not been shown to be effective in controlling ticks so far. However, Graham et al. (1972) and Thomson et al. (1981) showed that interspecific crosses between B. annulatus and B. microplus produce viable F1 progeny in which all males are sterile, but females are fertile when back-crossed to the male parent species. These sterile males were reported to mate with twice as many females as did the normal ones, and they also survived longer on the host, indicating that they could successfully be used for control (Davey et al., 1983). The females of the South African strain of B. microplus when mated with males of an Australian strain of the same species yielded 62% of viable progeny, whereas the reciprocal cross gave non-viable larvae.

Pheromones, chemicals released by an animal to influence the behaviour of other individuals of the same species, have been used in tick control programmes for Amblyomma spp. Gladney et al. (1974), Rechav et al. (1977), Rechav and Whitehead (1978; 1981) and Sonenshine et al. (1979) applied aggregation-attachment pheromone baited acaricide to single locations on bovine hosts, which attracted released ticks to this site where they were killed. Sex pheromones could also be used to confuse sexually mature males to such an extent that they would not be able to find the natural pheromone releasers (Sonenshine et al., 1979). Pheromones have also been used in tick traps where ticks are eliminated before they find hosts (Rechav et al., 1977; Rechav and Whitehead, 1978).

Sex pheromones of R. appendiculatus have been identified (ICIPE Annual Report, 1983, 1984). They are phenolic compounds including 2, 6-dichlorophenol which enable the sexes to come together for mating during the parasitic phase of adult life. However, mixtures containing artificial sex pheromones have not been shown to be effective in confusing the males and preventing mating in A. variegatum (Sonenshine et al., 1982).

Host resistance prevents ticks from feeding adequately due to immunologically-induced changes in the host (Roberts, 1968). In Australia, the control of B. microplus has been enhanced by the use of resistant

animals from within breeds with high ability to develop resistance, as part of an integrated control regimen. The ability to develop resistance to ticks is heritable (Hewetson, 1972; Seifert, 1984). It is stable over long periods although it can be altered by stress such as sickness, lactation or poor nutrition (Wharton et al., 1970; Seifert, 1971; Utech et al., 1978). Zebu cattle (B. indicus) and their crosses develop higher resistance to ticks than B. taurus breeds (Riek, 1962; Wilkinson, 1962; Seifert, 1971; Hewetson, 1979). Artificial immunization of animals against ticks is also being investigated (Mongi, 1980; Johnston et al., 1986).

Unfed stages of most species of ixodid ticks ascend plants in order to reach and cling on to a passing host, which often involves long periods of waiting. Any method which would decrease their life expectancy, or reduce the number of ticks at this waiting stage, would lower the subsequent population of the parasitic stage. The molasses grass Melinis minutiflora, and gamba grass, Andropogon sp. have been shown to reduce tick survival, resulting in lower infestations on cattle (Thompson et al., 1978). Some species of the tropical legume Stylosanthes have glandular trichomes which secrete a viscous fluid that immobilizes larvae of B. microplus (Sutherst et al., 1982). Zimmerman et al.(1984) obtained similar results with A. variegatum.

Biological control of ticks by using natural predators, parasitoids (insects which are internal parasites, but with a free living adult stage) and pathogens has not been investigated fully. Although isolated studies have been done on these natural enemies of ticks, very little work has been done on African ticks. Since the natural enemies of any pest are likely to differ between geographical regions, a study of natural enemies of important tick species in Kenya should be done, and then investigations made to find out how they could best be incorporated in an integtrated control system.

1.3. Objectives of this study

For effective control of ticks, a full understanding of their life-cycle, including causes of mortality, should be obtained. This information is needed for constructing computer models, which would, in future, become tools in guiding the development and use of integrated control packages. In this connection, therefore, it was thought necessary to make a thorough study of the role played by predators, parasitoids and pathogens in the regulation of natural populations of important ticks in Kenya. The work is based mainly on R.appendiculatus, with some investigations also on A.

variegatum and B. decoloratus. Data on ecological aspects of these ticks which have been encountered in the course of the study have also been reported. The possibility of using these natural enemies of ticks as biological control agents has also been examined.

For an effective tick control regime, whether by chemicals or by natural enemies, an understanding of the dropping-off rhythm of the engorged ticks from the host is also necessary, so that any control agent could be applied with maximum benefit. The information available on the drop-off rhythm of R. appendiculatus (Kitaoka, 1962; Minshull, 1982) is not adequate, and is conflicting, and so it was necessary to study the drop-off rhythm of all the three instars of R. appendiculatus. This study would also be important in the interpretation of the results obtained in the predation studies of this tick in the field, because the proportion of engorged ticks which are eaten by predators will depend partly on where they drop, and therefore also on what time of day or night they come off the host.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Predators of ticks

2.1.1. Predation on the host

During their life-cycle, ticks spend some time feeding on the host (parasitic stage) and the rest of the time off the host (free-living stage). For R. appendiculatus, the time spent on the host is 15-22 days in total (larvae 3-5 days, nymphs 5-7 days, females 7-10 days) (Hoogstraal, 1956; Tukahirwa, 1976). The rest of the tick life cycle is spent on the ground and the total could be up to 500 days (Hoogstraal, 1956; Branagan, 1973).

The red-billed oxpecker, Buphagus erythrorhynchus, and the yellow-billed oxpecker, Buphagus africanus prey on the parasitic stages of ticks as their main diet (Moreau, 1933; Van Someren, 1951; Olivier and Laurie, 1974; Stutterheim, 1976). The methods used to ascertain that these birds do prey on ticks were direct observations and examining their stomach contents. The birds were found to be eating ticks from horses, mules, cattle, donkeys, sheep, goats, pigs and camels, and also all the larger wild herbivores except elephants and hippopotamuses (Moreau, 1933).

Because of the importance of oxpeckers as predators of ticks, their ecological distribution has been studied in Southern Africa (Stutterheim and Brooke, 1981), while their host preference was reported by Attwell (1966), Stutterheim (1976), Grobler and Charsley (1978) and Grobler (1980).

With the advent of acaricides to control ticks in Africa, and the widespread elimination of large game, the numbers of oxpeckers were found to decrease, probably due to both acaricide poisoning and starvation (Van Someren, 1951; Brooke, 1963; Attwell, 1966; Stutterheim, 1976). This was based on visual estimates rather than actual counts. When there was abundant rainfall in Southern Africa over a period of eight years, the resulting good vegetation led to an increase in the numbers of large game, and of R. appendiculatus and B. decoloratus feeding on them, and consequently oxpeckers were also found to increase in Kruger National Park (Stutterheim and Stutterheim, 1980).

Cattle dipping in Kenya is enforced in most areas where R. appendiculatus is found, and as a result the numbers of oxpeckers are low and their impact would be considered negligible.

Other wild birds have been reported as predators of ticks on the host. The cattle egret, Ardeola ibis was found eating ticks by Rothschild and Clay (1952). Other

workers questioned their report, but McKilligan (1984) in a study on food and feeding habits of the cattle egret, confirmed that they ate ticks from cattle, but that ticks did not constitute a great part of their diet.

Other birds which have been reported to be predators of ticks include the pee-wee, Grallina cyanoleuca, and the starling, Sturnus vulgaris, in Australia (Legg, 1930); the magpie, Pica pica (Stelfox, 1968) in Canada, and the Indian myna, Acridotheres tristis (Page and Oately, 1979). There is also one record of water tortoises Pelomedusa subrufa, which were noticed removing ticks from a black rhino in a stream bed (Anon, 1962).

2.1.2. Predation off the host

Due to the fact that the free-living stages of ticks are well hidden on the ground, it is a difficult task to establish which animals eat them. With few exceptions, many of the reports are therefore casual or incidental observations.

In Australia, the ants Iridomyrmex detectus, Pheidole megacephala and Aphaenogaster longiceps have been reported to prey on B. microplus (Wilkinson, 1970a), giving an estimated 50% reduction in the numbers of engorged females. In Louisiana, U.S.A., the fire-ant Solenopsis invicta, preys on Amblyomma americanum, eating engorged females, eggs and larvae (Harris and Burns, 1977; Burns and

Melanchon, 1977). These studies were prompted by the observation that plots in which the ants were controlled had many ticks , while those with ants did not have as many ticks. This implies that ants do indeed affect natural populations considerably.

Butler et al. (1979) reported that the ant Solenopsis geminata was responsible for predation of 63% of gravid females of B. microplus within the period November to February in Mexico, and Diego et al. (1983) observed predation on Amblyomma cajennense by P. megacephala in Cuba.

Ants are not always active throughout the year and their effect on ticks would be expected to be seasonal (Wilkinson, 1970a; Butler et al., 1979). There has been no study of ant predation in relation to survival of ticks. Wilkinson (1970a) provided a hypothetical life-table, concluding that it would only require ants to reduce the ticks' rate of increase to zero for the residual population to be below the recovery level when conditions became favourable again. He therefore suggested that this might be a decisive factor in the "reputed tick scarcity areas" in Australia.

Murphy (1925) recorded that the spider Dysdera murphi preyed on the tick Ornithodoros amblus. After finding spiders' dens sometimes filled with shrivelled remains of ticks from which the body juices had been sucked,

he concluded that the spiders were the chief natural agency in restricting numbers of O. amblus. This conclusion was not shared by Wilkinson (1970a), who reported predation of ticks by the spiders Lycosa godeffroyi and Phidippus rimator, concluding that spiders were second to ants in importance as predators of ticks.

Predation of ticks by lizards has been reported (Norval, 1976; Norval and McCosker, 1983), involving the lizards Gerrhosaurus flavigularis and, Mabuyu quinquetaniata which were found eating engorged Amblyomma hebraeum females in Zimbambwe. In Peru, lizards belonging to the genus Tropidurus were reported to eat ticks (Clifford et al., 1980).

Although several workers have mentioned that rodents are predators of ticks, no actual studies had been carried out to confirm this until Wilkinson (1970b) set cameras with triggering mechanisms attached to tethered ticks, to investigate predation. He obtained pictures of the rodents Neotoma cinerea and Marmota flaviventris removing the ticks. Maywald (1987) also reported rodents to have been noticed eating ticks, although he did not do any actual studies on this . The only other study of rodents as predators of ticks was in the laboratory where individuals of Rhabdomys pumilo were offered engorged R. appendiculatus (Short and Norval, 1982), but they did not harm the ticks even in the absence of other foods.

Milne (1950) reported predation of ticks by shrews in an experimental plot, and Short and Norval (1982) found that the shrew Crocidura hirta dug up and ate engorged R. appendiculatus and B.decoloratus females buried in nylon bags in experimental plots. Subsequently, the shrews were found to eat ticks in the laboratory, and they were able to locate, apparently by smell, ticks which were buried beneath the soil, on the soil surface, or suspended by a thread above the soil surface.

Although there are a number of reports of birds pecking at ticks or removing ticks from the host, there are very few reports of birds eating free-living ticks. Milne (1950) suspected that birds removed ticks from experimental plots, and Hunter and Bishopp (1911) found domestic chickens eating engorged ticks which dropped from cattle. The American robin Turdus migratorius also preys on engorged ticks from the ground (Wilkinson, 1970b).

Various methods have been employed in studies of predation of free-living tcks. Wilkinson (1970a, b) tethered ticks by cotton thread stuck on to the scutum, or a polyester thread, respectively. He also tethered ticks by passing thread with a needle through the caudal end, in effect passing a suture under one millimeter of cuticle, taking care not to rupture the gut. A silver thread was used to tether Dermacentor albipictus tying them by one

leg (Darling, P. unpublished report) however, simply tied one leg of engorged Dermacentor albipictus using a silk thread. The tethering method is impratical for immature ticks due to their small size. Another method of studying predation of engorged ticks is by immobilizing engorged females in pairs by glueing their ventral sides together (Wilkinson, 1970a). This is definitely not a suitable method because movement and hiding from predators is prevented completely. The only mention of putting ticks out in experimental plots in nylon bags is by Short and Norval (1982), in their study on survival of ticks. No one has investigated the importance of smaller predators, by the use of differential protection methods.

The effect of predation of free-living stages of ticks on the regulation of natural populations has not been investigated for any tick species. In fact, predation has been considered to be of negligible effect, and has been omitted in the construction of computer tick models (Sutherst et al., 1978, 1979; Sutherst and Dallwitz, 1979; Norton et al., 1983; Haile and Mount, 1987). This aspect should certainly not be ignored, especially since high mortalities have been reported to be caused, for instance, by ants (Wilkinson, 1970a; Butler et al., 1979). As there is not yet a computer model for the major ticks in Kenya, it is important that the role played by predators be investigated and established.

2.2 Parasitoids of ticks

The first tick parasitoid to be identified was Ixodiphagus texanus (Howard, 1907). It was collected from the nymphal stages of the ticks Haemaphysalis leporispalustris, Dermacentor variabilis and Ixodes dentatus (Howard, 1907; Smith and Cole, 1943).

Since then, three other parasitoids belonging to the same genus have been identified namely: Ixodiphagus mysorensis, collected from an unidentified Ornithodoros in India, Ixodiphagus hirtus collected from Ixodes persulcatus; and Ixodiphagus biroi, the host of which is not known (Cole, 1965). There is very little information on I. mysorensis, I. hirtus and I. biroi apart from their initial identifications, a fact which makes some workers think they are all conspecific with I. texanus (Fiedler, 1953).

Howard (1908) identified and described a new hymenopteran parasitoid from Rhipicephalus sanguineus, erected a genus for it, and named it Hunterellus hookeri. Since then this species has been found in ten species of ticks. Wood (1911) collected it from Dermacentor parumapertus , Cooley and Kohls (1934) from Ixodes ricinus, Hyalomma aegyptum, Rhipicephalus oculatus, Rhipicephalus evertsi and R. appendiculatus, and Ushakova (1962) from Hyalomma asiaticum, Haemaphysalis concinna, Haemaphysalis japonica and I. persulcatus.

Two other parasitoids, first named as Ixodiphagus caucurtei and Habrolepis caniphia were later transfered to the species H. hookeri (Cole, 1965).

In South-west Africa, Fiedler (1953) descibed a second parasitoid in the genus Hunterellus, naming it Hunterellus theilerae. It was collected from a nymph of Hyalomma transiens which was feeding on a wild hare. It was recorded again emerging from Rhipicephalus oculatus in Transvaal, South Africa (Fiedler, 1953). The latest addition to the number of tick parasitoids is Hunterellus sagarensis , first discovered and described in India, where it was parasitizing Haemaphysalis bispinosa (Geevarghese, 1977), and later reported in Japan from Haemaphysalis longicornis (Tachikawa, 1980).

Although the genus Hunterellus has been obtained from five tick genera, there are only two possible records of it in Amblyomma ticks. Cooley (1930) mentioned that another parasitoid was noticed emerging from Amblyomma hebraeum in South Africa, but it was never identifed, and has not been mentioned again since that time. Graf (1979) however found a parasitoid from Amblyomma nutalli, which he reported to be closely related to H. hookeri. It was, however, absent from A. variegatum collected from the same neighbourhood as the A. nutalli.

Hunterellus hookeri is the most cosmopolitan of the parasitoids found, and has been recorded from England,

France, Siberia, India, China, Brazil, Cuba, Mexico and the U.S.A. (Philip, 1954). In Africa it was reported from Nigeria (Philip, 1931), South Africa (Cooley and Kohls, 1934) and in Kenya it was collected from R. sanguineus in the Mombasa area (Philip, 1954). The laboratory rearing of H. hookeri was first accomplished by Wood (1911). Later it was cultured in the laboratory (Larrouse et al., 1928; Cobb, 1942; Smith and Cole, 1943). Even among the tick parasitoids known today, it is only H. hookeri whose biology and life-cycle have been fully established. The female lays eggs in the host tick by piercing the integument with her ovipositor, and oviposition takes about 2-20 seconds (Wood, 1911; Cooley, 1928). The preferred stage is the unfed nymph and, less frequently, the engorged nymph (Cooley and Kohls, 1934). The phenomenon of latency, in which eggs laid in replete tick larvae stay through the process of moulting, pass the winter in the unfed nymphs, and later develop when the nymphs feed, has been reported by Cooley and Kohls (1928) and Cooley (1928, 1930).

It is believed that parasitoids attack ticks off the host animal, and also on the host during feeding. These aspects have been demonstrated by Wood (1911), Cooley and Kohls (1934) and Smith and Coles (1943). It has also been recorded that parasitoids infest ticks on the host (Philip, 1931; Cooley, 1934; Cobb, 1942; Fiedler, 1953) as parasitoids were found in the fur of animals to which ticks

had attached. The stage of tick found most suitable for infestation by H. hookeri in the laboratory was the unfed nymph (Wood, 1911; Cooley and Kohls, 1934; Smith and Cole, 1943), but the larval stage was said to be completely ignored. However, Bowman et al.(1986) working on I. texanus reported that engorged larvae, unfed nymphs and engorged nymphs were preferred by the parasitoid in that order.

There has not been a thorough laboratory study on the suitability of different stages of ticks and species of ticks for infestation with H. hookeri. Although such a study has recently been made for I. texanus (Bowman et al., 1986) using R. sanguineus, Amblyomma americanum and Amblyomma maculatus. They concluded that the Amblyomma ticks were not suitable hosts.

So far, parasitoid eggs have not been recovered from the bodies of ticks, but the eggs of H. hookeri were studied by dissecting the insect and crushing the ovaries (Cooley, 1928). The absolute numbers of eggs contained in females have not been counted, but estimates have been made from the number of adults that eventually emerged in the laboratory. Five to ten eggs are deposited with each insertion of the ovipositor (Cooley, 1928).

After a nymph is infested it continues to feed normally. The infestation only becomes apparent 8-15 days after repletion, when there is swelling, soon followed by an irregular striped appearance caused by larvae as seen through

the skin of the nymph. At emergence all the adult parasitoids come out through one or two holes which are made in the cuticle of the tick. Mating takes place immediately and lasts for only a few seconds (Cooley and Kohls, 1934).

The average numbers of adult parasitoids emerging from replete nymphs are reported for various ticks by Wood (1911), Cooley and Kohls (1934), Smith and Cole (1943) and Bowman *et al*. (1986).

In experimental infestations with *H. hookeri* using unfed nymphs, there was a 95% infestation rate for *I. ricinus*, 90% for *R. sanguineus*, 100% for *D. andersoni* (Cooley, 1928) and 61% for *D. variabilis* (Smith and Cole, 1934).

In the laboratory, the effect of temperature on the development of adult parasitoids was studied by Cooley (1928, 1930) who reported that 22°C was the optimum temperature for development in *H. hookeri*, with emergence taking about 44 days. However Smith and Cole (1943) found that at 27°C the period before the parasitoids emerge was reduced to 20-30 days. Survival of the emerged parasitoids was 2 days at 27°C. Neither hibernation during winter nor aestivation during unfavourable summer conditions were reported for the emerged parasitoids.

For *H. hookeri*, different sex ratios have been reported in different tick species (Cole, 1965), the reasons

for which are not clear. For mass rearing of H. hookeri nymphal ticks have been used. The most successful method was to infest while they were engorging on the host (Wood, 1911). For mass rearing of I. texanus, it was found that exposing parasitoids to ticks while they were off the host worked better (Bowman et al., 1986).

Following the laboratory rearing and successful mass production of H. hookeri, attempts were made to control different ticks in the field. The methods used for release of the parasitoids are described by Cooley and Kohls (1934), and Smith and Cole (1943). Larrouse et al. (1928) released H. hookeri in Naushon Island, Massachusetts, to combat D. variabilis. Apparent success was reported initially , but later the parasitoids were overwhelmed by the number of ticks in the field. Nevertheless, a recent study on Naushon Island showed that one third of the nymphs of the deer tick, Ixodes dammini, were still being parasitised by H. hookeri (Mather et al., 1987). This was attributed to descendants of the original parasitoids released in 1928. Cooley (1928) also released 4 million H. hookeri to attempt control of D. andersoni in Colorado and Montana. Bishopp (1934) released H. hookeri to combat D. variabilis, and Smith and Cole (1943) released 100,000 H. hookeri in Martha's Vineyard Island, Massachusetts. In Leningrad province, USSR, Alfeev (1940) also released H. hookeri to reduce numbers of I. ricinus.

All the attempts were reported to be successful at first, with proven reduction in tick numbers, but later the ticks increased again. No one, however, has attempted either to release parasitoids over a long period to maintain success, or to couple parasitoid effects with other methods of control. These should be tested.

In Kenya, apart from the report of H. hookeri found in R. sanguineus (Philip, 1954), there has been no work done on the parasitoids of important ticks. The role they play in regulating natural tick populations should therefore be established.

2.3. Pathogens of ticks

Since the adverse consequences of chemical pesticides as pollutants in the environment have become well known, the development of alternative methods for effective pest and vector control has been an important subject for research. Microbial control involves the use of viruses, rickettsiae, protozoa, bacteria and fungi or their metabolic products in order to kill the target pests or vectors (Calberg, 1986). These disease-causing organisms are referred to as pathogens, and their effect on various pests and vectors of agricultural, medical,and veterinary importance has been investigated with a view to exploiting them for

biological control. A few bacteria have been isolated from dead, or obviously sick, engorged ticks. Proteus mirabilis was isolated from dead D. andersoni (Brown, 1970). Klebsiella pneumoniae, Pseudomonas mirabilis and a Staphylococcus sp. were isolated from dead ticks in a laboratory colony of B. decoloratus (Hendry and Rechav, 1981).

Experimental infection of ticks with bacteria was tried using Serratia marcescens (Steinhaus, 1959). In all the cases where bacteria were either isolated from ticks in natural infections or used in artificial infections, the symptoms were reported to be a change in colour from grey to black, with death following within days after repletion (Brown, 1970; Hendry and Rechav, 1981). It has been suggested that the bacteria are acquired after the engorged ticks have dropped to the ground, since no blackening ticks are found while they are feeding. However it is possible that infection could be acquired from the skin of the host since a sick tick will probably fall off the animal before blackening. The only exception was the infection by P. mirabilis (Brown, 1970) which was reported to be transovarially transmitted, and disease was triggered in otherwise healthy ticks by ingestion of blood.

To check whether bacteria which are naturally acquired are pathogenic or merely saprophytic, the method of injecting them back into engorged ticks has been tried (Hendry and Rechav, 1981). There is no mention of topical

application of bacteria to the feeding surfaces on the hosts. In vitro feeding of ticks in the laboratory is another method that has not been tried which could be useful in testing pathogenicity of bacteria. There is however a general lack of enthusiasm to fully investigate bacteria as pathogens of ticks, as mentioned by Hendry and Rechav (1981), probably due to the difficulties in application, and also because bacteria are not specific to ticks, and might be pathogenic to man and domestic animals. Recently Kaaya and Dirji (1989) also demonstrated that several serotypes of B. thuringiensis as well as other species of bacteria are pathogenic to adult tsetse.

An alternative method might be to use metabolic products of bacteria to kill ticks. The exotoxin of Bacillus thuringiensis has been extracted and tested for safety to humans, and it has been used against Hymenoptera, Coleoptera and Orthoptera (Calberg, 1986) and also Acarina (the mite Dermanyssus gallinae) by Lavrenuik & Uzdenov (1977). Using the exotoxin of B. thuringiensis var. berliner, 80-96% of the mites were killed at 27°C. The exotoxins of B. thuringiensis have not been tried on ticks, however.

Fungi have also been isolated from naturally sick ticks. Lipa (1971) reported that Aspergillus fumigatus, Beauveria bassiana and Penicillium insectivora were found in unnamed ticks. In Korea, other unnamed ticks were found to die of mycoses, probably caused by Torrubiella sp.

(Steinhaus and Marsh, 1962). These were casual observations, and the only organised study of fungi which are infective to ticks was done by Samsinakova (1974) in Czechoslovakia. She isolated 17 species of fungi from Dermacentor marginatus, Dermacentor reticulatus and I. ricinus from the field. Of these, five were obligate parasites, five were facultative parasites and seven were saprophytic. The fungi most commonly found were Aspergillus parasiticus, B. bassiana, Beauveria tenella, Cephalosporium coccorum and Paecilomyces fumosoroseus.

There are no reports of laboratory trials on ticks using live fungal spores or mycelia, although such studies have been done for other arthropods. Metarhizium anisopliae, B. bassiana and a Hirsutella sp. were used to artificially infect tsetse (Poinar et al., 1977). Kaaya (1988) tested B. bassiana, M. anisopliae, P. fumosoroseus and P. farinosus on the same insect and found the former two species to be highly pathogenic to adult tsetse while tha latter two species were only mildly pathogenic.

Work has also been done using metabolic products of fungi to kill ticks. Krylova (1977) used toxins of B. bassiana, M. anisopliae and P. fumosoroseus against the soft tick Argas persicus. The ticks were immersed for 15 seconds in the extracted fungal toxins, but were found to be resistant, with only 10% mortality 96 hours after treatment.

Toxins of B. bassiana and M. anisopliae have been well studied, and their mode of action and toxin production in different strains of fungi have been shown to be correlated with virulence to the target pest (Ferron, 1981). It would therefore be necessary to start by studying the effect of the live fungi on the target pest. Since the penetrating hyphae of live fungi might also contribute to causing mortality in ticks, the effect of the extracted toxins would have to be evaluated on their own.

Although the development of resistance in ticks to these toxins might arise, cross resistance with chemicals which are currently being used as acaricides is unlikely, because they belong to different chemical groups.

Like fungi, viral infections in ticks have only been reported to occur naturally and no laboratory trials have been conducted. Sidorov and Shcherborok (1973) investigated mass epizootics occurring in laboratory colonies of Ornithodoros lahorensis, Ornithodoros pavlova, Ornithodoros moubata and A. persicus. Viruses were found to be the cause of ulcers in different body areas, particularly deformities of the mouthparts, cuticular ulcerations, tumour-like outgrowths and papillomas (with or without ulcerations) but with changes in shape, size and number of mouthparts, and gangrene of the extremities. The source of the viral infection was thought to be rabbits from a breeding farm.

Other virus-like particles (VLP) pathogenic to the salivary glands of B. microplus were observed by Megaw (1978). The VLP damaged the cytoplasm of the infected cells and the ultrastructure of the VLP in granule-secreting cells of the salivary glands was observed.

There are no reported experiments involving protozoa as pathogens of ticks. However, Rickettsia prowazeki was used to artificially infect females of D. marginatus and Dermacentor albipictus (Rehacek, 1965). Infected females died prematurely and egg production was reduced. Large numbers of rickettsiae were found in all the organs.

Another rickettsia, Wolbachia persicus, was successfully put into the gut of O. moubata where it multiplied and had damaging effects (Weyer, 1973). The ticks died after a few weeks, as multiplication took place in the haemolymph and the rickettsiae were excreted in coxal fluids, and were found also in oviposited eggs.

Wolbachia-like symbiotes were injected into D. andersoni intracoelomically (Burgdorfer, 1973). They produced infection of the haemocytes, hypodermal tissues, salivary glands and connective tissues surrounding the midgut, malphighian tubules and ovary. These massive invasions invariably killed the ticks.

In all the above-mentioned studies of tick pathogens there has been no investigation of the role played by the pathogens in regulating natural populations, except

for the report by Samsinakova (1974) on fungi of field ticks, which were found to cause 5% mortality in winter, and 45-53% in summer. It is likely that ticks feeding on different host species would encounter different pathogens when these organisms are acquired from the host, and so a study of the pathogens from different hosts should be done.

2.4 Drop-off rhythm of R. appendiculatus from cattle

The drop-off rhythm of engorged females has been studied for A. hebraeum (Rechav, 1978), H. leporispalustris (George, 1971), Hyalomma excavatum (Hadani and Rechav, 1969; 1970), Ornithodoros gurneyi (Doube, 1975), Haemaphysalis bispinosa and R. appendiculatus (Kitaoka, 1962), I. persulcatus and I. ricinus (Balashov, 1954; Kheisin and Lavrenenko, 1956). There are some conflicting results, however, in the literature. Kitaoka (1962) reported that there was no definite drop-off rhythm of R. appendiculatus, whereas Minshull (1982) demonstrated definite drop-off rhythms for all stages of R. appendiculatus. Drop-off rhythm studies have been done on larvae and nymphs of Hyalomma anatolicum and H. leporispalustris (Serdyukova, 1954, quoted by Kitaoka, 1962) and R. appendiculatus (Minshull, 1982).

Several reasons have been advanced to explain the occurrence of drop-off rhythms in ticks. Balashov (1954) and Kheisin & Lavrenenko (1956) independently reported that the activity of the host and its feeding regime influence the drop-off rhythm of Ixodes ticks. They demonstrated this by

reversing the drop-off time, by reversing the feeding and activity regime of the cattle. Kitaoka (1962) however differed from this opinion, but suggested that other factors such as external physical conditions, physiological state and movement of the infested cattle may all effect the dropping-off of ticks in concert.

Working on H. bispinosa and B. microplus, Kitaoka (1962) postulated that the rhythmic changes of light intensity during day and night was responsible for rhythm in dropping off. He demonstrated this by causing instant drop-off of fully fed ticks by shining a torch on them.

Minshull (1982) agreed with the theory of the effect of light on drop-off of engorged females. She, however, suggested that it could be the combination of light intensity and temperature that caused the early morning drop-off of R. appendiculatus females. For larvae and nymphs, she explained that their drop-off rhythm was governed by three oscillators, two of which are in the tick and are light sensitive, and one is in the host animal. Her findings supported and amplified those of George (1977) for H. leporispalustris , Doube (1975) for O. gurneyi and Rechav (1978) for A. hebraeum.

The third theory put forward by Kitaoka (1962) was that the drop-off rhythm is affected by the type of mouthparts of the ticks. Ticks with long mouthparts which are deeply inserted into the host, such as I. ricinus and I. persulcatus, need stronger stimuli to cause drop-off than

those whose mouthparts are only superficially inserted during feeding, such as species of Haemaphysalis and B. microplus. The latter are therefore easily afffected by slight changes of light intensity and temperature.

Although the drop-off rhythm of R. appendiculatus has been studied by Kitaoka (1962) and Minshull (1982) there are three reasons that warrant a repeat study for this tick in Kenya. First, the two results reported previously are in conflict.

Secondly, both studies were done in countries which experience strong seasonality, which affects both light intensity and day length, factors which are quoted as important in regulating the drop-off rhythms of ticks. It is therefore necessary to make a study in a country like Kenya where the ticks experience little variation in the length of day and night, throughout the year.

Thirdly, one purpose of studying the drop-off rhythm is to help in determining the time to apply tick control to the greatest effect. Both previous studies were done in the laboratory in open stalls, where conditions of light intensity, temperature, shade and activity of the hosts were not natural. A new study should therefore mimic field conditions and use animals which are kept according to the system of local husbandry.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Ticks

The Rhipicephalus appendiculatus ticks used in predation, pathogen and drop-off rhythm experiments were received from the tick colony at the I.C.I.P.E., established from a strain originally kept at the East African Veterinary Research Organization, Muguga, Kenya. It had been maintained in the laboratory since 1952, using methods described by Bailey (1960) and modified by Branagan (1974). The Amblyomma variegatum adults used in predation experiments, and the nymphs for infecting with parasitoids, were also received from a laboratory colony kept at the I.C.I.P.E. for several years, with occasional boosting with ticks collected from the Trans-Mara area of Kenya. The Boophilus decoloratus females examined for pathogens came from colonies which have been kept at the Veterinary Laboratories, Kabete, Kenya, for seven years. The engorged R. appendiculatus and A. variegatum nymphs examined for parasitoids were collected from cattle in the Trans-Mara and Rusinga Island areas in Kenya (Figure 1). They were all maintained in the laboratory at 28oC and 80% r.h. The humidity was maintained by putting a saturated solution of potassium chloride in an open tray in the incubator.

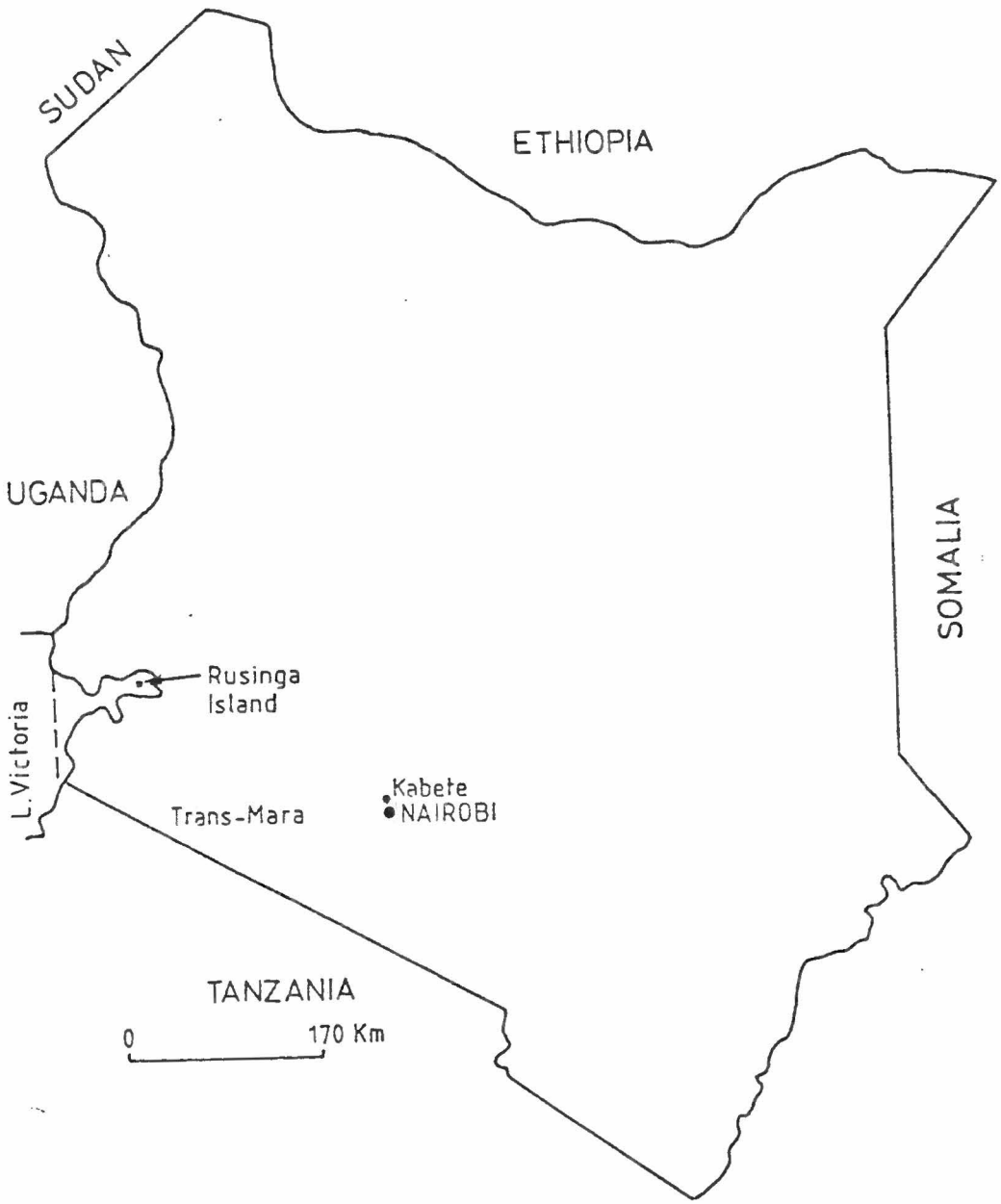

Figure 1 Map of Kenya showing the tick collection sites (Trans-Mara and Rusinga Island) and location of the study plot (Kabete).

3.2 Hosts

New Zealand White rabbits were used for feeding all R. appendiculatus and A. variegatum instars. The rabbits were obtained from Sasumua Farm, Njoro, Kenya,and were maintained on commercial pellets and water.

The cattle used for feeding B. decoloratus were crosses of Friesian x Zebu and Sahiwal x Zebu, obtained from a herd maintained at the Veterinary Laboratories. The cattle used for the experiments to investigate the drop-off rhythm of R. appendiculatus were pure Friesian calves aged 6-9 months, which were tick naive, obtained from the University of Eastern Africa, Eldoret, Kenya. All the cattle were fed on commercial concentrates and hay.

3.3 Parasitoids

After emergence from parasitized nymphs, adult parasitoids were maintained in perspex boxes (Plate 1) which were kept in an incubator at 28o C. Inside the boxes, the humidity was raised by placing a pad of moist cotton wool at one corner. The parasitoids were provided with grass blades for resting on, and cotton wool soaked with a 0.5% sugar solution was placed on the top of a glass tube and placed in the box to provide food.

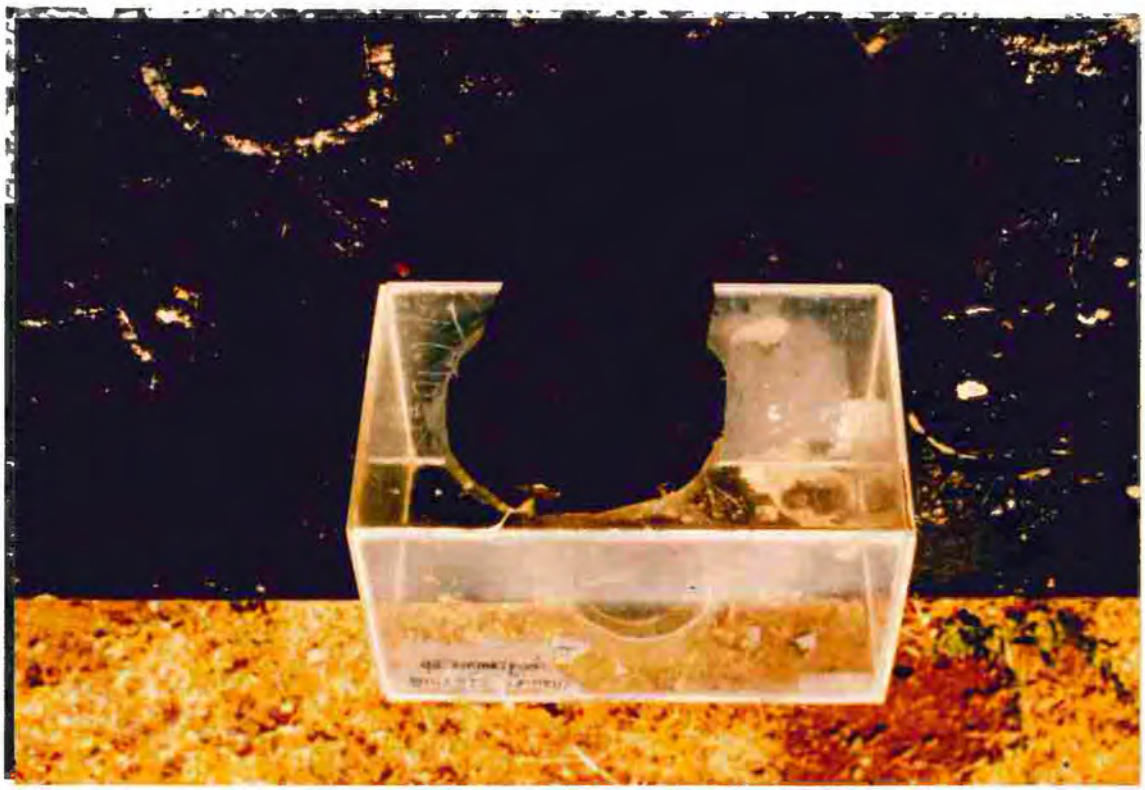

Plate 1 Perspex box measuring 30 x 18 cm, and fitted with cotton sleeve used for holding adult parasitoids.

3.4 Grass plot used for predation experiments

The grass plot used for predation experiments was 50 x25 m, situated in pasture at the Veterinary laboratories (Plate 2). It is in ecological Zone II (Pratt & Gwynne, 1977) and the grass species found in the plot were Cynodon dactylon, Sporobolus pyramidalis, Chloris gayana, Setaria verticillata, Chloris virgata and Bromus uniloides.

3.5 Predation experiments

3.5.1. Confined females in grass. Three groups each of 42 engorged R. appendiculatus females (meausuring about 1.0 cm width and 1.2 cm length) 3 were put on the ground once a month at 0730 hours in the grass plot, plus 42 controls left in the laboratory at 28°C and 80% r.h. In each group, 21 ticks were put out in grass 6-10 cm high (maintained at that height by regular manual cutting), and 21 ticks in grass 40-60 cm high, at a density of 3 ticks per 16 m^2. The plot was subdivided into sub-plots so that each sub-plot was used only once every five months.

Group 1 Tethered ticks

Using a fine nylon thread 45 cm long, which was removed from nylon gauze (Nybolt, gauge PA 236142, Swiss Bolting Silk Company, Zurich, Switzerland), each engorged female was tethered by one leg (Plate 3) to a wire marker 80 cm long, which was pushed into the soil. The ticks were watched for a

Plate 2 Grass plot used for predation experiments at Kabete Veterinary grounds.

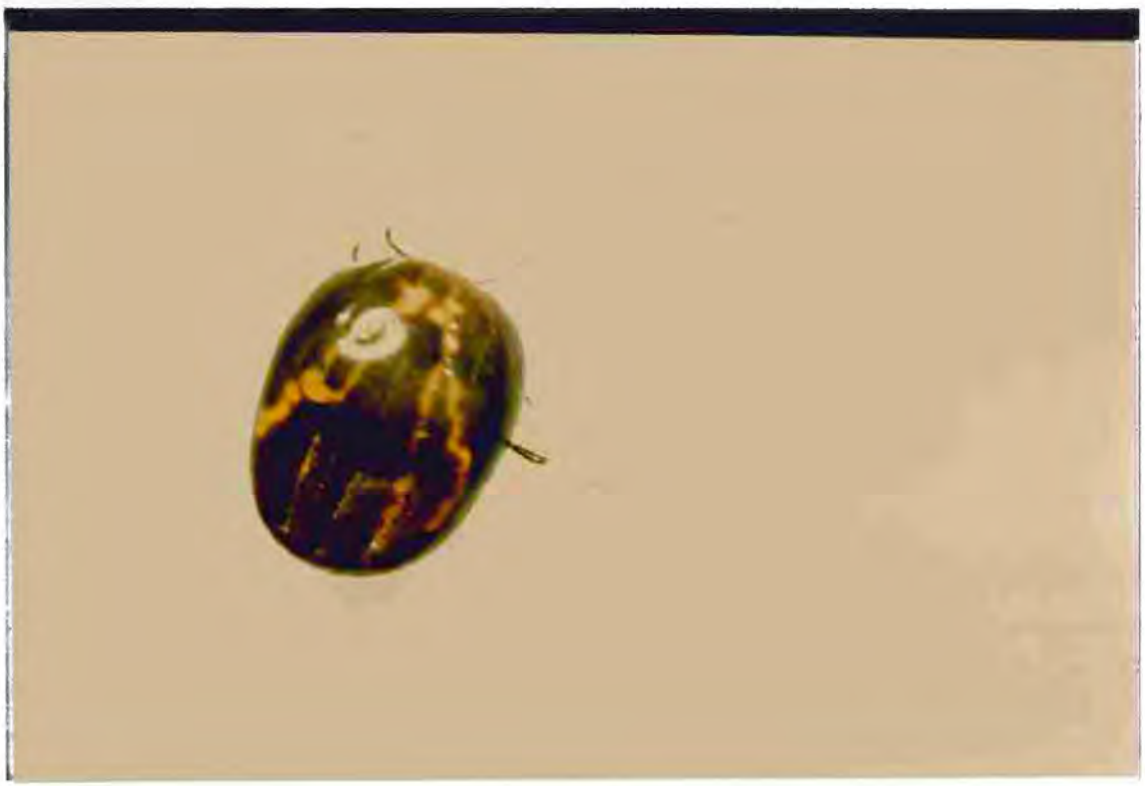

Plate 3 Engorged female R. appendiculatus with nylon thread tied to one leg.

period of 8 hours on the day were put out to look out for any predators using a pair of binoculars with the observer sitting on a 2.5 meter high fence..

Group 2 Caged ticks

Engorged females (about 1.2 cm long and 1.0 cm wide) were placed singly in wire mesh cages measuring 8.0 x 3.0 cm with a 0.5 x 0.5 cm mesh (Plate 4). They were placed in the grass at soil level.

Group 3 Ticks in nylon bags

Engorged females were placed singly in bags measuring 4.0 cm x 2.0 cm (Plate 5) made from nylon gauze (see above) and were heat sealed using a small electric soldering iron. The bags were then placed in the grass at soil level.

All three groups were checked every day for predation at 0730 hours, for a period of 8 days. Tick remains were brought to the laboratory and any predators found eating the ticks were collected and identified. Temperatures in the plot were monitored at 0730 hours and 1400 hours at soil level, 30 cm above the ground, and 60 cm above the ground. Rainfall was recorded daily.

3.5.2. Free engorged R. appendiculatus in grass. Once every two months, for a period of one year, 21 engorged R. appendiculatus females were placed free on the ground in an area which was tick free. Each tick was placed in the

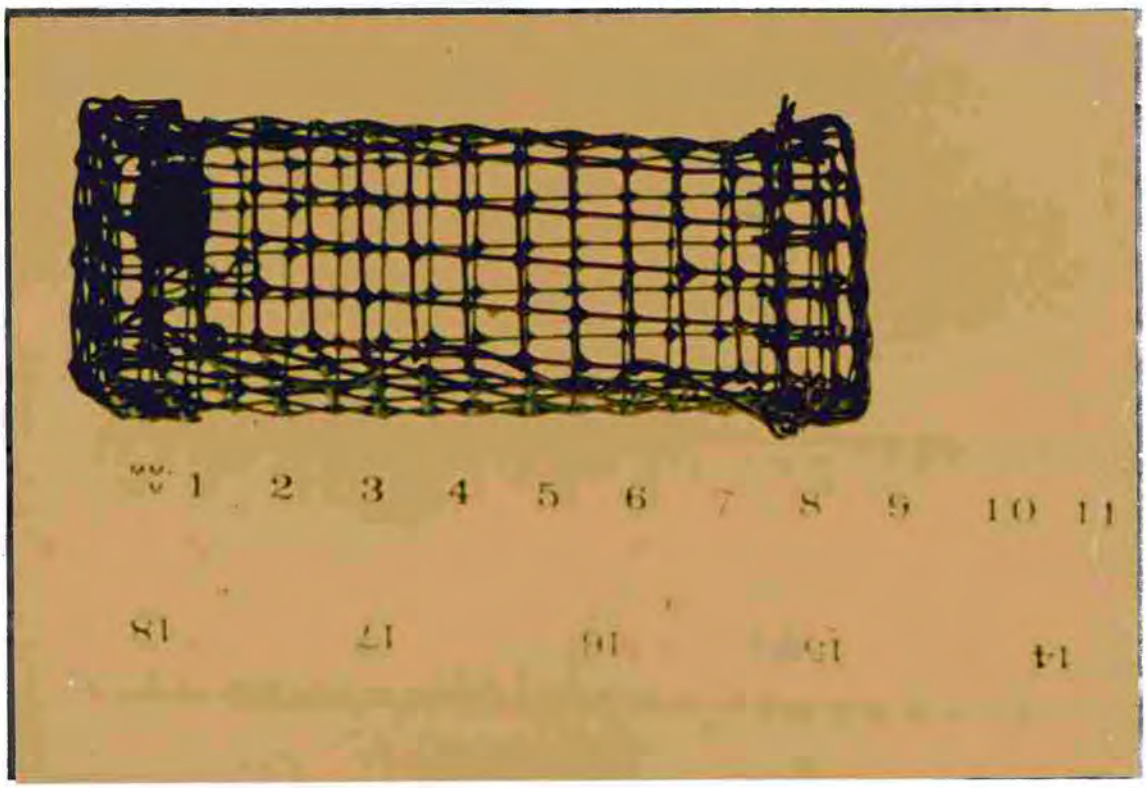

Plate 4 Wire mesh cage for putting out engorged *R. appendiculatus* females in grass

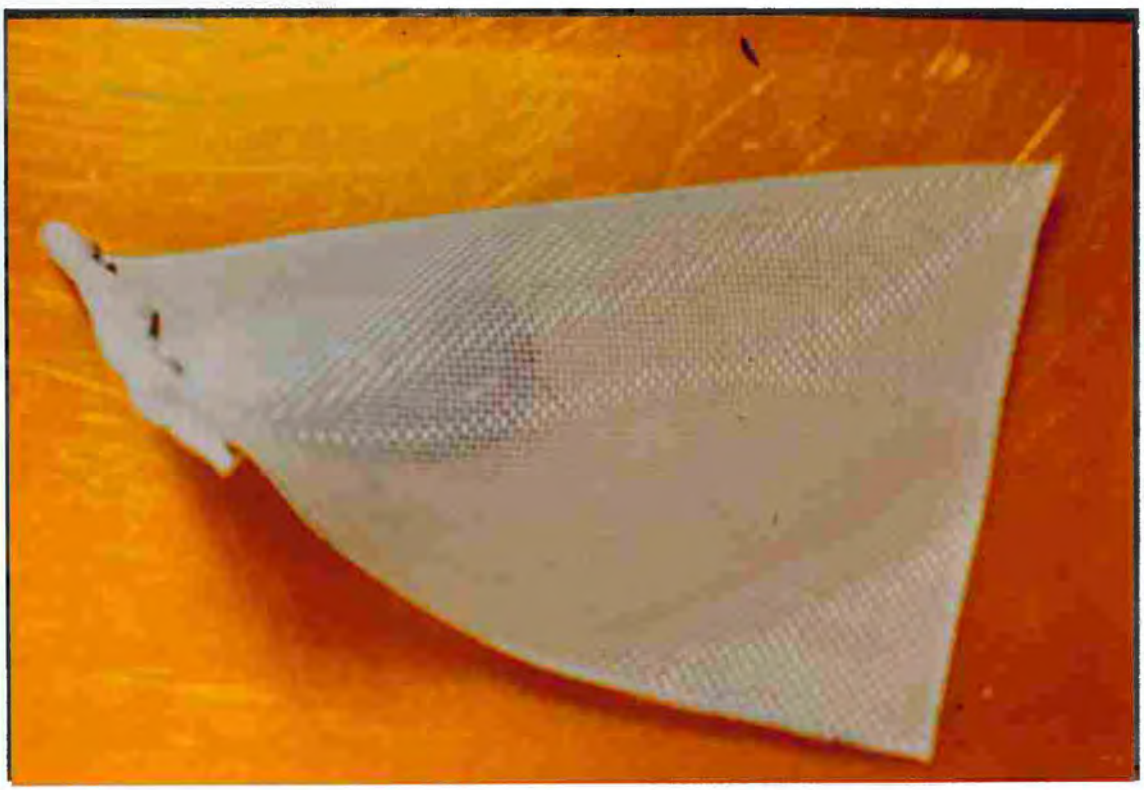

Plate 5 Nylon bag measuring 4.4 x 2.1 cm used for putting out engorged ticks in grass

centre of a square metre, identified by four metal markers 80 cm high pushed into the soil at the corners. Adjacent to each tick, a similar control tick was placed in a nylon bag inside a metal cage. Ticks were left undisturbed to lay eggs, and the eggs to hatch. The controls were left until their larvae had hatched, and were used as indicators of the right time to look for larvae on the top of grass blades. The score for the combined survival of the females and eggs was made from clusters of larvae which later climbed the grass blades. The presence of even a few larvae scored positive.

3.5.3 Confined R. appendiculatus females in a cattle boma (cattle holding ground). Ticks were put out in a cattle boma, 25 m x 25 m in size (Plate 6), situated in the Karen area of Nairobi, Kenya. They were tethered by one leg using nylon gauze threads to metal rods which were pushed into the soil, arranged at random, and slightly buried. Two groups of 30 ticks each were used in February 1988, and another two groups in the rainy season, in April 1988. All the ticks were watched continuously for 8 hours after they were put out on day 1, and any predators attacking them were recorded.

3.5.4 Confined R. appendiculatus nymphs in grass. For a period of one and a half years, two groups of 56 nymphs each were put out monthly in the grass. There were 28 nymphs in

Plate 6 The cattle boma used in predation studies of engorged R. appendiculatus females.

grass 40-60cm high and 28 in grass 6-10 cm high. They were laid out at a density of four nymphs per 16 m2. Predation was monitored daily for a period of eight days. A control group of 56 nymphs was left in the incubator at 28oC and 80% r.h., and was monitored for moulting.

Group 1 Tethered engorged nymphs

Engorged nymphs were tethered, as before, by one leg using a fine nylon thread removed from nylon gauze (Nybolt gauge PA-8-1801XX, Swiss Silk Company, Zurich, Switzerland). The thread was attached using a dissecting microscope at X60 magnification, whilst the nymphs were immobilised by placing them on plasticine.

Group 2 Engorged nymphs in nylon bags

Engorged nymphs were put in pairs in nylon bags measuring 4.0 x 2.0 cm, and placed in the grass at soil level. The nylon bags were placed in metal cages.

3.5.5 Confined A. variegatum females in grass. Each month, for a period of 4 months, 42 tethered engorged A. variegatum females were put out (Plate 7) on the ground in the grass plot. The same tethering method was used as described for female R.appendiculatus (see section 3.5.1), and they were put out at the same time and place as the female R. appendiculatus.

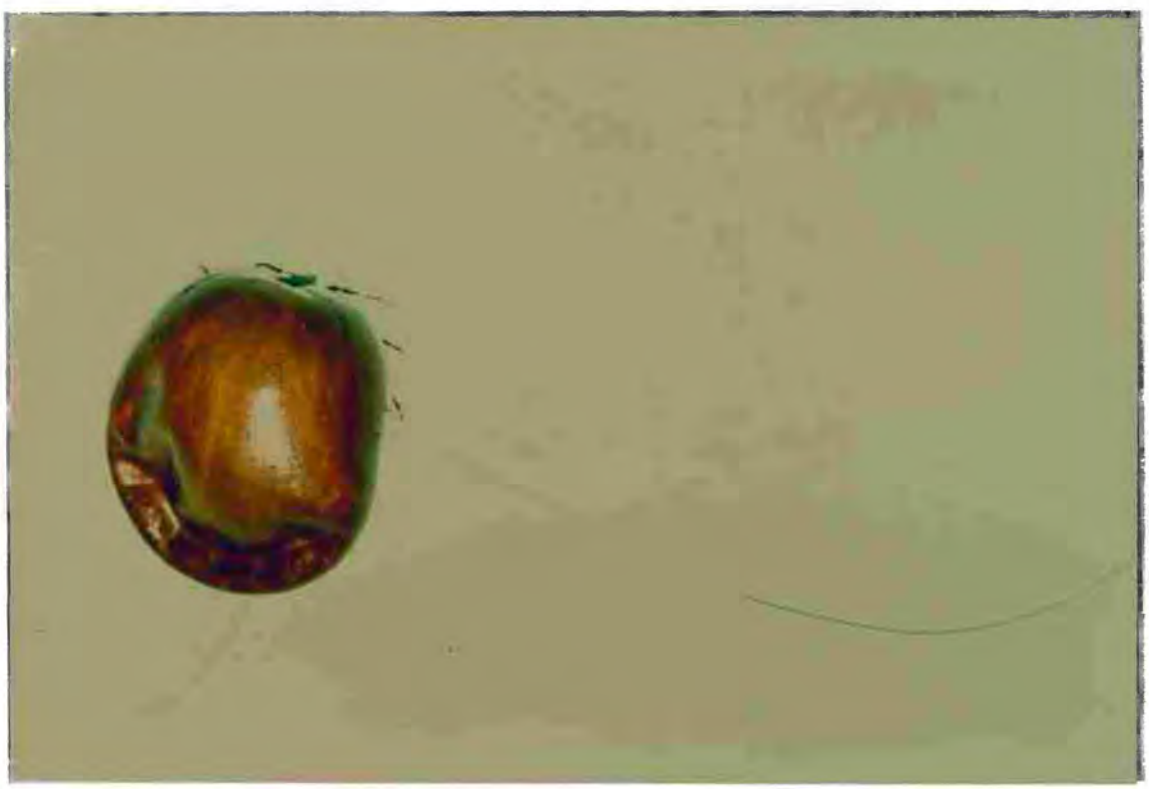

Plate 7 Engorged female A. variegatum with nylon thread tied to one leg.

3.6. Sampling tick predators from the grass

Potential predators were sampled from the grass once a month. A metal frame measuring 50 x 50 x 45 cm (Plate 8) was placed on the ground and the grass thus enclosed was cut to soil level. All spiders, ants, beetles, cockroaches and crickets were collected and preserved in 70% alcohol for identification and counting. The plot was also dug 2 cm deep to look for burrowing beetles and crickets.

Each month, for a period of four months, starting from September 1988, rodents and other small mammals were trapped using 30 live traps (Plate 9), set in five rows of six each in the study plot. The trap spacing was four metres within rows and four metres between rows. All the traps were baited with a piece of buttered bread and a carrrot. Forty-four traps were used for a further four months.
The traps were left out for three days. Trapped rodents were taken to the laboratory and identified, marked by clipping hair on a patch 2 cm by 2 cm on their backs, and then released back to the field. Marked rodents were not counted again if recaptured..

Five pitfall traps (Plate 10) which were metal cylinders closed at the bottom (30 cm deep with a diameter of 20 cm) were used to catch other potential predators like shrews and lizards. The traps were left open for 3 days. At the end of this period their contents were recovered and any mammals or lizards found were identified.

Plate 8 The metal frame measuring 50 x 50 x 45 cm used for sampling predators of ticks in the grass plot.

Plate 9 Wire mesh live trap measuring 40 x 18 cm for trapping small mammals.

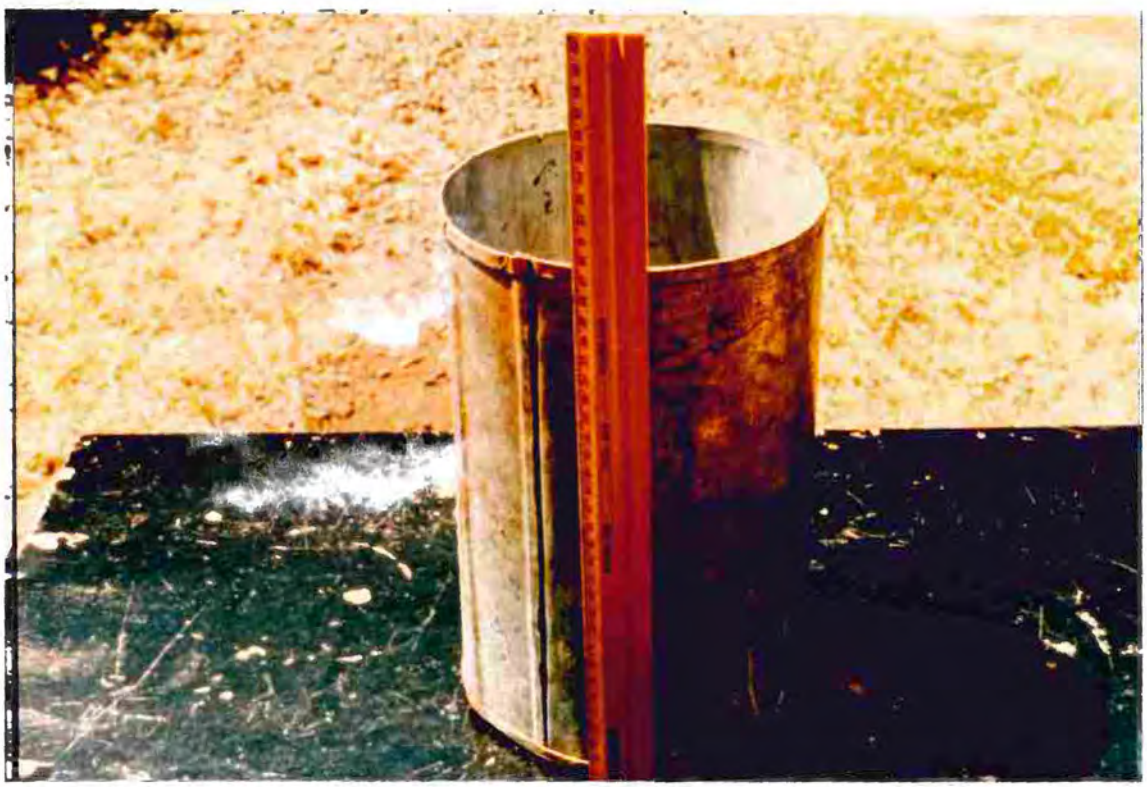

Plate 10 Metal cylinder used as a pitfall trap for trapping ground animals.

3.7. Predation studies in the laboratory

Predators and potential predators (spiders, rodents, cockroaches and beetles) were tested in a perspex box measuring 45 x 45 x 45 cm, with the top covered by nylon gauze, and containing grass growing in soil (Plate 11). Members of one group at a time were placed in the box and left for 24 hours to acclimatise, and then they were offered engorged R. appendiculatus females. Records of whether they attacked or ate the ticks were kept, but quantitative data were not collected.

3.8. Tick parasitoids

3.8.1 Examining ticks for parasitoids. Engorged nymphs of R. appendiculatus and A. variegatum collected from the field were kept in the laboratory at 28°C and 80% r.h. and watched for any changes in colour or appearance, or symptoms of parasitization, until moulting or emergence of parasitoids occurred.

3.8.2. Infecting A. variegatum with parasitoids in the laboratory. After emergence, the adult parasitoids were transferred to an incubator at 22°C and kept in a perspex box in which unfed nymphs of A.variegatum were released at a ratio of parasitoid:nymphs of 1:3, and maintained together until parasitoids died. The nymphs were then fed to engorgement on rabbit ears, incubated, and examined for signs of parasitization. Those that were parasitised were

Plate 11 Perspex box measuring 45 x 45 x 45 cm used for predation studies in the laboratory.

later used as a source of more parasitoids to infest other healthy ticks.

3.8.3 Identification of parasitoids. Emerged adults were fixed in 70% alcohol and then dehydrated and cleared by leaving them for 1 hour in each of 80% alcohol, 90% alcohol, absolute alcohol and xylene, and then mounted on glass slides in DPX. They were then examined under the microscope and measurements of lengths of body, head, thorax, abdomen, and length and width of wings were recorded using 30 males and 50 females.

The measurements were taken using an eyepiece graticule of X40 magnification in a Wild M3B dissecting microscope and compared with four specimens of H. hookeri and four I. texanus from the U.S.A. Drawings of the parasitoids were made using a camera lucida attached to a compound microscope.

3.9 Pathogens

3.9.1 Screening ticks for bacteria. Rhipicephalus appendiculatus engorged females which had been out in the grass for 8 days were incubated at 27°C and 80% r.h., and examined for symptoms of disease. Those which became sick, together with any from the laboratory colonies of R. appendiculatus and B. decoloratus that were sick, were examined for bacteria.

All the sick or dead ticks were surface sterilized as follows:

(a) in 70% alcohol for three seconds

(b) in 5% sodium thiosulphate for five minutes

(c) in 10% sodium hypochlorite for five minutes

(d) three minutes in each of 3 changes of sterile water.

The ticks were then cut open using sterile instruments, and a drop of the gut contents was streaked onto a plate of Trypton Soya Agar. Identification of the growing bacteria was done using the Analytical Profile Index (Api System, La Balme Les Grottes, 38390, Montalieu, Verciew, France).

3.9.2. Examination of healthy R. appendiculatus for bacteria. Five groups of 10 surface sterilized engorged females, which had dropped from rabbits on the same day, and 5 groups of 10 engorged females which had dropped one week previously, were each surface sterilised, cut open and a drop of their gut contents streaked onto a plate of Trypton Soya Agar. Five groups of 10 unfed adult R. appendiculatus (mixed sexes) were also examined for bacteria using haemolymph, which was extracted by amputating one leg, and taking one drop of the haemolymph oozing from the cut surface, by lightly touching the agar with the cut surface.

All the plates were examined for bacteria after 48 hours.

3.9.3 Screening ticks for fungi. Engorged female R. appendiculatus which had been placed on the ground in the grass for 8 days, together with engorged female R.appendiculatus and B. decoloratus females from the laboratory colonies, were incubated at 28°C and 80% r.h. for 14 days. Any ticks which developed mycoses, as evidenced by mycelial growth on the surface of the cuticle, were surface sterilised cultured in plates of Sabourand's Dextrose Agar and examined after 14 days.

3.9.4 Preparation of fungi for identification:
The slide culturing method. Two glass rods were placed in a glass petri dish (diameter 9 cm.), lined with filter paper. A microscope slide was placed on top of the glass rods, and the whole apparatus was sterilised. An agar block (SDA) measuring 1.0 x 0.5 x 0.25 cm was placed on the slide, and the fungus was inoculated at the four sides using a loop. A sterile cover slip was placed on top of the agar, and a few drops of sterile water added on the filter paper. (Plate 12). The whole apparatus was incubated at room temperature. The cover slip was then removed after 2-3 days, and the fungal growth on it stained with lactophenol blue and examined under the microscope.

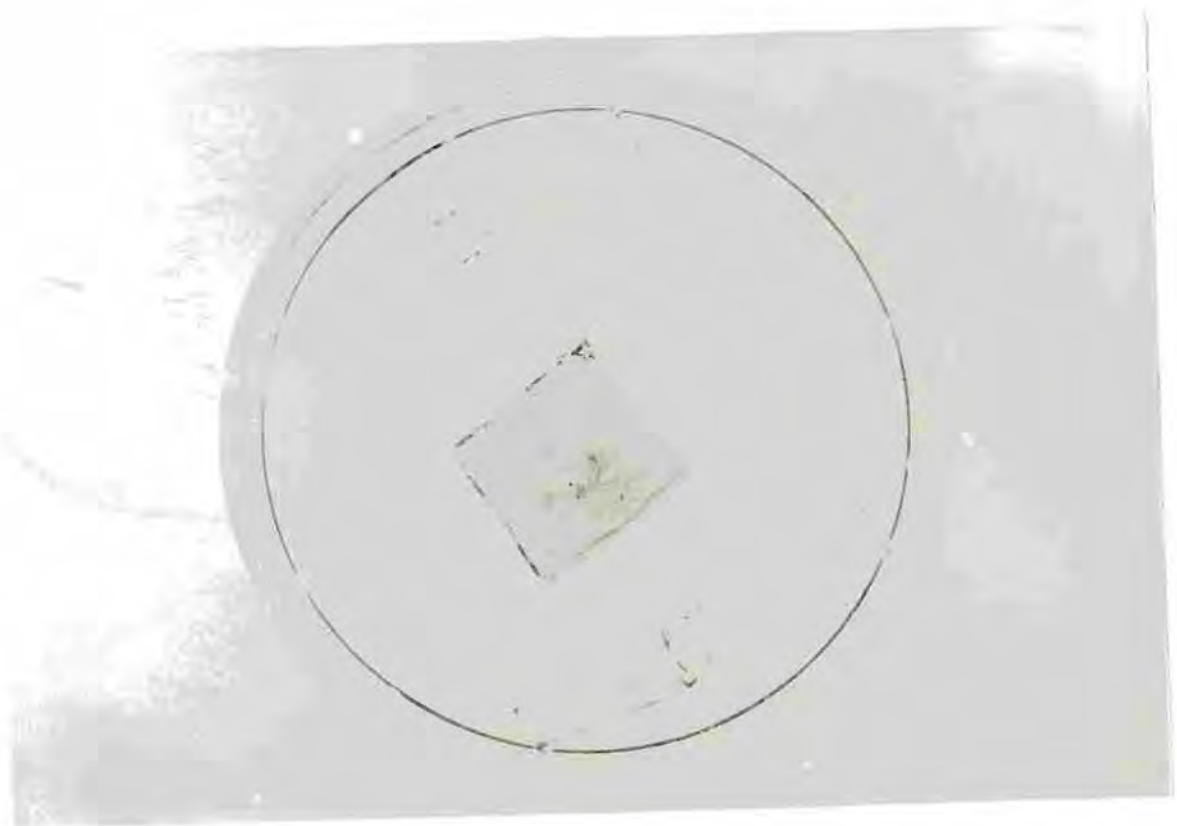

Plate 12 Apparatus used for identification of fungi "slide culture method".

3.9.5 Artificial infection of R.appendiculatus with the fungi B. bassiana and M. anisopliae.

Infection with spore suspensions. Spores of B. bassiana and M. anisopliae were removed from agar by washing with 0.05% Triton-X 100 solution. They were centrifuged at 2000 r.p.m. for 10 minutes and resuspended in distilled water. This process was repeated, and the final clean pellet was resuspended in distilled water and spore counts made using Thoma cell counting chamber (Weber Scientific International Ltd.,England). A working concentration of 10^9 spores per ml was used as it was found to most suitable in a trial run with a wide range of concentrations.

In experiments to investigate pathogenicity of B. bassiana to R. appendiculatus, 5 groups of 30 engorged and unfed adults and nymphs were each immersed in a B. bassiana spore suspension for 3 seconds, while 5 groups containing 500 engorged larvae were immersed in the spore suspension. All the fed stages were kept until egg laying or moulting took place. These together with the unfed stages were observed for signs of fungal infection for two weeks. For each category equal numbers of ticks immersed in distilled water served as controls.

To check whether B. bassiana spores interfered with feeding of ticks on the host, 5 groups of 30 of adults and nymphs of R. appendiculatus were immersed in the spore suspension and immediately transferred to rabbit ears,

enclosed in ear-bags. For this experiment, 5 groups of 200 larvae were used. After engorgement they were incubated and observed for two weeks for signs of infection. Untreated ticks were placed on the other ears of the same rabbits to act as controls.

3.9.6 Pathogenicity of fungi in the laboratory. For laboratory-infected ticks which died of a fungal infection, the infective organisms were recovered by culturing in SDA. Fungi growing from inside the tick were identified.

3.10. Drop-off rhythm of R.appendiculatus

Nine Friesian calves, 6-9 months old, were used in this experiment. They were kept in a paddock at the Veterinary Laboratories, Kabete.

3.10.1 Adults. All nine calves (using three at a time) were infested with 100 male and 100 female adult R. appendiculatus on the right ear at 0900 hours on day 1, (referred to as first feeding) and with the same number of ticks on the left ear at 2000 hours, using nylon gauze ear bags which were removed 24 hours after infestation and replaced on the animals at 0600 hours on day 5. Tick drop-off was monitored by opening the bags and removing detached ticks every two hours starting at 0600 hours and continuing until 2000 hours, for days 6, 8, 9 and 10. On day 7, the

same procedure was followed, but checking continued throughout the night at 2200, 2400, 0200 and 0400 hours.

3.10.2 Nymphs. Each animal was infested with 800 nymphs on day 2, (since adult ticks were put on the aniamls) using a 20 x 10 cm area on the right side of the animal. The area was covered by a nylon gauze patch, glued in place and fittted with a zippered window through which the detached engorged nymphs_were collected. They were then counted and weighed in bulk, at similar times as for adults on days 6, 7, 8 and 9 (see section 3.10.1.).

3.10.3. Larvae. On the left side of each animal, a batch of larvae consisting of those hatching from 0.5 gm of eggs was put to feed on day 3, (since the adults were put on the animals) using a similar patch as for nymphs. Starting with day 6, engorging larvae were collected, counted and weighed in bulk at the same times as for adult ticks. Ambient temperature was taken each time the dropped ticks were collected.

3.10.4 Adults on tick sensitised animals. 100 male and 100 female R. appendiculatus (referred to as second feeding) were put to feed on each ear of six animals which had previously been used for feeding all stages of ticks (see Sections 3.10.1, 3.10.2 and 3.10.3). Dropping engorged females were collected on days 6, 7, 8, 9 and 10 and at same

hours as described for adults in Section 3.10.1.

3.11. Data analysis

The data were analysed using analysis of variance (General Linear Model) for comparison of more than two means, followed by Duncan's Multiple Range Test where variation was found. All percentages were first transformed using the arc sine transformation before the analysis was done.

4 CHAPTER 4

PREDATION OF TICKS

4.1 RESULTS

4.1.1 Mortality of engorged R. appendiculatus. The mortality patterns of engorged female R. appendiculatus put out in grass which was 40-60 cm high are shown in Figures 2, 3 and 4 (see Appendices 1, 2 and 3) for tethered, caged and ticks in nylon bags respectively. The mortality due to predation was much higher (in tethered but not so high in metal cages) than that caused by other environmental factors both for tethered and caged ticks, but not for those in nylon bags.

Figures 5, 6 and 7 show the mortality of tethered, caged and ticks in nylon bags respectively, put out in grass which was 6-10 cm high, (see Appendices 4, 5 and 6 respectively). The overall predation for tethered ticks was lower in short grass than in long grass, and the difference was found to be statistically significant using Analysis of Variance ($P > 0.05$).

The three experimental treatments used for adults, namely; tethered, caged and in nylon bags, were found to influence mortality, with the tethered group having the highest mean mortality (39.8%), followed by those in cages (7.0%), and then those in nylon bags (0.7%), and all of them were found to be statistically different for predation (Appendix 7a). However for deaths due to other causes, those

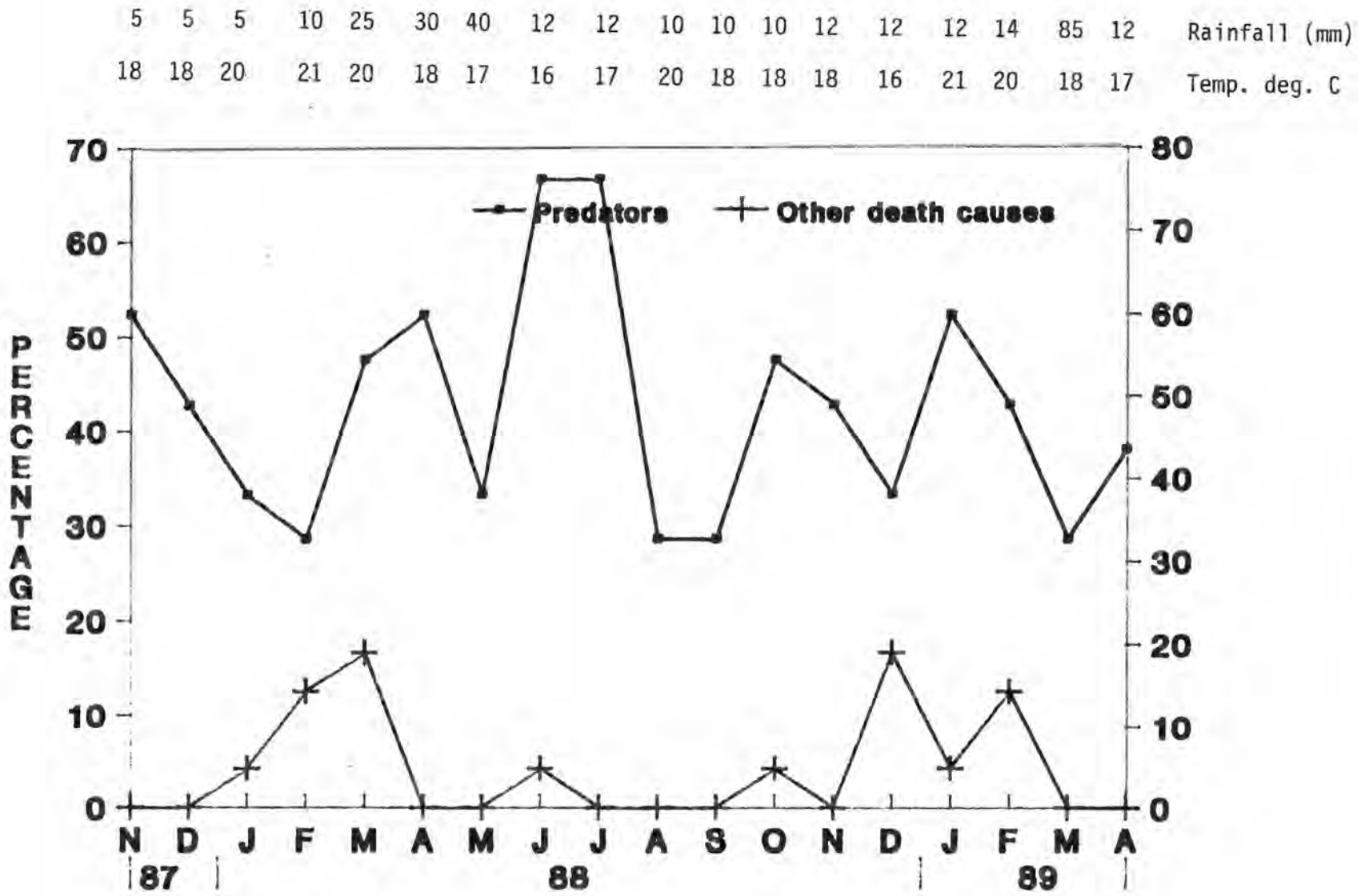

Figure 2 Percent mortality of tethered engorged R. appendiculatus in long grass (40-60 cm) for 8 days.

5	5	10	25	30	40	12	12	10	10	10	12	12	12	12	14	85	12	Rainfall (mm)
18	18	20	21	20	18	17	16	17	20	18	18	18	16	21	20	18	17	Temp. Deg. C

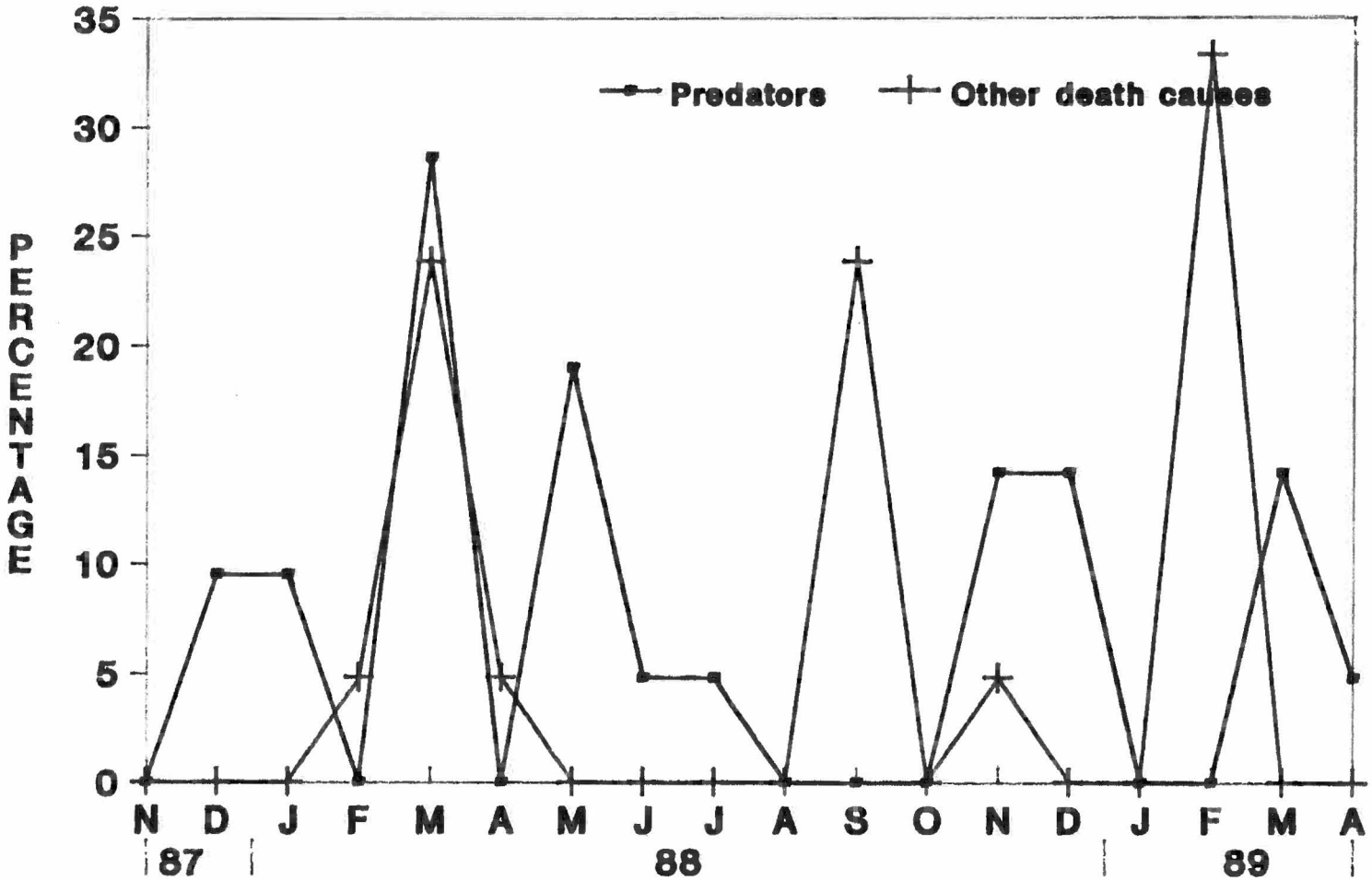

Figure 3 Percent mortality of engorged female R. appendiculatus put out in metal cages in long grass (40-60 cm) for 8 days.

5	5	5	10	25	30	40	12	12	10	10	10	12	12	12	14	85	12	Rainfall (mm)
18	18	20	21	20	18	17	16	17	20	18	18	18	16	21	20	18	17	Temp. Deg. C

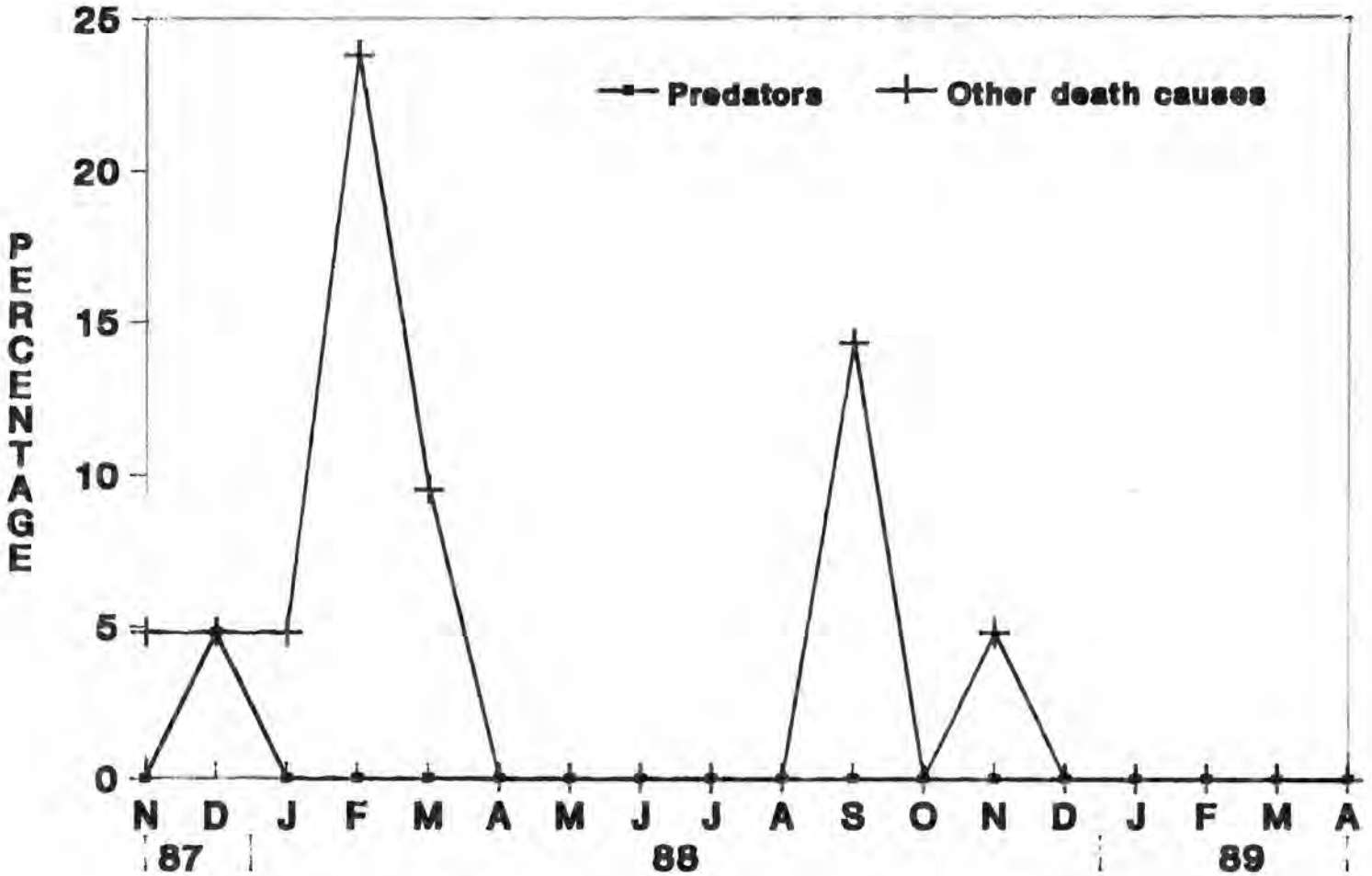

Figure 4 Percent mortality of engorged female R. appendiculatus put out in nylon bags in long grass (40-60 cm) for 8 days.

5	5	5	10	25	30	40	12	12	10	10	10	12	12	12	14	85	12	Rainfall	(mm)
18	18	20	21	20	18	17	16	17	20	18	18	18	16	21	20	18	17	Temp. Deg.	C

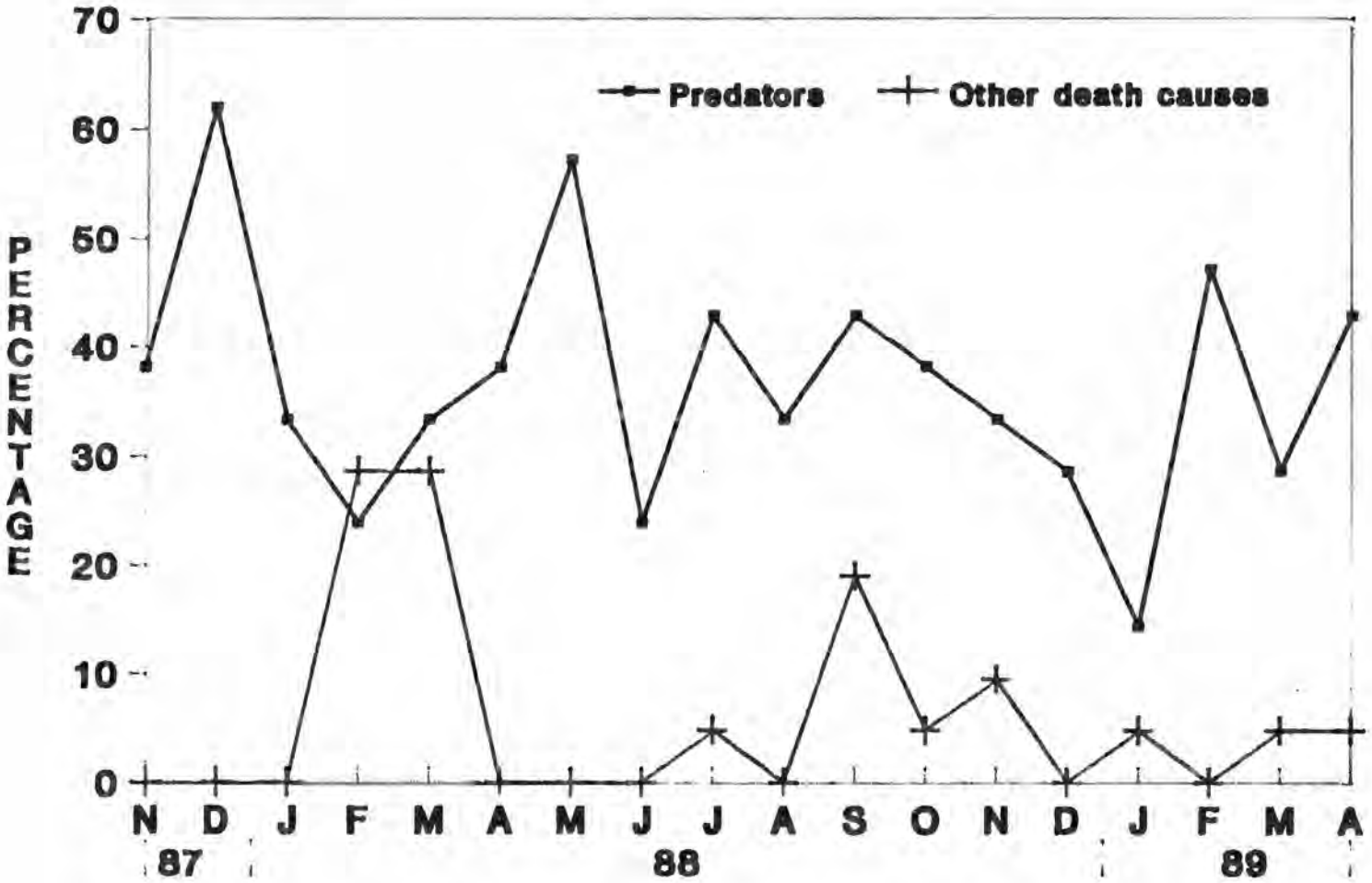

Figure 5 Percent mortality of engorged female R. appendiculatus tethered in short grass (6-10 cm) for 8 days.

5	5	5	10	25	30	40	12	12	10	10	10	12	12	12	14	85	12	Rainfall (mm)
18	18	20	21	20	18	17	16	17	20	18	18	18	16	21	20	18	17	Temp. Deg. C

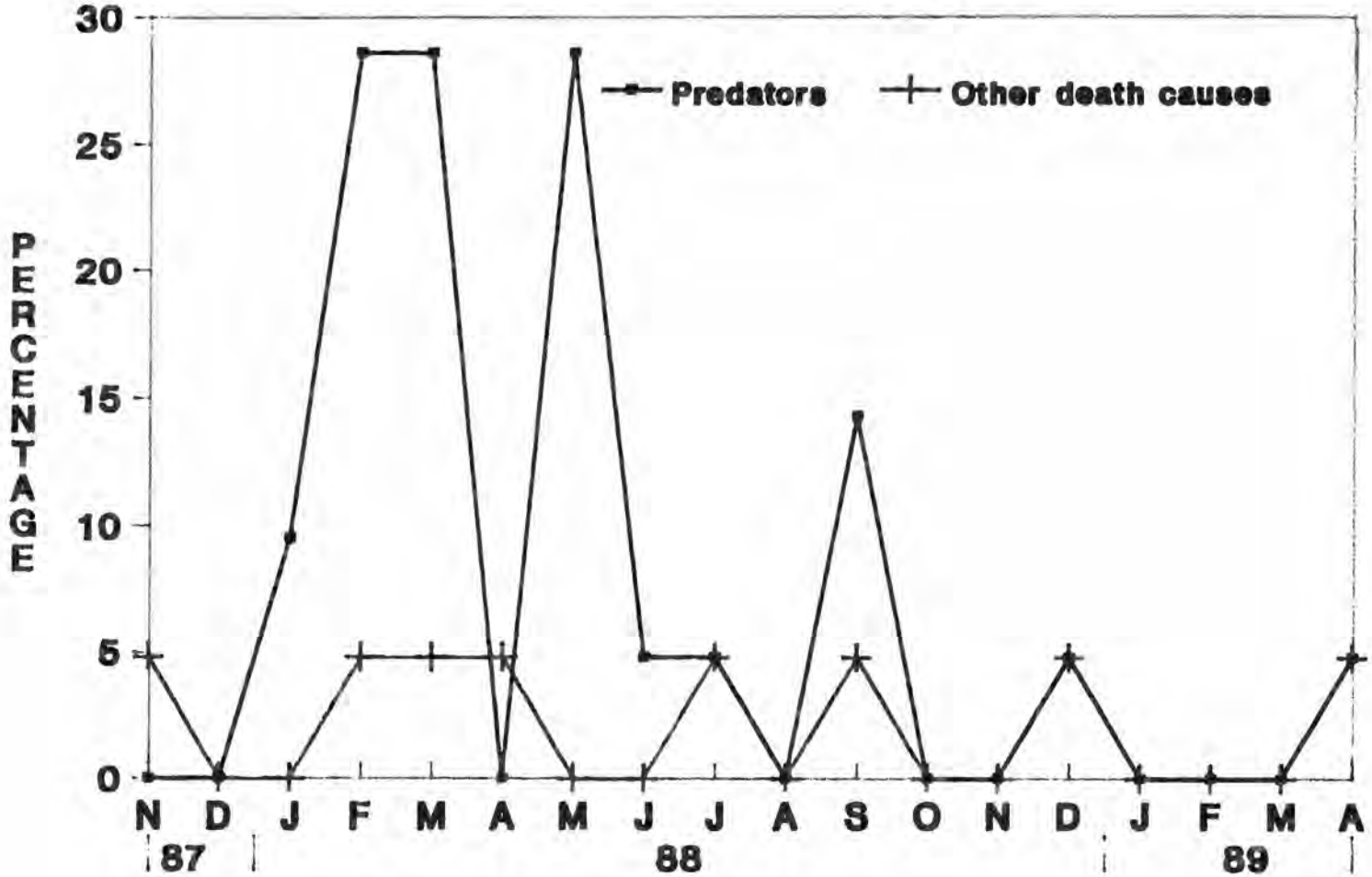

Figure 6 Percent mortality of engorged female R. appendiculatus put out in metal cages in short grass (6-10 cm) for 8 days.

5	5	5	10	25	30	40	12	12	10	10	10	12	12	12	14	85	12	Rainfall (mm)
18	18	20	21	20	18	17	16	17	20	18	18	18	16	21	20	18	17	Temp. Deg. C

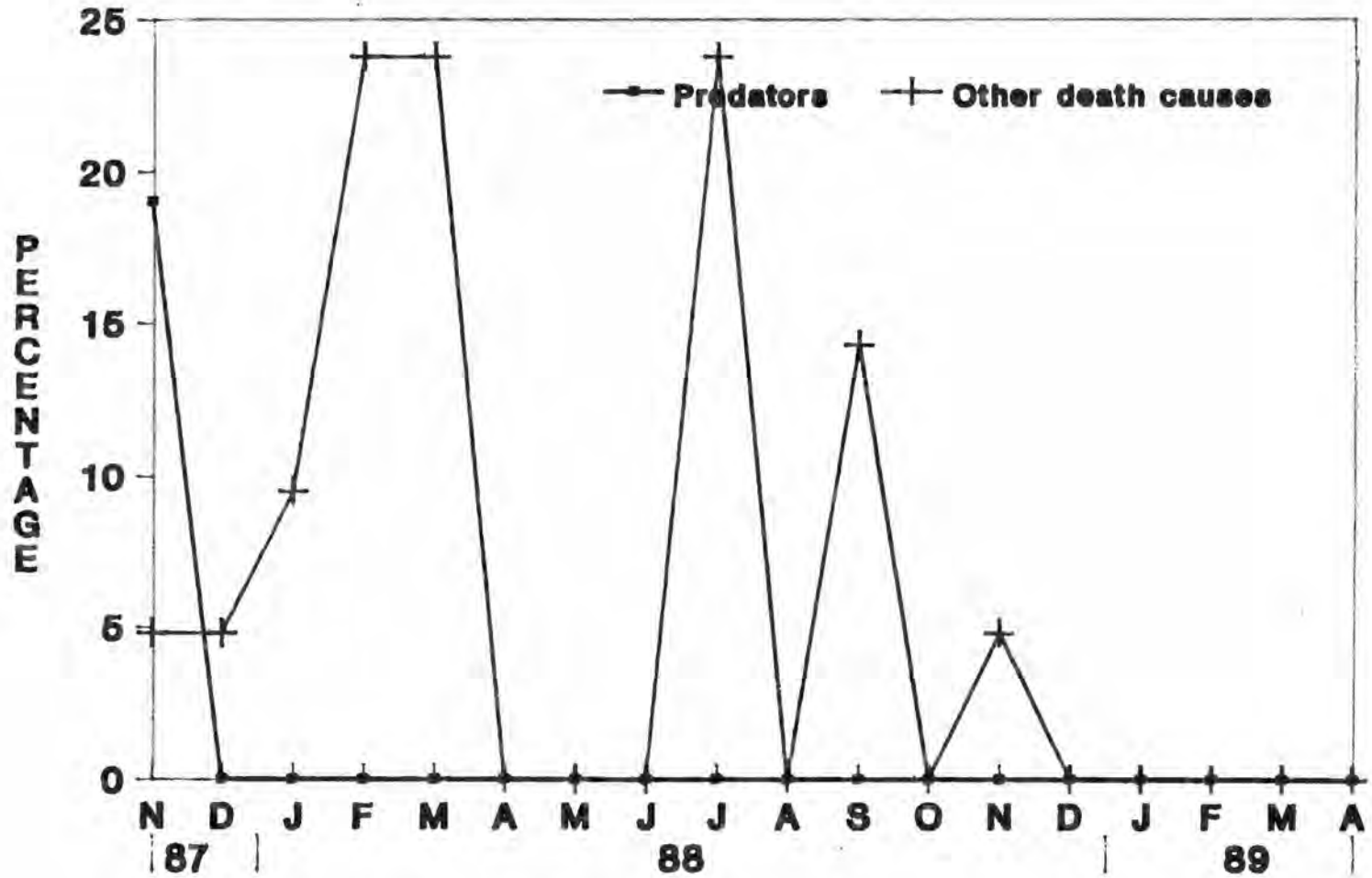

Figure 7 Percent mortality of engorged female R. appendiculatus put out in nylon bags in short grass (6-10 cm) for 8 days.

in nylon bags, and those in cages were not different from each other, but were both different from the tethered ones (Appendix 7b).

In all the 3 experimental categories, death due to other causes was mainly due to the ticks drying up, with no physical injuries. There were also three ticks which died due to Serratia marcescens infection.

Overall, there was more mortality due to predation in long grass (16.5%) than in the shorter grass (14.8%) for all the three experimental treatments combined. The difference was found to be statistically significant (Appendix 8a). Conversely the mortality due to other causes was on the whole lower in the long grass (4.5%), than in the short grass (6.6%), and the difference was statistically significant (Appendix 8b).

4.1.2 Combined mortality of females and eggs of R. appendiculatus put out free in the grass. Table 1 shows the mortality of females and eggs of R.appendiculatus throughout the incubation period. Forty-six percent of engorged females and their eggs died due to predation and other environmental factors, while in the control group only 13% of engorged females and their eggs died due environmental factors. Clusters of larvae started to appear on the top of the grass from day 54 onwards.

TABLE 1

Mortality of engorged R. appendiculatus females put out in long grass (40-60 cm) until their larvae were found on top of the grass (54 to 73 days later)

	Mortality	
	Free ticks	Controls
Replicate 1	46.6	0.0
Replicate 2	39.1	4.8
Replicate 3	47.6	19.0
Replicate 4	52.4	28.6
Replicate 5	46.4	13.1
Mean±SE	46.4±2.1	13.1±5.1

4.1.3. Mortality of engorged A. variegatum. Table 2 shows the mortality of female A. variegatum tethered in long grass. All the ticks which died due to predators were found punctured and bleeding. There were no deaths due to other causes. Predation of A. variegatum was slightly higher than that of R. appendiculatus put out in the same months and at the same place. The difference was, however, not statistically significant.

4.1.4. Mortality of engorged nymphs of R. appendiculatus. The mortality of R. appendiculatus nymphs put out in long grass is shown in Figures 8 and 9 for tethered and those in nylon bags respectively (Appendices 9 and 10), while that for nymphs in short grass is shown in Figures 10 and 11 respectively (Appendices 11 and 12 respectively). Unlike the adults, the difference between the overall means of those eaten by predators for long grass and short grass was not found to be statistically significant.

When the results were pooled for long-grass and short grass, the overall mortality of tethered nymphs due to predation was 36.1± 3.3 % while mortality due to other causes in the same group was 9.4± 4.1% (Appendix 13). In the group put out in nylon bags there was virtually no mortality due to predation, but there was mortality due to other causes (Appendix 14).

TABLE 2

Percent mortality of engorged A. variegatum females tethered in long grass (40-60 cm.) for 8 days

		Mortality	
Month	N	Predation	Other causes
Jan. 1989	21	47.0	0.0
Feb. 1989	21	41.0	0.0
Mar. 1989	21	51.8	0.0
Apr. 1989	21	46.6	0.0
Mean±SE	21	46.6±2.2	0.0

5	5	5	10	25	30	40	12	12	10	10	10	12	12	12	14	85	17	Rainfall (mm)
18	18	20	21	20	18	17	16	17	20	18	18	18	16	21	20	18	17	Temp. Deg. C

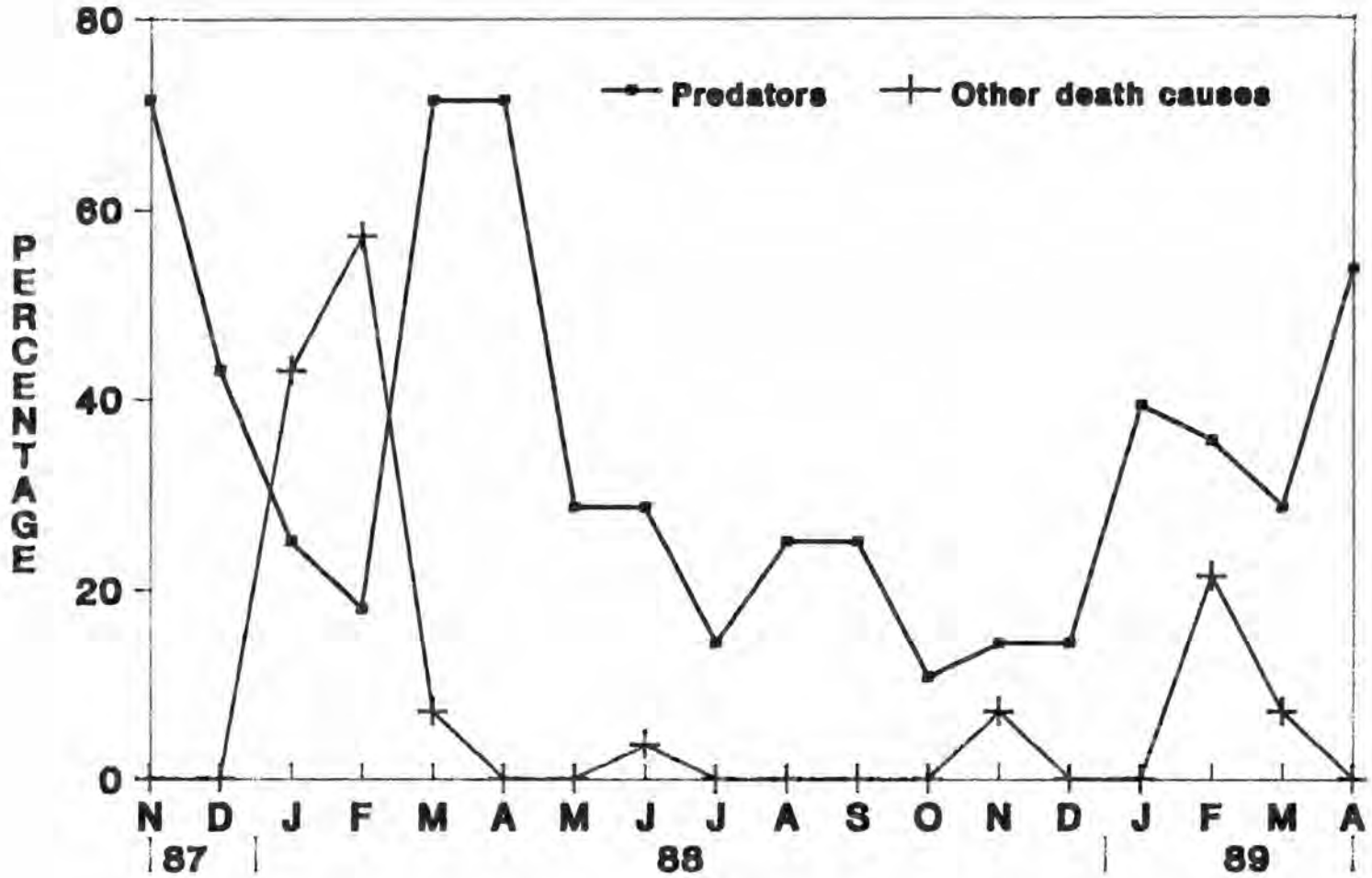

Figure 8 Percent mortality of engorged R. appendiculatus nymphs tethered in long grass (40-60 cm) for 8 days.

5	5	5	10	25	30	40	12	12	10	10	10	12	12	12	14	85	12	Rainfall (mm)
18	18	20	21	20	18	17	16	17	20	18	18	16	21	20	18	18	17	Temp. Deg. C

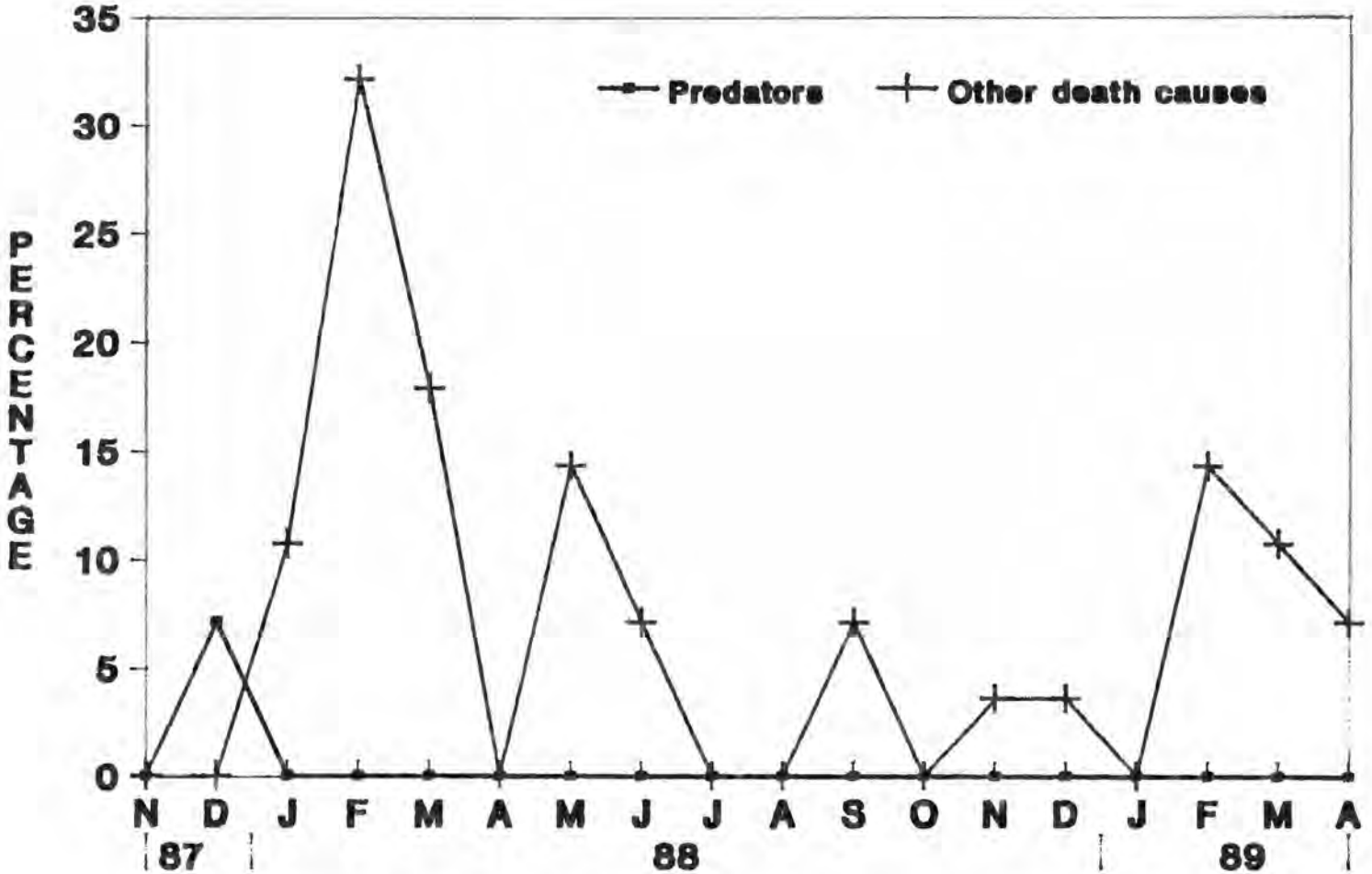

Figure 9 Percent mortality of engorged R. appendiculatus nymphs put out in nylon bags in long grass (40-60 cm) for 8 days.

5	5	5	10	25	30	40	12	12	10	10	10	12	12	12	14	85	12	Rainfall (mm)
18	18	20	21	20	18	17	16	17	20	18	18	18	16	21	20	18	17	Temp. Deg. C

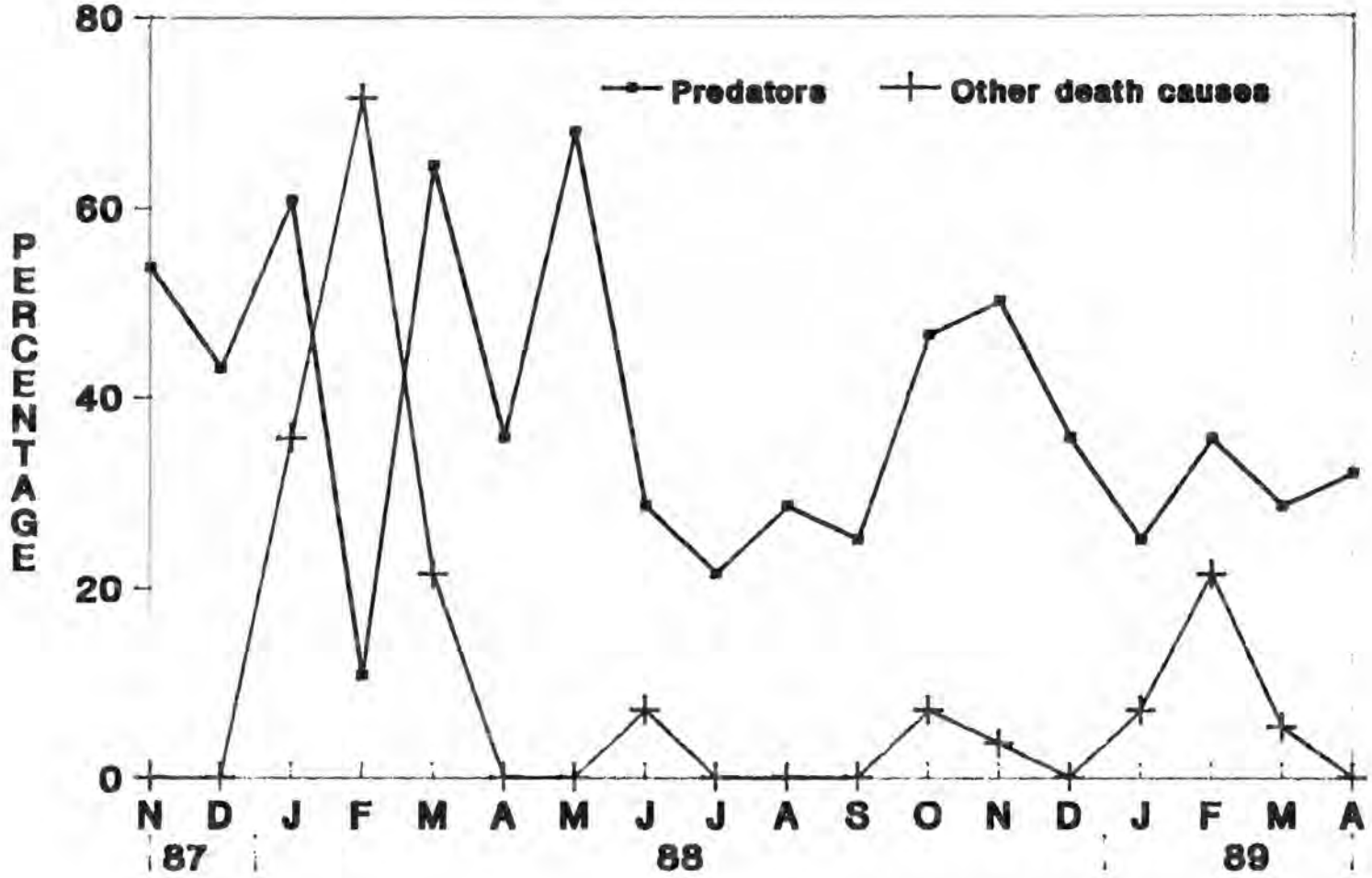

Figure 10 Percent mortality of engorged R. appendiculatus nymphs tethered in short grass (6-10 cm) for 8 days.

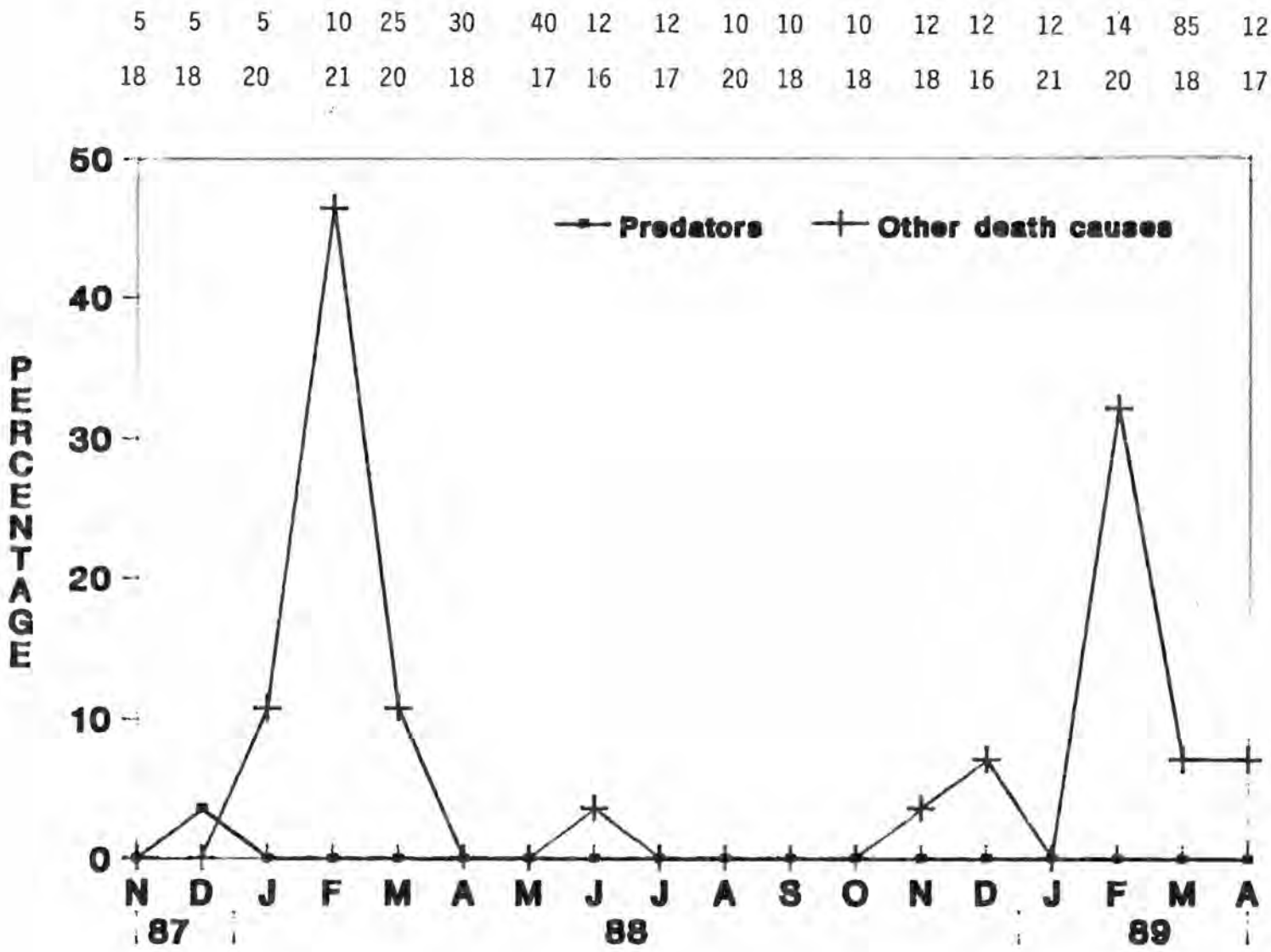

Figure 11 Percent mortality of engorged R. appendiculatus nymphs put out in nylon bags in short grass (6-10 cm) for 8 days.

4.1.5 Temperature and Rainfall. There were temperature fluctuations throughout the study period. Higher temperatures were recorded in short grass than in long grass at 1400 hours but not at 0730 hours. Months which had higher temperatures also had higher mortality of adults and nymphs due to the environmental factors as seen in the periods January to March for both 1988 and for 1989 (Figures 2-11). The rainfall recorded for the whole period was mainly distributed in 2 rainy seasons in the months of April to May 1988 and March to April 1989 (Figures 2 -11).

4.1.6 Tick remains after predation. After predation of female ticks, four types of remains were collected from the grass from the tethered group. Sixty-five percent of them had one puncture, so that the greater part of the tick was still intact, except for blood coming out of the punctured area (Plate 13). Other ticks looked fresh, but had been torn apart into two or more pieces, with most of the blood spilled or consumed (Plate 14). The third category was ticks which were dry or semi-dry, whose mouthparts and legs had been removed, and which had one or more holes made in them (Plate 15). A fourth category was ticks whose inner contents had been consumed, leaving the empty cuticle almost intact (Plate 16). The remains of nymphs which were attacked by predators were very small and were therefore not collected for examination in the laboratory.

Plate 13 Remains of an engorged female A. variegatum after being puntured by predators.

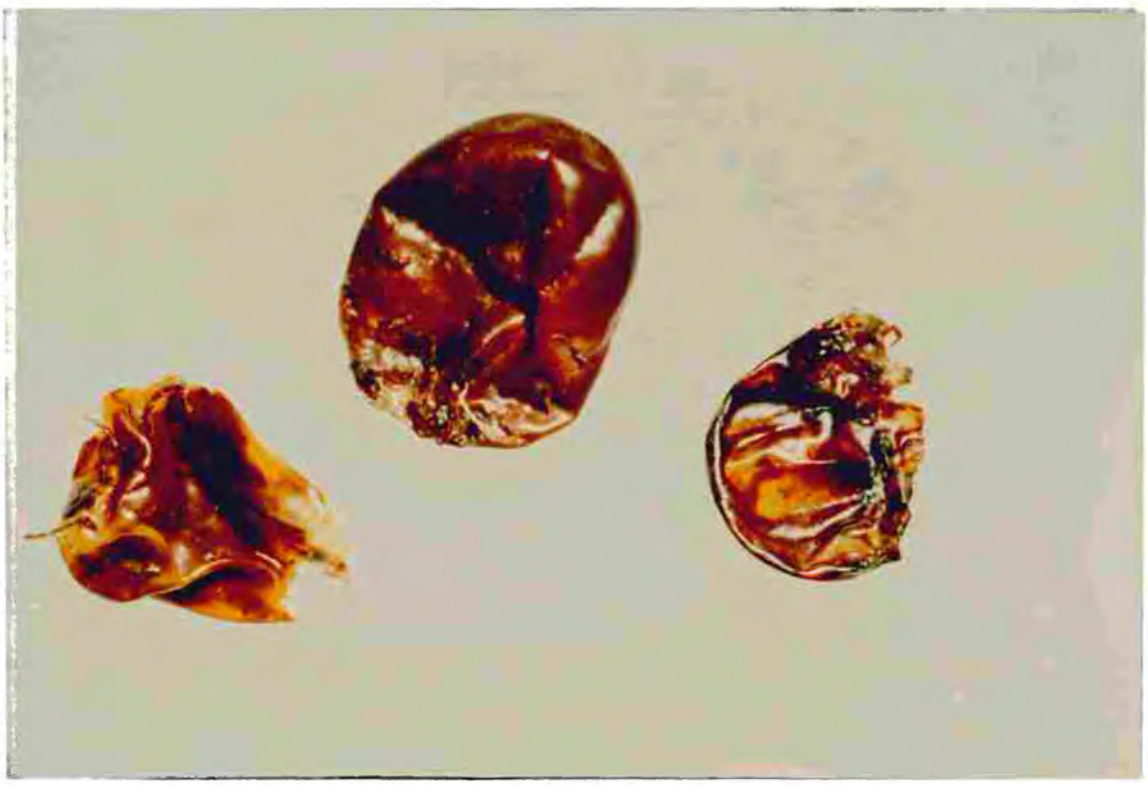

Plate 14 Remains of R. appendiculatus engorged females after predation by rodents.

Plate 15 Remains of engorged female R. appendiculatus after predation by ants.

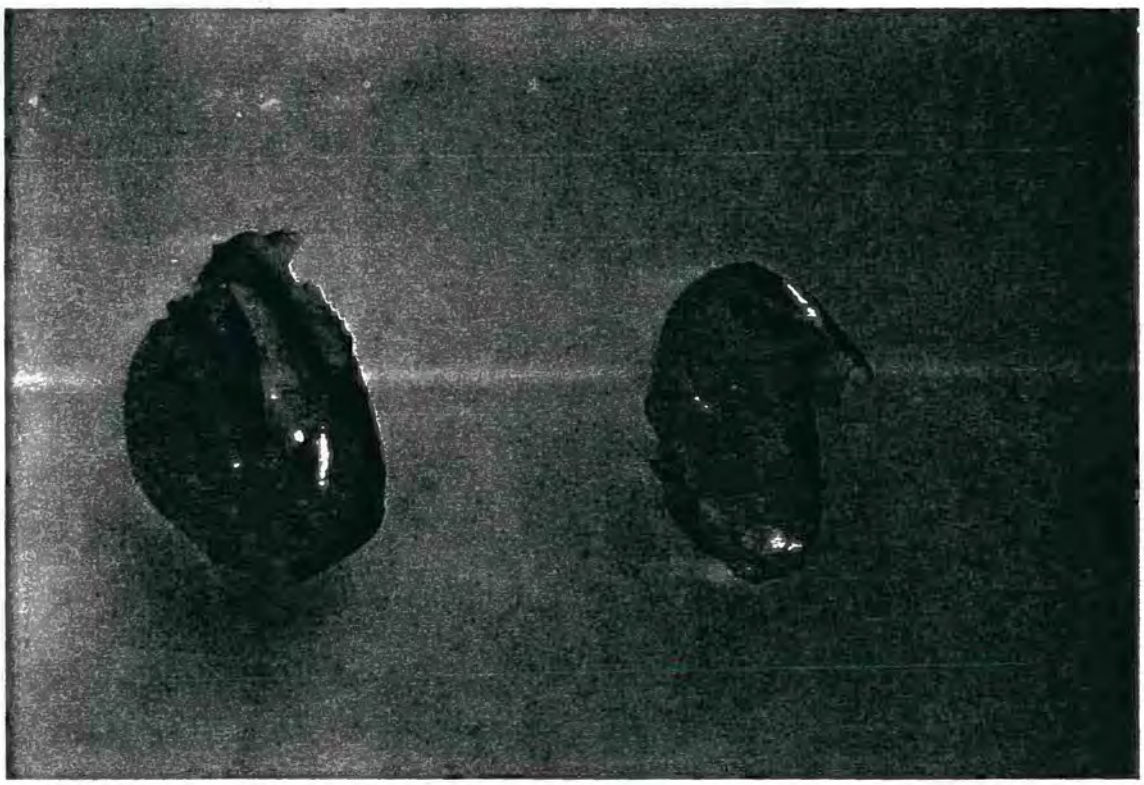

Plate 16 Remains of R. appendiculatus engorged females after predation by rodents or spiders.

4.1.7. Predators and potential predators. The animals which were observed eating ticks in the field were the ant, Pheidole megacephala, the rodents Mastomys natalensis and Lemniscomys striatus and also two birds, the African pied wagtail, Motacilla aguimp, and the superb starling, Spreo superbus. As soon as the ticks were placed in the grass, there was increased activity of the ants near the ticks, walking back and forth and on the ticks. The worker ants were observed to pierce the tick at one or more positions, leaving it to bleed. The ants started to take away the tick in tiny bits after it was dry and dead, starting with the dried blood at the puncture. They also removed the legs and mouthparts.

Rodent activity was observed in the field. If they came across the tethered ticks, they either bit them and left them, or tore them into pieces as they ate them. They avoided eating the cuticle of the ticks in all cases. Spiders were not observed eating ticks in the field.

Ants, spiders, beetles and cockroaches were sampled every month, and the numbers per square meter are shown in Table 3. The numbers did not show any seasonality, except for ants which were more abundant in the months of January, February, March and May. More ticks in the caged category were also eaten by ants in the same months (Appendices 2 and 5). Sampling of small mammals was done and the mammals caught are shown in Table 4. They were the rodents Mus minutoides

TABLE 3

Tick predators and potential predators collected from grass and soil over a period of 9 months using nine replicates

Month	Mean number per square meter				
	Hymenoptera (ants)	Blattodea (cockroaches)	Arachnida (spiders)	Orthoptera (crickets)	Coleoptera (beetles)
Jan.88	216.0	16.0	24.0	0.0	8.2
Feb.88	234.0	18.0	29.0	0.2	10.8
Mar.88	128.0	28.6	25.8	0.2	4.4
Apr.88	136.8	22.6	19.8	0.6	2.2
May.88	386.4	28.2	17.0	1.0	3.2
Jun.88	21.0	15.0	10.2	0.8	2.2
Jul.88	68.0	29.6	25.6	2.0	1.4
Aug.88	71.0	27.0	26.4	0.8	1.6
Sep.88	87.6	25.0	21.4	1.0	2.0
Mean±SE	149.9±12.5	23.3±1.9	22.1±1.9	0.7±0.2	4.0±1.1

TABLE 4

Numbers of small mammals trapped in the experimental plot. They were marked and released back to the plot after counting, but not counted again if recaptured.

	1988		1989				
Mammal	Nov.	Dec.	Jan	Feb.	Mar.	Apr.	Total
Otomys irroratus	1	1	5	3	3	0	13
Mastomys natalensis	2	2	4	0	2	0	10
Lemniscomys striatus	0	0	1	3	3	1	8
Laphuromys sikapusi	1	0	0	0	0	0	1
Mus minutoides	0	1	0	0	0	0	1
Crocidura nigrofusca	0	0	2	1	0	2	5
Total	4	4	12	7	8	3	38

(Plate 17), Mastomys natalensis (Plate 18), Otomys irroratus (Plate 19), Lemniscomys striatus (Plate 20) Lophuromys sikapusi and the shrew, Crocidura nigrofusca. Two striped lizards, Mobuya striatus (Plate 21) were caught in the study plot but were not observed eating ticks in the field.

4.1.8 Predation of ticks in the laboratory. When offered ticks in the laboratory, all the mammals attacked the ticks, killing them immediately, with the exception of Otomys irroratus. In most cases they bit the ticks and left them bleeding, or consumed the blood leaving only the cuticle. In the laboratory, the lizard M. striatus which was caught in the experimental plot, also ate ticks readily swallowing them whole.

When spiders were offered ticks, they transferred them to the centre of the box and punctured them, and then slowly ate them leaving a drying, wrinkled shell behind. The beetles and cockroaches did not attack ticks at all.

4.1.9 Predation of ticks in a cattle boma. Table 5 shows mortalities of engorged R. appendiculatus tethered in the cattle boma. Domestic chickens ate 86.7% of the ticks, and a further 2% were eaten by pied wagtails M. aguimp. The remainder of the ticks were killed by the sun even though

Plate 17 The rodent Mus minutoides .

Plate 18 The rodent Mastomys natalensis.

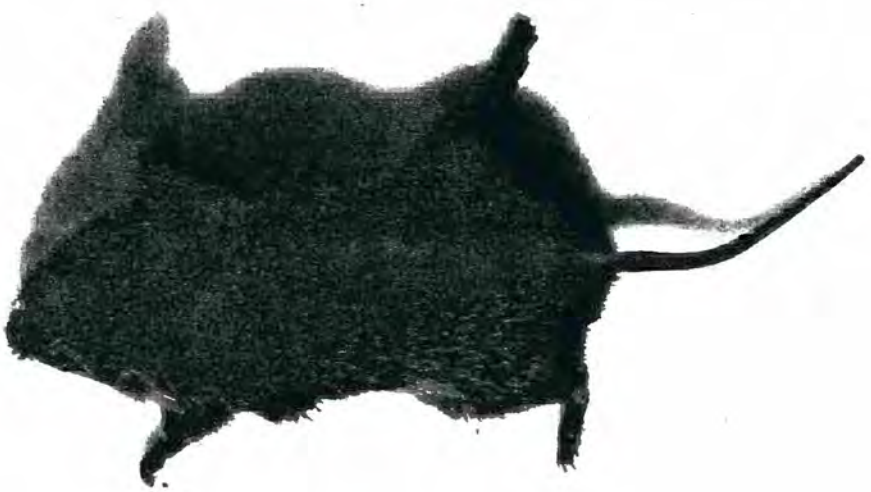

Plate 19 The rodent Otomys irroratus.

Plate 20 The rodent Lemniscomys striatus.

Plate 21 Two lined lizard Mabuya striatus.

TABLE 5

Mortality of engorged R. appendiculatus tethered in a cattle boma for 3 days; replicates 1 and 2 in Feb. 1988, and replicates 3 and 4 in Apr. 1988

	N	Domestic chickens	other causes
Replicate 1	30	86.7	13.3
Replicate 2	30	100.0	0.0
Replicate 3	30	83.3	16.7
Replicate 4	30	80.0	20.0
Mean±SE	30	87.5±4.3	12.5±4.3

they hid in the hot soil. Ground temperatures went as high as 45°C in the afternoon in the hot season.

The chickens normally searched through the cattle boma looking for items of food. Any ticks found were immediately eaten. In most cases, the ticks were eaten by more than one chicken, as the others were alerted by the calls made by the one that found the ticks.

4.2 DISCUSSION

Predation of tethered R. appendiculatus was there throughout the year . This could be explained by the fact that there were six types of animals involved in eating ticks, namely: ants, spiders, birds, lizards, rodents and shrews. With such diversity of predators, ticks were likely to be eaten throughout the year. This is different from what Wilkinson (1970a) found on predation of ticks by ants and spiders in Australia, where there was definite seasonality. In Mexico, Butler et al. (1979) found that the ant, Pheidole megacephala was active in the period between December to February, and ate 63% of gravid B. microplus. The 42.5% mortality of adult R. appendiculatus found in this study (Appendix 1) is low compared to the 50%

predation by ants alone estimated by Wilkinson (1970a) in the dry season in Australia.

The purpose of including the caged ticks was to investigate the role played by small predators such as ants, small spiders and beetles. The 6.9% and 7.2 % predation in this group in long grass and short grass respectively (Appendices 2 and 5) was caused by ants as indicated by the tick remains. The other small predators may have been put off by the presence of metal cages. The predation by ants however did not show marked seasonality in that it was higher in the months of February and March when there was also higher temperatures, but there was also high predation in other low temperature months.

The mortality experienced in November and December 1987 for ticks put out in nylon bags was due to experimental error (Appendices 3 and 6), because the bags were not placed in metal cages, and so they were gnawed open by rodents, and the ticks were eaten. There was otherwise virtually no mortality in this group due to predation. The purpose of this category was to give an indication of the effect of environmental factors such as temperature and humidity. In all the three experimental categories, mortality due to the enviroment remained low throughout the study time, except in the months of higher temperature in February , March and September. Only three ticks in total died due to pathogens, which was found to be infection with the bacteria Serratia marcescens. However, about 10% of all the ticks which survived initial exposure in

the field later died in the incubator, and were found to have bacterial and fungal infections (see Chapter 5). The fungi must have been acquired before the ticks were removed from the field, but bacteria could have been acquired either from the hosts during feeding, or later from the environment.

There was slightly higher predation of the tethered females in the long grass compared to the short grass. The predation by rodents, which was mainly found in the longer grass, could be the cause for the difference, as they are not normally found in short grass.

The predation of tethered nymphs was on the whole of the same level as adults, except that nymphs suffered bigger fluctuations from month to month. Higher mortalities were realised in the dry season than was the case for adults. Due to their small size, nymphs were not put out in metal cages. No attempt was made in the present study to quantify predation of engorged larvae because of their small size. Predation of all the unfed stages of R. appendiculatus was also not investigated, partly because of their size, and also because it was expected that predators would be more interested in the engorged stages. An attempt was made to quantify predation of eggs, but was abandoned later because of lack of a good method of distinguishing between predation and losses caused by factors like being washed away by rain or eggs being blown off by wind.

The purpose of the work done with A. variegatum was to investigate the preference of predators when R. appendiculatus was put out together with A. variegatum. The results obtained indicate that most of the predation was by larger predators, as the ticks were mostly torn apart. There was no predation by ants probably because the cuticle of A. variegatum is too tough for ants to pierce.

The level of predation of adults found in this study could be taken as representative of what happens in the field. However tethering ticks might have made them more available to predators, and that was the reason the fourth group of ticks put out freely in the grass was included. The 46% overall mortality in this group included death due to predators, and death due to environmental factors, and therefore the results from this group compare well with those obtained from the tethered group, plus deaths due to other environmental factors. The method of tethering ticks used in this study gave a 90 cm diameter circle for the movement of the tick to look for shelter. The ticks were found to move without difficulty, and could hide from predators. The fact that they were found hidden under grass-tufts, in cracks and even slighly buried under a thin layer of soil showed that the thread did not inhibit movement.

The results obtained in the present study would be useful in making a computer model for R. appendiculatus and

suggests that predation in the field is an important cause of mortality which should not be ignored.

The aim of the work done in the cattle boma was to investigate whether there are other predators of ticks apart from those found in pastures. In the cattle boma there was no grass, but there was fine soil and dry dung in the hot season and deep mud in the rainy season. The chickens observed eating ticks were from the homestead, and normally searched for food all over the boma and the rest of the compound. They were even able to find ticks which had were slightly under the soil because of their scratching activity while looking for food. In a separate study (unpublished), I have also observed that domestic chickens eat ticks which drop off from animals naturally. On Rusinga Island, domestic chickens have also been found to be important predators of the ticks which drop off the animals, as well as picking them directly from the animals when they are lying down (H. Shawgi, personal communication).

In almost all cases mortality due to other causes was due due drying up with no visible injuries. Deaths to other factors like water-logging, flooding or trampling were not observed. Although many predators of ticks have been found by different workers, (Murphy,1925; Butler et al., 1979; Norval, 1976), no attempts have been made to use them for biological control of ticks. Wilkinson (1970a) suggested that ants might be responsible for the absence of ticks in the reputed tick scarcity areas in Australia. Most of the other predators have

not been put to use because introducing predators like rodents and spiders could be harmful in other ways and could probably offset natural balances. Predators would also be difficult to produce in large enough numbers to give significant impact. The best way to use predators for biological control would be to conserve and foster those already existing.

5 CHAPTER 5

PATHOGENS OF TICKS

5.1 RESULTS

5.1.1 Natural occurrence of bacteria and fungi in ticks.

Table 6 shows bacteria and fungi isolated from the 423 engorged ticks which had been left in the field for periods of 8 days, and died after return to the laboratory, before completion of egg-laying. The bacteria were Proteus mirabilis, a Pseudomonas sp. and a non-chromogenic Serratia marcescens. Out of 210 controls which had been left in the incubator, only three died due to a Pseudomonas sp. From R. appendiculatus laboratory colony, the bacteria Enterobacter cloacae and Escherichia coli were isolated from dead ticks, while Staphylococcus aureus was isolated from dead B.decoloratus from a laboratory colony. In the instances of bacterial infection in laboratory colonies, there was 100% infection with these organisms for particular batches of ticks dropping from the same animal. The ticks turned from grey to black and died within three days after dropping from the hosts; sometimes with swelling, before death. When infected with S. aureus, the ticks became swollen and turned pink, with thick pinkish-white liquid oozing from the mouth.

TABLE 6 Cases of bacteria and fungi isolated from engorged females of R appendiculatus which died after 8 days of exposure on the ground.

Month	Proteus mirabilis	Pseudomonas sp.	Serratia marcescens	Aspergillus sp	Mucor sp.	Fusarium sp.	Nos. exposed	% mortality
May 1988	1	2	3	0	0	0	84	7.1
Jun.1988	0	3	6	0	0	0	80	11.3
Jul.1988	5	4	6	2	0	1	96	18.8
Aug.1988	2	3	0	0	0	0	82	6.2
Sep.1988	2	0	4	2	2	0	82	12.2
Total	10	12	19	4	2	1	423	11.1
Percentage	2.4	2.8	4.5	0.9	0.5	0.2		

Out of the 423 ticks examined (Table 6) only 1.7% (7 ticks) had fungi. The fungi isolated were an Aspergillus sp, a Mucor sp. and a Fusarium sp. No fungi were found in the laboratory colonies of ticks.

In the Fusarium and mucor infections, the ticks were covered with white hyphae, which turned pinkish and blackish respectively after about 2 weeks. The Aspergillus infections had grey hyphae, which turned greenish after about 2 weeks. Total mortality in the field ticks due to bacteria ranged from 6.2% (Aug. 1988) to 15.6% (Jul. 1988). The overall average was 9.7%, compared with 1.7% for fungi. No organisms were recovered from 150 healthy ticks examined in the gut and the haemolymph.

5.1.2. Experimental infection of R. appendiculatus. The mean mortality for unfed adults immersed in a 10^9 spores per ml of B. bassiana suspension was 73.0% (Table 7), and 26.7% for those immersed in a similar concentration of M. anisopliae (Table 8). The egg-laying capacity of engorged females which survived the B. bassiana infection was greatly reduced as shown in Plate 22. Eighty and 100% of unfed nymphs and larvae respectively were killed by immersing them in a spore suspension of B. bassiana (Table 9 and Table 10). The fungus did not affect the moulting processes of those immersed in the spore suspension after engorgement. When adults, nymphs and larvae were fed on rabbits after immersion in the fungal

TABLE 7

Mortality in groups of 30 adult unfed R. appendiculatus observed for 14 days after immersion in a 109 spores pe suspension of B. bassiana, compared with controls in distilled water

	Spores	Controls
	% mortality	% mortality
Replicate 1	83.3	10.0
Replicate 2	60.0	6.7
Replicate 3	70.0	13.3
Replicate 4	63.3	13.3
Replicate 5	86.7	10.0
Mean±SE	72.7± 5.2	10.7±1.7

TABLE 8

Mortality in groups of 30 adult unfed R. appendiculatus, obse
for 14 days (mixed sexes) after immersion in a 10^9 spores per
suspension of M. anisopliae, compared with controls in distil
water

	Spores	Controls
	% mortality	% mortality
Replicate 1	6.7	10.0
Replicate 2	13.3	13.3
Replicate 3	43.3	16.7
Replicate 4	13.4	6.7
Replicate 5	30.0	3.3
Mean±SE	21.3±5.8	10.0±2.4

TABLE 9

Mortality in groups of 30 unfed nymphs of R. appendiculatus, observed for 14 days after immersion in a 10^9 spores per ml suspension of bassiana, compared with controls in distilled water.

	Spores % mortality	Controls % mortality
Replicate 1	86.6	13.3
Replicate 2	70.0	10.0
Replicate 3	90.0	3.3
Replicate 4	76.7	6.7
Replicate 5	76.7	10.0
Mean±SE	80.0±3.6	8.7±1.7

TABLE 10

Mortality in groups of 500 unfed larvae of R. appendicula observed for 14 days after immersion in a 10^9 spores per suspension of B. bassiana, compared with controls in distilled water.

	Spores	Controls
	% mortality	% mortality
Replicate 1	100	24.6
Replicate 2	100	15.8
Replicate 3	100	16.2
Replicate 4	100	30.0
Replicate 5	100	24.8
Mean±SE	100.0	22.3±2.7

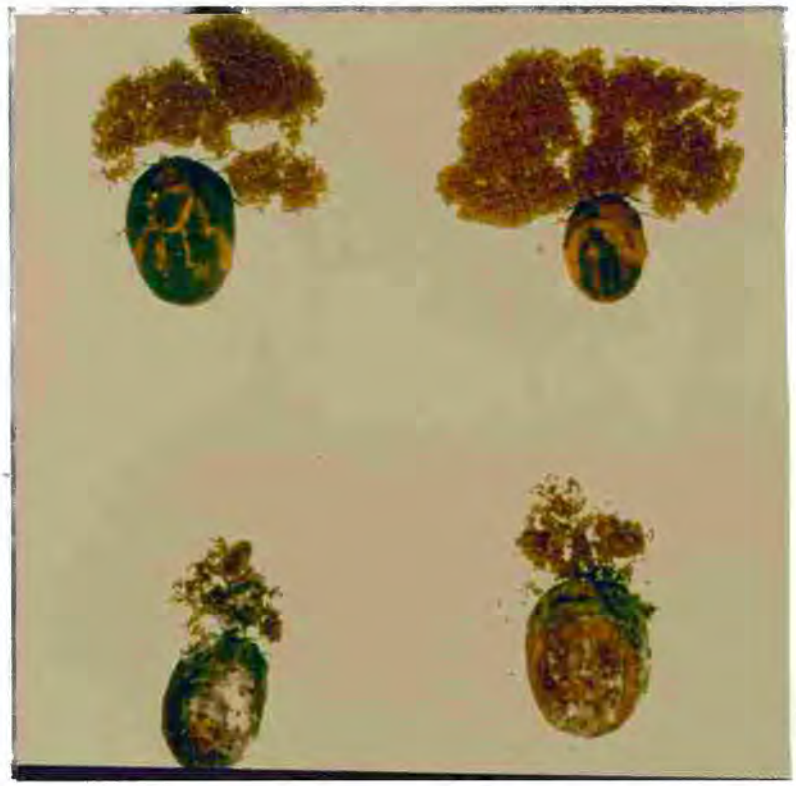

Plate 22 Female R. appendiculatus and their eggs laid after immersion in a spore suspension of B. bassiana compared to controls.

suspension, the duration of feeding was not altered. However on incubation at 28°C and 80% r.h., 77.3% of adults, 69.9% of nymphs and 75.6% of larvae died within a period of two weeks after they dropped off from the rabbits (Tables 11, 12 and 13).

5.2. DISCUSSION

Bacterial infections were found to be 6 times more frequent than fungal infections in naturally infected ticks. It is possible that more ticks would have died due to fungi if the exposure time was longer than eight days. Engorged ticks which were kept in the field for 8 days could have acquired the bacterial infections on the ground, because only 1.4% of the incubator controls had bacteria. It has been suggested that ticks acquire bacterial infections while on the ground after falling off from the hosts, as no blackening of ticks is noticed while they are on the animals (Brown , 1970). However, for laboratory colonies examined in the present study, the infections could have come from the surface of the animal during feeding, as the ticks were all found to have the same organism, even when kept in separate containers. The overall mortality of 11.3% (Table 6) is an indication of the proportion that die in natural populations at the coolest part of the year. However these ticks were only kept in the field for periods of 8 days, and exposure throughout the period of oviposition might have shown a

TABLE 11

Mortality in groups of 30 engorged females of R. appendiculat immersed in a 10^9 spores per ml suspension on B. bassiana anc then fed on rabbits, and observed for 14 days after drop-off, compared with controls immersed in distilled water

	Spores	Controls
	%	%
	mortality	mortality
Replicate 1	83.3	16.7
Replicate 2	70.0	33.3
Replicate 3	60.0	33.3
Replicate 4	86.6	13.3
Replicate 5	76.6	43.3
Mean± SE	77.3±4.8	28.0±5.6

TABLE 12

Mortality in groups of 30 engorged nymphs of R. appendiculatus observed for 14 days after immersion in a 10^9 spores per ml suspension of B. bassiana when unfed,and then f on rabbits, compared with controls immersed in distilled water

	Spores % mortality	Controls % mortality
Replicate 1	53.3	20.0
Replicate 2	83.3	26.7
Replicate 3	86.6	3.3
Replicate 4	86.6	20.0
Replicate 5	70.0	26.7
Mean±SE	69.9±6.4	19.3±4.3

TABLE 13

Mortality in groups of 200 engorged larvae of R. appendiculatu immersed in a 10^9 spores per ml suspension of B. bassiana when unfed, and then fed on rabbits. They were observed 14 days aft drop-off, and were compared with controls immersed in distilled water.

	Spores % mortality	Controls % mortality
Replicate 1	75.0	25.0
Replicate 2	83.0	17.0
Replicate 3	62.5	37.5
Replicate 4	71.5	28.5
Replicate 5	86.0	14.0
Mean±SE	75.6±4.2	24.4±4.2

higher degree of infection. In her study on pathogens of Dermacentor marginatus, Dermacentor reticularis and Ixodes ricinus, from the field, Samsinakova (1974) commented that fungal infections alone could cause about 50% mortality of the ticks in summer in Czechoslovakia. In the present study, a much lower proportion (2.0%) was found to be infected with fungi, probably due to drier conditions and the short period of exposure.

The ticks which died of bacterial infections changed in colour from grey to black and then death followed after one to two days. These symptoms are the same as reported by Brown (1970) and Hendry and Rechav (1981). In contrast, ticks which are infected with viruses and rickettsias were reported to have ulcers, deformities of the mouthparts and gangrene of the extremities (Sidorov and Shcherborok, 1973). Megaw (1978) also found that other virus-like particles were pathogenic to the salivary glands and damaged the cytoplasm of infected cells in Boophilus microplus. These ticks which are infected with viruses and rickettsias survive but probably lay fewer eggs than normal. Those infected with bacteria and fungi however do not live long enough to lay eggs.

In experimental infections in the laboratory, B.bassiana was found to be more pathogenic to R. appendiculatus adults than M. anisopliae.

The process of oogenesis inside the female, and subsequent laying takes about 2-3 weeks after completion of

engorgement. This is roughly the same as the time required for B. bassiana spores to germinate and begin to cause mortality, and this is also why the egg-laying capacity of the tick is affected.

The unfed nymph and larvae showed a mortality of 80% and 100% respectively, but as they had not been incubated singly because of their small size these results might have been reinforced later by tick-to-tick contact in the tubes.

The moulting process of the immatures was not interfered with by the B. bassiana, probably because the cuticle hardens within a few days of engorgement, thus protecting the moulting tick inside. However it seems that some of the germinating hyphae had already penetrated inside to infect the tick, as 60% and 85% of the moulted larvae and nymph respectively, died after moulting. It is possible also that the new soft ticks could be infected by contact with spores on the surface of the exuviae as they come out. In insect studies, Ferron (1986) observed that B. bassiana is only effective if the new cuticle of the moulting insect is reached,otherwise the fungus would be shed off with the old cuticle.

Beauveria bassiana did not interfere with the feeding process of any of the three instars of R. appendiculatus, when the unfed forms were immersed in a spore suspension before feeding. This is because the feeding period of larvae is normally 3-4 days, 4-5 days for nymphs and 6-8 days for adults, while B. bassiana takes about 14 days to

cause mortality.

The control methods which are currently being used against ticks are mainly aimed at the parasitic stages. The use of bacteria and fungi as biocontrol agents has not been tested, probably due to the fact that these organisms are not specific to ticks. Some of the organisms isolated in the present study are also pathogenic to human beings and domestic animals.

Entomopathogenic fungi belonging to the genera Beauveria, Metarhizium, and Paecilomyces have been studied to investigate their use as biological control agents (Gillespie et al., 1986; Roberts and Wright, 1986; Zimmerman and Simons, 1986; Anderson et al., 1988). Some of them have been put into commercial production for crop protection (Roberts and Wright, 1986; Storey and Gardner, 1987; 1988). Extracting the toxins of micro-organisms and using them instead of the live fungi is an alternative way of using them for control of pests. Toxins of B. bassiana have been extracted and are in use against some arthropods (Ferron, 1986). However, when tested against the soft tick Argas persicus they were found to be ineffective. It could be assumed that they would be effective against R. appendiculatus because the live fungus is effective, and if so, they would be a better alternative for killing ticks as compared to the synthetic acaricides which are currently in use, and which are not bio-degradable. However, more work would be needed to develop a good method of controlling

ticks using any toxins that were found effective, depending on their mode of action.

The bacteria isolated from ticks in this study are not good candidates for use in biological control of ticks, because of the pathogenicity to domestic animals and human beings. Trials should however be done to see if Bacillus thuringiensis, which has been found pathogenic to a variety of arthropods (Goldberg and Margalit, 1977; Calberg, 1986), will kill ticks. Toxins of B. thuringiensis have been extracted and are in use against Diptera, Coleoptera, Hymenoptera and Orthoptera (Calberg, 1986). They have also been tested against the mite Dermanyssus gallinae, but not, so far, against ticks.

6 CHAPTER SIX

PARASITOIDS OF TICKS

6.1 RESULTS

Out of 140 fully engorged A. variegatum nymphs collected from cattle in the Trans-Mara over the period October 1987 to November 1989, 46.4% were infested with parasitoids, while 39.3% moulted successfully into adults, and the remaining 14.3% died due to other causes (Table 14). Post mortem of the dead ones showed no parasitoid infestations. There were no collections during the months of April, June and August 1988. Parasitoids were found in 6 out of the 10 months the study was done. Over the same period, 592 engorged R. appendiculatus nymphs were collected from the same cattle and, on incubation in the laboratory, no parasitoids were found in them and 86% moulted successfully, while 14% died due to other causes (Table 15).

From Rusinga Island, 230 engorged A. variegatum nymphs and 338 engorged R. appendiculatus nymphs were collected from cattle in seven months over the period December 1987 to August 1989, and taken to the laboratory to moult (Tables 16 and 17), but they had no parasitoids at all. Eighty percent of the A. variegatum and 81.7% of the R. appendiculatus moulted to adults, and the remainder died.

TABLE 14

Development of engorged A. variegatum nymphs collected from the Trans-Mara

Month	N	% moult	% dying (other causes)	% with parasitoids
Oct. 1987	30	50.0	0.0	50.0
Nov. 1987	7	71.4	28.6	0.0
Dec. 1987	3	66.7	33.3	0.0
Jan. 1988	13	53.9	23.1	23.0
Feb. 1988	24	-	100.0*	-
Mar. 1988	3	33.3	66.7	0.0
Apr. 1988	-	-	-	-
May 1988	6	50.0	50.0	0.0
Jun. 1988	-	-	-	-
Jul. 1988	16	50.0	18.8	31.2
Aug. 1988	-	-	-	-
Sep. 1988	36	27.8	16.6	55.6
Oct. 1988	17	23.5	0.0	76.5
Nov. 1988	9	0.0	0.0	100.0
Total	140	39.3	14.3	46.4

* All ticks died in incubator; not used in calculations.

"-" No ticks collected.

TABLE 15

Development of engorged R. appendiculatus nymphs collected from the Trans-Mara

Month	N	% moult	% dying (other causes)	% with parasitoids
Oct. 1987	43	93.0	7.0	0.0
Nov. 1987	36	80.6	19.4	0.0
Dec. 1987	32	93.8	6.2	0.0
Jan. 1988	120	90.8	9.2	0.0
Feb. 1988	84	75.0	25.0	0.0
Mar. 1988	15	100.0	0.0	0.0
Apr. 1988	-	-	-	-
May. 1988	60	95.0	5.0	0.0
Jun. 1988	-	-	-	-
Jul. 1988	45	77.8	22.2	0.0
Aug. 1988	-	-	-	-
Sep. 1988	51	78.4	21.6	0.0
Oct. 1988	63	95.2	4.8	0.0
Nov. 1988	43	86.0	14.0	0.0
Total	592	86.0	14.0	0.0

"-" No ticks collected

TABLE 16

Development of engorged *A. variegatum* collected from Rusinga Island

Month	N	% moult	% dying (other causes)	% with parasitoids
Dec. 1987	20	60.0	40.0	0.0
Feb. 1988	58	86.2	13.7	0.0
May 1988	10	80.0	20.0	0.0
Jul. 1988	11	81.8	18.2	0.0
Sep. 1988	25	80.0	20.0	0.0
Mar. 1988	10	60.0	40.0	0.0
Aug. 1989	12	50.0	50.0	0.0
Sep. 1989	23	87.0	13.0	0.0
Oct. 1989	61	86.9	13.1	0.0
Total	230	80.0	20.0	0.0

TABLE 17

Development of engorged R.appendiculatus nymphs collected from Rusinga Island

Month	N	% moult	% dying (other causes)	% with parasitoids
Dec. 1987	43	90.7	9.3	0.0
Feb. 1988	40	85.0	15.0	0.0
May 1988	56	89.3	10.7	0.0
Jul. 1988	10	80.0	20.0	0.0
Sep. 1988	29	79.3	20.7	0.0
Mar. 1989	33	72.7	27.3	0.0
Aug. 1989	30	73.3	26.7	0.0
Sep. 1989	22	77.3	22.7	0.0
Oct. 1989	75	78.7	21.3	0.0
Total	338	81.7	18.3	0.0

Infested nymphs showed symptoms of parasitization from day 9 onwards. The cuticle either turned from grey to a light brown colour or it developed a mottled appearance. The hardened cuticle became more rounded and swollen (Plate 23). The time taken for parasitoids to emerge varied from nymph to nymph, even when they dropped from the host on the same day. They took 29-42 days to emerge and the mean was 35.3±0.5 days (Table 18), while healthy adults took about 23 days to emerge. The mean number emerging from one nymph was 22.4±1.3 with a range of 7-40 (Table 19), and the sex ratio of the adults was 1 male: 5 females. Emergence invariably took place at night. They gnawed a small hole, or sometimes two holes, in the dorsal side of the nymph, through which they came out, leaving the empty cuticle of the nymph otherwise intact (Plate 23). Mating was observed to occur immediately, as soon as the parasitoids were out. After a few hours, the females were ready to lay eggs on unfed nymphs.

The parasitoid was identified as a hymenopteran wasp belonging to the super family Chalcidoidea, family Encyrtidae.

6.1.1 Description of the Trans-Mara parasitoid

Female. Figure 12 and Plate 24 show the female parasitoid, and the measurements are given in Table 20. It is brownish black and 1.1 mm long. The antennae (Figure 13a) are 0.41

TABLE 18

Mean (± SE) number of days to emergence of the Trans-Mara parasitoid at 27°C and 80 % r.h.

	N	Mean	Range
Replicate 1	25	34.5	29 - 42
Replicate 2	25	33.9	29 - 36
Replicate 3	25	36.2	29 - 40
Replicate 4	25	37.0	30 - 41
Replicate 5	25	35.0	30 - 39
Mean±SE	25	35.3 ± 0.6	29 - 42

TABLE 19

Mean (± SE) number of parasitoids emerging from one nymph

	N	Mean	Range
Replicate 1	25	22.4	7 - 40
Replicate 2	25	23.0	9 - 36
Replicate 3	25	26.8	11 - 30
Replicate 4	25	19.5	10 - 36
Replicate 5	25	20.2	14 - 40
Mean±SE	25	22.4± 1.3	7 - 40

Plate 23 A. variegatum engorged nymphs infected with parasitoids compared to normal controls.

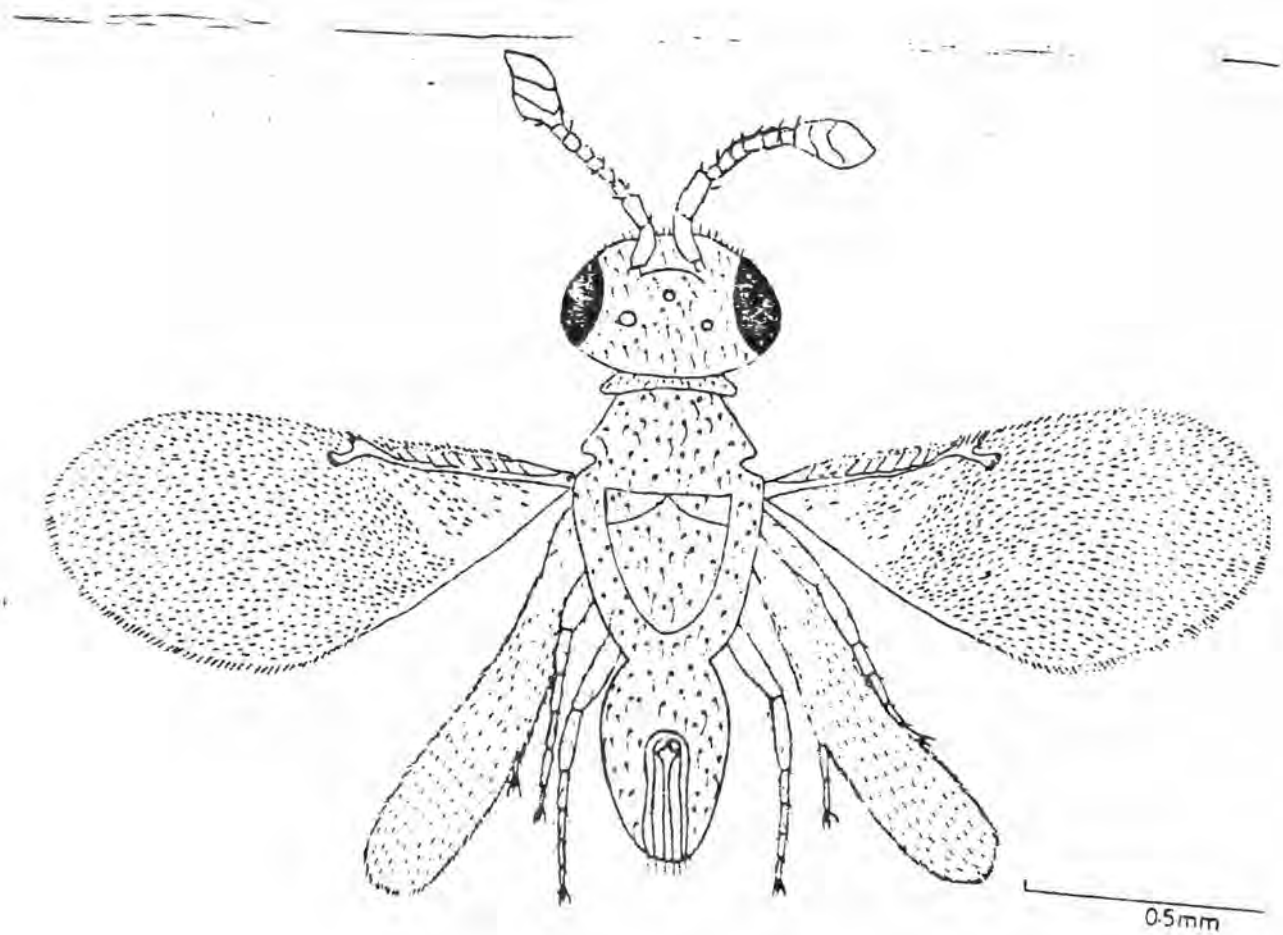

Figure 12 Dorsal view of a female parasitoid from A. variegatum from the Trans-Mara.

Plate 24 Female parasitoid of A. variegatum from the Trans- Mara.
Magnification X 100.

Table 20.

Mean measurements (mm) of *I.texanus*, *H.hookeri*, *H.theilerae* and the Trans-Mara parasitoid

	*I.texanus**	*H.hookeri**	*H.theilerae**	Trans-Mara parasitoid
Female				
Length of body	1.55	1.40	1.40	1.10±0.02
Length of head	0.25	0.30	0.25	0.24±0.05
Length of thorax	0.60	0.60	0.50	0.44±0.02
Length of abdomen	0.70	0.50	0.65	0.33±0.03
Length of antennae	0.70	0.60	0.45	0.41±0.03
Length of forewing	1.10	1.30	1.05	0.95±0.10
Maximum width	0.50	0.60	0.45	0.50±0.03
Length of hindwing	NA	NA	NA	0.59±0.02
Maximum width	NA	NA	NA	0.51±0.01
Male				
Length of body	1.25	1.25	1.65	0.92±0.05
Length of head	0.25	0.30	0.30	0.2010.02
Length of thorax	0.45	0.55	0.60	0.42±0.10
Length of abdomen	0.55	0.44	0.75	0.30±0.12
Length of antennae	0.70	0.80	0.55	0.56±0.06
Length of forewing	0.75	1.10	0.35	0.80±0.12
Maximum width	0.33	0.50	0.10	0.30±0.01
Length of hindwing	NA	NA	NA	0.55±0.02
Maximum width	NA	NA	NA	0.12±0.03

* Figures from Fiedler (1953)

NA - Not available

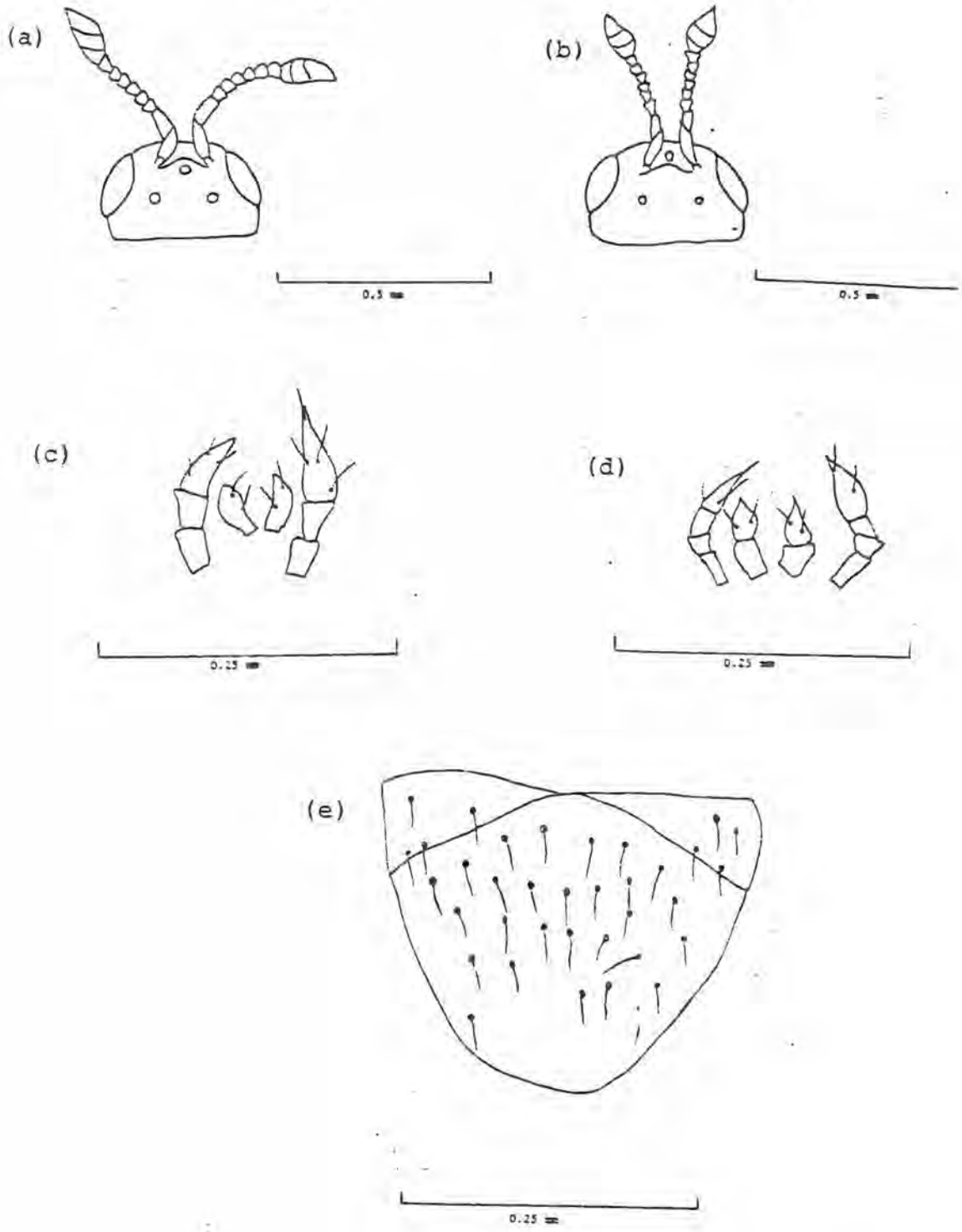

Figure 13 (a) Dorsal view of the head of a female Trans-Mara parasitoid

(b) Dorsal view of the head of a female H. hookeri

(c) The maxillary and labial palps of the majority of parasitoi from the Trans-Mara

(d) The maxillary and labial palps of H. hookeri and I. texanus

(e) Dorsal view of the scutellum of the Trans-Mara parasitoid

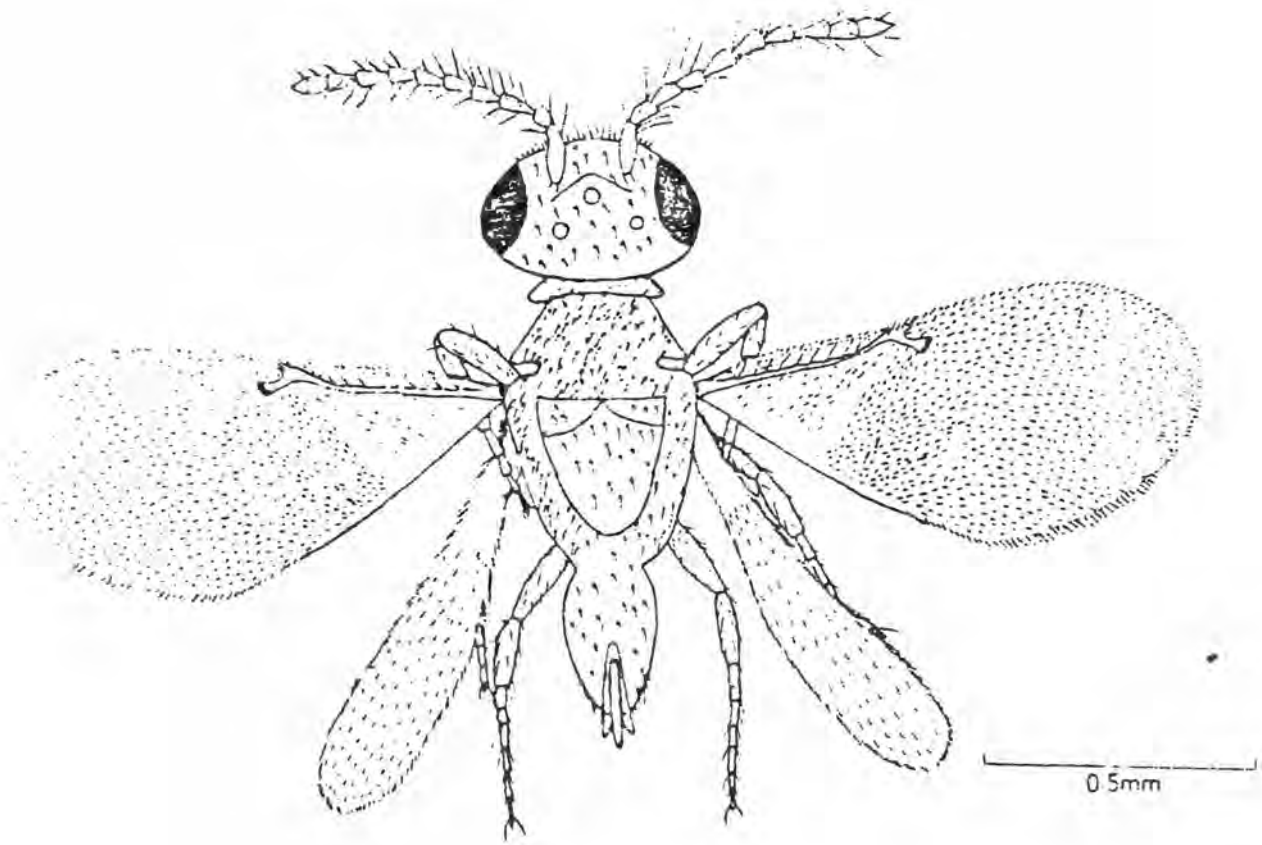

Figure 14 Dorsal view of a male parasitoid from A. variegatum from the Trans-Mara.

mm long, have nine segments, and are moderately hairy. The last antennal segment is much wider than the others and club-shaped, and is divided into 3 parts. The scapes of the antennae are inserted 0.14 mm apart, and incline inwards. The distance between the two lateral ocelli is 0.14 mm (Figure 13a) and that between the median ocellus and either lateral ocellus is 0.08 mm. The compound eyes are 0.13 mm wide. The maxillary palps usually have three (sometimes four) segments, while the labial palps have one segment in the majority of cases (Figure 13c). The scutellum has 3-5 bristles in each triangular lobe (Figure 13e).

Male

Figure 14 and Plate 25 show the male parasitoid, and measurements are given in Table 20. It is 0.92 mm long. The antennae have long hairs, and are 0.56 mm long with 10 distinct segments. The last antennal segment is not divided. All the funicle segments are longer than they are broad. The scapes of the antennae are inserted 0.14 mm apart, but they do not incline to the mid-line of the head as in the females.

6.1.2. Comparison of the Trans-Mara parasitoid with Ixodiphagus texanus and Hunterellus hookeri. The ocelli of I. texanus examined in this study resembled those of the parasitoid from the Trans-Mara (Figure 13a) while those of

Plate 25 Male parasitoid from A. variegatum from the Trans-Mara.

Magnification X 100.

H. hookeri that were examined had the ocelli arrangement as shown in Figure 16b. The maxillary palps of both H. hookeri and I. texanus had four segments, while their labial palps had two segments (Figure 13d). The triangular lobes of the scutelli of H. hookeri and I. texanus had from 3-5 bristles.

6.1.3. Infestation of A. variegatum with parasitoids in the laboratory. When kept in a container with unfed nymphs, parasitoids were observed ovipositing on nymphs only a few hours after emergence. The ovipositor was inserted into the dorsal side of the nymph for periods of 2-3 minutes at a time. When the ratio of parasitoids to nymphs was 1:3, there was 40% parasitization, as evidenced by symptoms of parasitization and subsequent emergence of parasitoids when those nymphs were fed on rabbits and allowed to moult. Seven per cent of the nymphs which showed apparent parasitization had no subsequent emergence of parasitoids, but died. On dissection however, fully formed adult parasitoids were found inside, but they too were dead.

6.1.4. Effect of temperature on parasitoid development and maintenance. In the group of infected nymphs kept at 28°C, the earliest emergence of parasitoids was on day 29, while

for those at 22°C it was on day 34. The emerged parasitoids were observed to be less active at 28°C, and died after 1-2 days. Those placed at 22°C were active, and died after 3-4 days.

Of the three types of containers used, the perspex box was found to be the most suitable, as the parasitoids survived for up to 4 days, while they died within 1-2 days in the other two types of containers.

When the parasitoids were offered a 0.5% sugar solution, they were observed visiting the sugar container for brief periods. Their life-span at 28°C was not altered by the presence of the nutrient, but at 22°C they lived for 3-6 days. Thus their mean period of survival was longer with sugar, as long as the temperature was 22°C.

6.2. DISCUSSION

All the engorged nymphs were collected from the cattle by forcibly detaching them; only those judged to be fully engorged, or nearly so, were retained to moult. This treatment could have damaged some of them, and could explain why 14.3% of the nymphs died without moulting. The Trans-Mara is a wet and humid area, with a well distributed rainfall of 1300 mm per year and these conditions themselves might favour survival of the adult parasitoids, together with the fact that A. variegatum nymphs for them to infest

are present in most months of the year. Rusinga Island is rather drier, with a rainfall of 1060 mm per year, but this is restricted to two distinct rainy seasons.
The intervening dry periods might not be conducive to the survival of the adult parasitoids. The nymphs are only abundant in the months of March and October (D.K. Punyua, personal communication) and so offer restricted opportunities for oviposition. If on the other hand the parasitoids are able to survive on Rusinga Island, it is possible that they were not able to spread from the mainland because there was very little movement of animals before 1983 when a causeway was built linking the Island with the mainland. The tendency of tick parasitoid populations to remain isolated has been reported for H. hookeri in South Africa where parasitoids were found in some farms and not in others in the same neighbourhood (Cooley, 1934).

Identity of the Trans-Mara parasitoid

The morphological features used by previous workers to distinguish between Encyrtidae have been the arrangement of the ocelli, the distance between the antennae at insertion, the numbers of bristles on the triangular lobes of the scutellum, the length of the thorax in relation to that of the abdomen, the size of the eyes, and the number of segments in the maxillary and labial palps (Fiedler, 1953). In the present work, the arrangement of the ocelli in the

majority of the specimens from the Trans-Mara resembles that of I. texanus, although a few individuals have the pattern found in H. hookeri. According to Fiedler (1953) H. hookeri and I. texanus have two bristles in each of the triangular lobes of their scutelli, and he used this feature to separate H. theilerae, which he reported to have only one bristle. The number of bristles in the parasitoid from Trans-Mara, and also in material of both H. hookeri and I. texanus examined in this study was found to vary between 3 and 5, even in members of the same population, and therefore this feature is not reliable for use in taxonomy.

The number of segments in the maxillary palps of both H. hookeri and I. texanus is four (Fiedler, 1953). This was confirmed in the material of the same species collected from the USA examined in the present study. The majority of the parasitoids from the Trans-Mara, however, was found to have only three segments. Moreover, most of the parasitoids from the Trans-Mara were found to have one segment in the labial palps, while the other two species have two. Three segments in the maxillary palps were reported by Fiedler (1953) in H. theilerae, but its labial palps had two segments. The size of eyes was used by Fiedler (1953) to distinguish H. theilerae, because the male has eyes which are only half the size of those of the female. Some males of the Trans-Mara parasitoid have the smaller eyes, but males of H. hookeri and I. texanus were found to have only big eyes. The Trans-Mara parasitoid therefore has features

in common with H. hookeri and I. texanus but very little in common with H. theilerae, especially because the latter has distinct characteristics not shared with any other reported encyrtid parasitoid, which include a forked processus on the head of the male, rudimentary wings, and a very short and stubby first pair of legs. It is therefore possible that the Trans-Mara parasitoid is a distinct species, especially since it is the only one so far reported from A. variegatum. In his study in Ivory Coast, Graf (1979) reported a parasitoid from Amblyomma nutalli which closely resembled H. hookeri. A. variegatum nymphs collected from various hosts in the same neibourhood were reported not to be infected by this parasitoid. The parasitoid from A. nutalli was also different from the Trans-Mara one in that numbers emerging from one nymph were as high as 262, while the highest recorded for the Trans-Mara parasitoid was 40. However this might be a local characteristic rather than a taxonomic feature. Attempts to infest A. variegatum nymphs in the laboratory were unsuccessful, using the parasitoid from A. nutalli (Graf, 1979). Hunterellus hookeri has been reported in 10 species of ticks (but not in A. variegatum) and it has also been reported to infest R. appendiculatus under field conditions in South Africa (Cole,1965).

The parasitoid from Trans-Mara is a much smaller insect than the other parasitoids as shown in Table 20. The size could however be influenced by the number emerging from each nymph, and may therefore not have much significance in

taxonomy. Graf (1979) found that the number of wasps emerging from A. nutalli depended on the engorgement weight of the nymph.

Identification of tick parasitoids has so far been done using morphological features only. More modern methods such as isoenzyme profiles, breeding, or DNA mapping should be used for more conclusive results.

Biology of the Trans-Mara parasitoid

At 28°C and 80% r.h., the pre-emergence period of the Trans-Mara parasitoid was 29-42 days. This differed from I. texanus and H.hookeri both of which emerge after 20-30 days under the same conditions. The pre-emergence period in the laboratory could be influenced by the conditions the parasitoid was exposed to in the field for a long time in the life of the species. Such conditions could be temperature, humidity and alternation of hot days and cold nights. Parasitoids from different geographical localities could therefore have different pre-emergence periods.

The mean number of adults emerging from one nymph was 35.3±0.6 for the Transmara parasitoid. An important factor influencing the number emerging must be the number of eggs laid in the tick. The size of the engorged host itself does not seem to be important in influencing numbers emerging, according to Bowman et al., (1986). They found that engorged larvae of Dermacentor andersoni and D. variabilis carried a mean of 54.0 and 51.0 I. texanus respectively,

while their engorged nymphs only had 41.0 and 25.0 parasitoids respectively. This could be due to physiological differences between the instars. Graf (1979), however reported that the number of wasps emerging increased with increasing weight of the nymphs. This suggests that there could be some form of density dependent mortality occuring when the nymph does not become very big. Cole (1965) reported that several H. hookeri parasitoids were observed laying eggs in one nymph, which would result in higher numbers of parasitoids emerging. For mass rearing of parasitoids therefore, it is safer to have a higher proportion of parasitoids to nymphs, to ensure maximum oviposition in each nymph.

Lowering the temperature from 28°C to 22°C increased the pre-emergence time by 5 days for the Trans-Mara parasitoid. The phenomenon of latency in the U.S.A., where the parasitoid passes the winter months in the unfed nymph and develops when the nymph feeds, has been reported for H. hookeri (Cooley, 1928; 1930). The tropical climate is totally different. Nevertheless the host species shows marked seasonality in parts of its range, and some form of latency may still occur. Temperature had an effect on the longevity of the adult parasitoids, as those kept at 22°C survived 3 more days than those at 28°C.

Parasitoids which were kept in the perspex box survived longer and were more vigorous than in the smaller containers. The reasons for this are not clear, since it

would be expected that if they have only a short distance to fly they would have more food reserves to invest in egg production. The provision of artificial diet for parasitoids has not been mentioned in previous tick parasitoid work, and it is not clear whether adult tick parasitoids feed under field conditions. Other insect parasitoids have however been offered artificial diets (R. Ochieng, personal communication). These diets have been found to increase longevity. The 0.5% sugar solution offered to the parasitoid in this work helped prolong the life-span at 22°C. However, at 28°C, it made no difference which indicates that temperature is more critical than supplementary feeding in survival.

Infestation of A. variegatum in the laboratory

If the Trans-Mara parasitoids are to be used for control of ticks in the field, they would have to be mass reared in the laboratory and then released in the field. To effect this , a technique giving maximum infestation of A. variegatum in the laboratory is needed. The proportion of infested nymphs achieved in the present study could be raised if larger numbers of adult parasitoids were available. Successful infestation of D. variabilis in the laboratory has been reported for I. texanus (88%) (Bowman et al.,1986) and over 80% for H. hookeri on Rhipicephalus sanguineus (Cole, 1965).

7. CHAPTER 7

DROP-OFF RHYTHM OF R. APPENDICULATUS

7.1 RESULTS

7.1.1 Engorged females. Table 21 shows the percentages of females dropping off at intervals of 2 hours throughout the day and night. There were some ticks at all collecting times, but the peak drop-off was between 0800 and 1000 hours, and the next highest period was 0600 to 0800 hours (Figure 15). Analysis of the data showed that the percentages of ticks dropping between 0800 and 1000 and also between 0600 and 0800 did not differ significantly, but were both significantly higher than the other collecting times (Appendix 15). Out of a total of 1279 female ticks which dropped from the 9 animals, only 6 (0.47%) dropped during the period 2000 to 0600 hours. There was no significant variation in the drop-off pattern between the individual animals (Appendix 15).

Ticks started to drop in the morning of day 6 if they were put on the animals at 0900 hours on day 1 and continued for 5 days (Table 22 and Figure 16), with maximum drop-off on day 8.

7.1.1.1 Engorged female ticks from sensitised animals. Table 23 shows the pattern of drop-off for female ticks that fed on tick-sensitised animals. The time of maximum drop-off was also 1000 hours (Figure 17), which was again found

TABLE 21

Percentage distribution of drop off for 1279 engorged R. appendiculatus females, according to time of collection. They were put on the animals at 0900 hours.

	Time of collection							
Animal	0600	0800	1000	1200	1400	1600	1800	2000
1	0.0	8.5	45.2	15.8	5.6	19.8	5.1	0.0
2	0.0	4.5	73.7	9.6	4.5	4.0	3.7	0.0
3	0.0	22.4	52.4	16.3	4.7	0.6	1.8	1.8
4	2.4	22.7	49.7	5.5	7.4	4.9	4.3	3.1
5	0.9	20.4	51.5	10.9	3.9	5.9	4.5	1.9
6	0.5	19.8	49.0	7.9	6.9	8.4	4.9	2.5
7	0.0	76.8	10.5	10.5	1.1	1.1	0.0	0.0
8	0.0	37.9	37.9	2.1	7.6	10.3	0.6	3.5
9	0.0	56.1	0.0	0.0	43.9	0.0	0.0	0.0
Mean ±SE	0.4 ±0.3	29.9 ±7.8	41.1 ±7.5	8.7 ±1.9	9.5 ±4.4	6.1 ±2.1	2.7 ±0.7	1.4 ±0.5

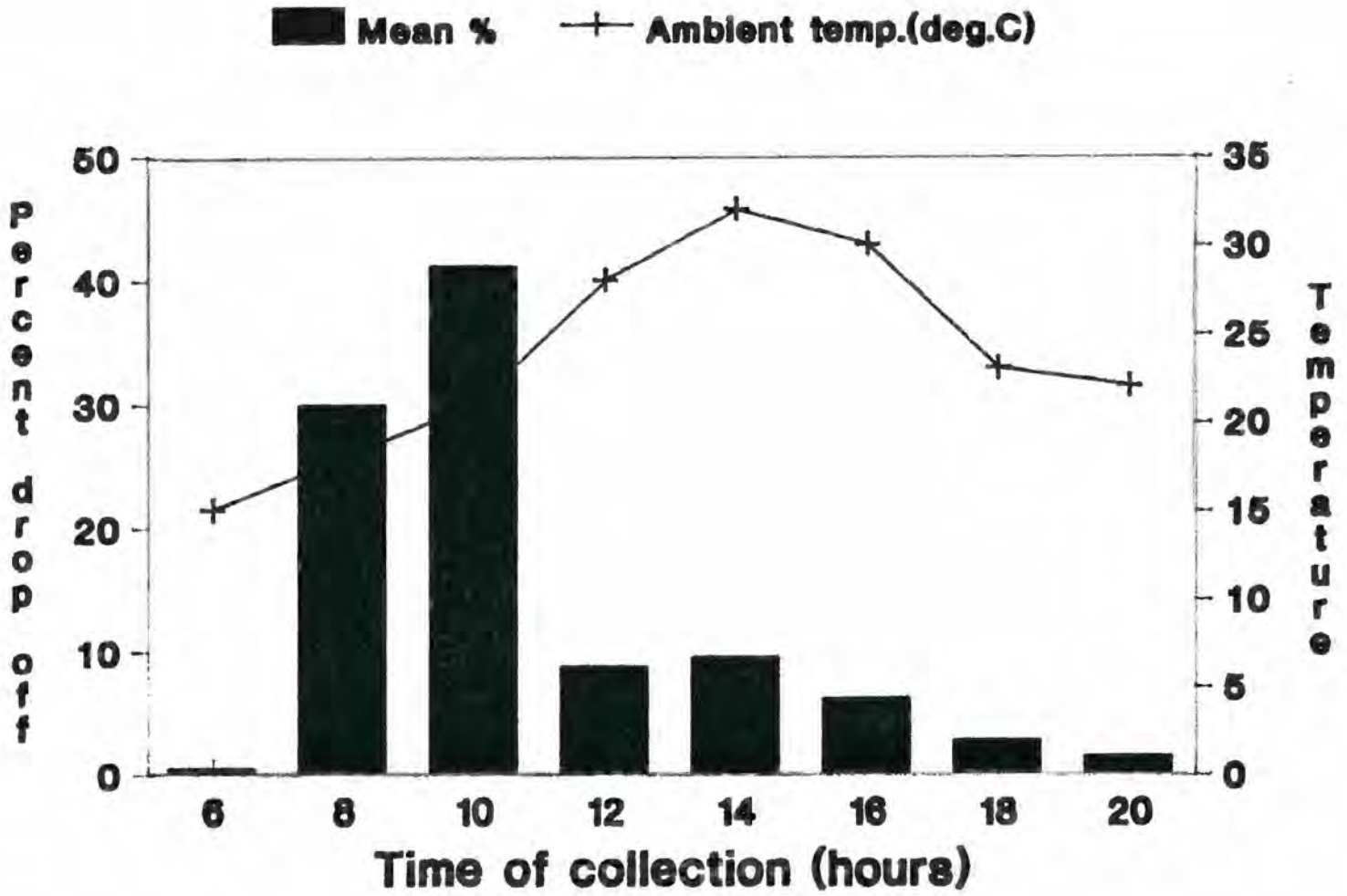

Figure 15 Distribution of drop-off by time of collection for female engorged R. appendiculatus from cattle.

TABLE 22

Percentage distribution of 846 engorged R. appendiculatus female dropping off by animal, and duration of feeding, for ticks put on the animals at 0900 and 2000 hours on day 1

Day since infestation	Time of application	Animals 1	2	3	4	5	6	Mean+SE
6	0900	19.1	22.3	8.7	1.6	4.7	0.0	9.4±3.8
	2000	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0900	48.9	64.0	24.3	15.6	58.8	0.0	35.3±10.5
	2000	22.9	45.2	59.4	0.0	34.7	0.0	27.0± 9.9
8	0900	27.7	6.9	47.5	50.0	32.9	58.1	37.2± 7.6
	2000	74.3	45.5	17.5	22.6	55.9	0.0	36.0±11.2
9	0900	4.3	6.8	19.5	32.8	3.6	41.9	18.2± 6.6
	2000	2.7	8.2	16.3	77.4	9.4	34.8	22.3±12.0
10	0900	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	2000	0.0	1.1	7.8	0.0	0.0	65.2	12.4±10.6

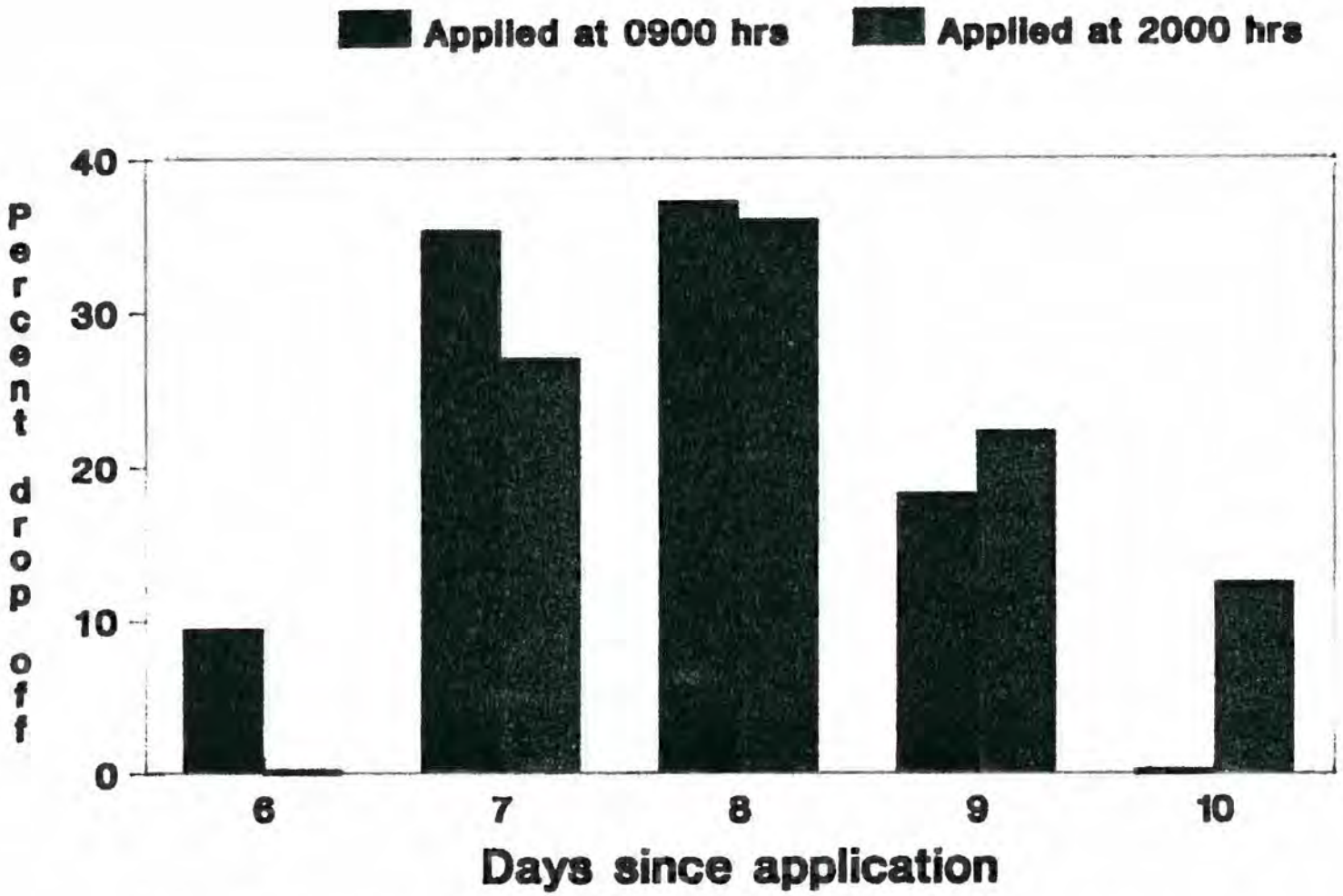

Figure 16 Duration of feeding of female R. appendiculatus on cattle, applied at 0900 and 2000 hours.

TABLE 23

Percentage distribution of 840 engorged R.appendiculatus females droppping off by time of collection, from 6 tick-sensitised animals

	Time of collection							
Animal	0600	0800	1000	1200	1400	1600	1800	2000
1	0.0	18.9	44.3	21.7	8.5	1.9	4.4	0.0
2	0.0	15.1	30.0	15.3	32.1	4.4	3.1	0.0
3	0.0	13.9	40.9	12.3	13.0	0.0	5.2	14.7
4	0.0	22.6	27.8	6.3	20.1	6.3	0.0	16.9
5	0.0	33.5	44.3	2.9	5.4	4.3	7.8	1.8
6	0.0	31.9	35.4	5.7	4.1	4.1	18.0	0.8
Mean ±SE	0.0	22.7 ±3.4	37.1 ±2.9	10.7 ±2.9	13.9 ±4.4	3.5 ±0.9	6.5 ±2.5	5.7 ±3.2

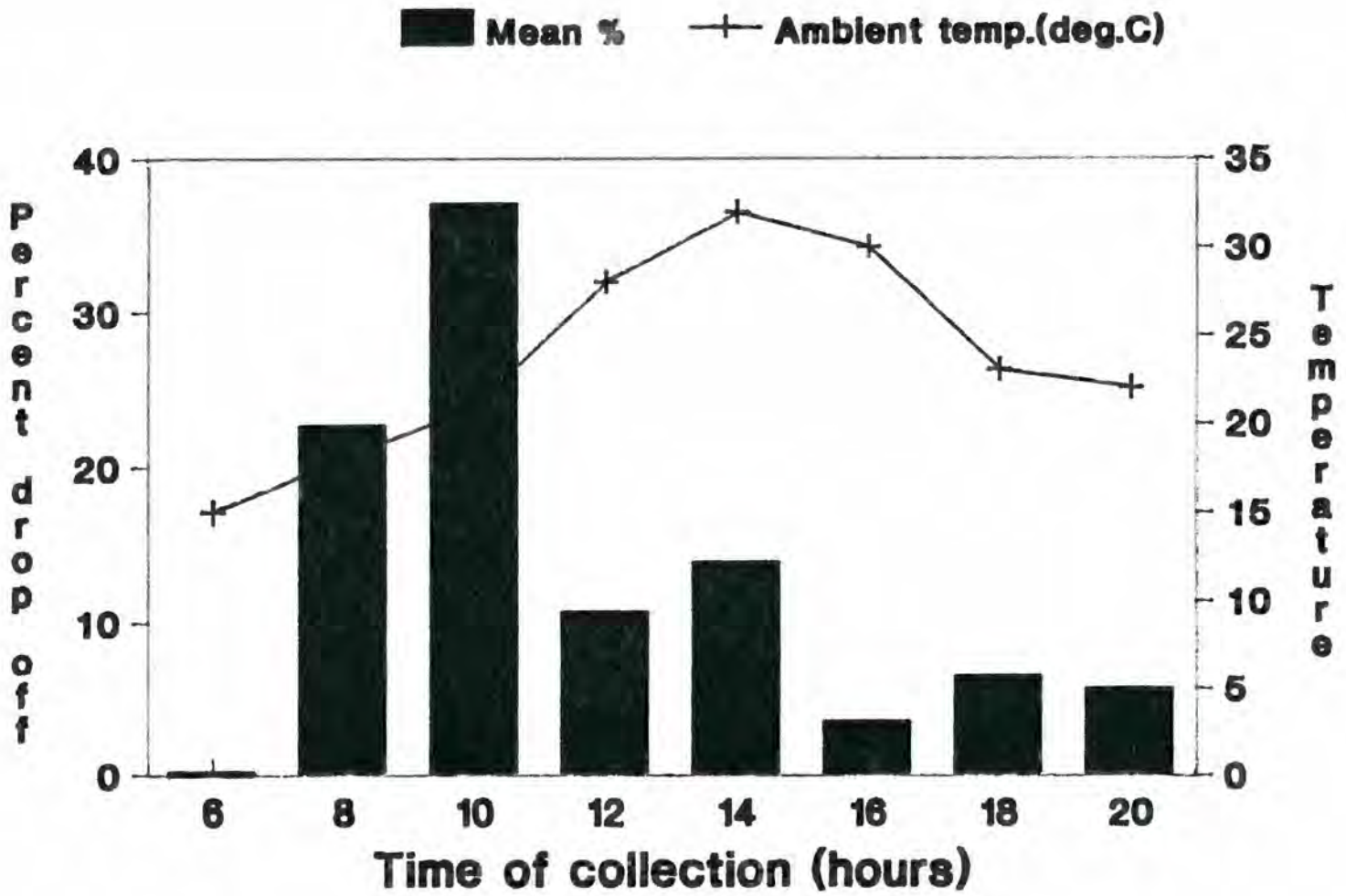

Figure 17 Distribution of drop-off by time of collection for engorged female R. appendiculatus from tick-sensitised cattle.

to be significantly different from all the other times (Appendix 16)

7.1.1.2. Weight of engorged females. Table 24 shows the distribution of numbers and weights of ticks dropping off for the first and for the second infestations. The ticks from the first infestation weighed slightly, but significantly, more (0.41± 0.0021 g) than those from the second infestation (0.38± 0.001), (Appendix 17a). When the weights of ticks dropping at different times were compared, no difference was found between the eight collecting times, for either the first or the second infestations.

7.1.2. Engorged nymphs

The distribution of nymphs dropping from the animals throughout the day is shown in Table 25 and Figure 18. The maximum drop-off was between 1600 and 1800 hours, which was significantly different from all other collecting times, with 1400 to 1600 hours next (Figure 18 and Appendix 18). Drop-off during the night (2000 - 0600 hours) was only 0.40%. Ticks began to drop on day 5 and continued up to day 7, with the maximum drop on day 5 (Table 26). The mean weights of engorged nymphs dropping at different times of the day are shown in Table 27. Analysis of data showed no significant variation in weight due to time of dropping.

Table 24

Total numbers and the mean weights of females of R. appendiculatus dropping from 6 animals at first and second feeds; 200 ticks applied to feed in each case

Animal	Infestation	Total ticks	Mean wt.±SE (g)
1	1	177	0.41 ± 0.006
	2	124	0.38 ± 0.004
2	1	198	0.42 ± 0.001
	2	159	0.39 ± 0.004
3	1	170	0.41 ± 0.005
	2	115	0.39 ± 0.01
4	1	95	0.41 ± 0.003
	2	159	0.39 ± 0.001
5	1	145	0.42 ± 0.001
	2	167	0.38 + 0.001
6	1	66	0.41 ± 0.001
	2	122	0.38 + 0.001
Mean±SE	1	141.8±20.9	0.41 ± 0.001
Mean±SE	2	140.0± 9.4	0.38 + 0.001

TABLE 25

Percentage distribution of 2736 engorged R. appendiculatus nymphs dropping off according to time of collection

	Time of collection							
Animal	0600	0800	1000	1200	1400	1600	1800	2000
1	1.7	5.6	7.9	13.7	1.3	7.6	57.5	4.7
2	0.0	7.2	11.6	14.0	2.3	0.0	63.5	1.4
3	0.4	7.5	39.9	14.4	4.2	9.3	24.1	0.2
4	0.0	3.9	0.0	0.0	8.7	35.6	50.5	1.2
5	0.0	1.3	0.0	0.0	5.3	14.3	73.6	5.5
6	0.0	5.3	0.0	0.0	6.9	0.0	61.4	26.4
Mean ±SE	0.4 ±0.3	5.1 ±0.9	9.9 ±6.3	7.0 ±3.1	4.7 ±1.1	11.1 ±5.4	55.1 ±6.9	6.6 ±4.1

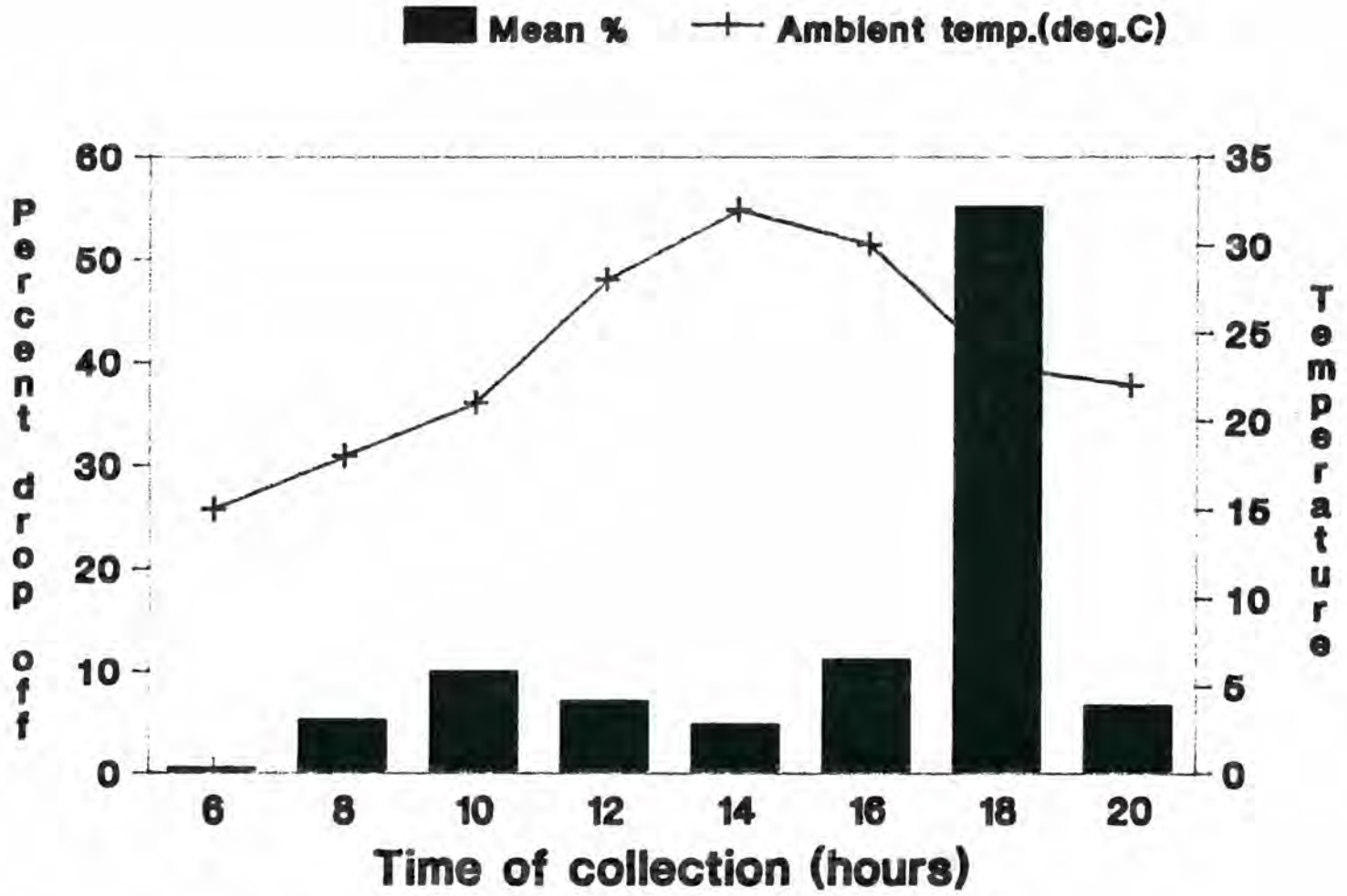

Figure 18 Distribution of drop-off by time collection of engorged R. appendiculatus nymphs from cattle.

TABLES 26

Percent distribution of engorged R.appendiculatus nymphs dropping from animals and their duration of feeding

Days post infestation	Animals 1	2	3	4	5	6	Mean±SE
5	70.8	75.1	83.9	87.2	93.5	87.5	83.0±3.5
6	26.9	23.7	16.0	12.9	6.6	12.2	16.4±3.1
7	2.3	1.2	0.4	0.0	0.0	0.0	0.7±0.4

Table 27

Mean weights (mg) of engorged nymphs of R. appendiculatus dropping-off by time of collection

	Time of collection							
Animal	0600	0800	1000	1200	1400	1600	1800	2000
1.	8.2	8.1	8.9	9.1	9.0	9.1	8.7	8.5
2.	8.2	8.3	8.5	9.3	9.2	9.0	9.1	9.1
3	8.5	8.6	9.1	9.2	8.5	8.5	8.5	8.3
4	8.6	8.7	8.7	9.0	9.0	9.0	9.0	9.3
5	9.3	8.7	8.8	8.9	8.9	9.0	9.1	8.9
6	9.1	9.2	9.3	8.5	8.6	8.6	8.7	8.5
Mean	8.6	8.6	8.8	9.0	8.8	8.8	8.8	8.7
±SE	+0.2	+0.2	+0.1	+0.1	+0.1	+0.1	+0.1	+0.2

7.1.3 Engorged larvae

Table 28 shows the distribution of engorged larvae by time of collection from the host animals. There was no definite dropping-off pattern, although the period of maximum drop-off was between 1600 to 1800 hours (Figure 19). Analysis of the data showed that collection at 1400 and 0600 hours were significantly lower than all the others (Appendix 19). A higher proportion of larvae dropped overnight (4.1%) than for nymphs or adults. Drop-off started on day 4 and continued to day 5, with maximum drop-off on day 4 (Table 29). The mean weights of larvae are shown in Table 30. The weights were found to be virtually the same regardless of the dropping time.

All unengorging ticks and all the males were ignored.

7.2. DISCUSSION

A drop-off rhythm was found in both nymphs and female R. appendiculatus but not in larvae. Minshull (1982) found a drop-off rhythm for all instars of the same tick. For females the time of maximum drop-off was between 0800 and 1000 hours, while Minshull (1982) reported maximum drop-off just after sunrise, soon after 0600 hours. She suggested that drop-off could be a response to increasing temperature or light intensity, or a combination of the two. Maximum drop-off of the females in this study however occurred between about 2 and 4 hours after sunrise, and the appearance of day light alone was not effective in causing heavy drop-off. A combination of light and increasing

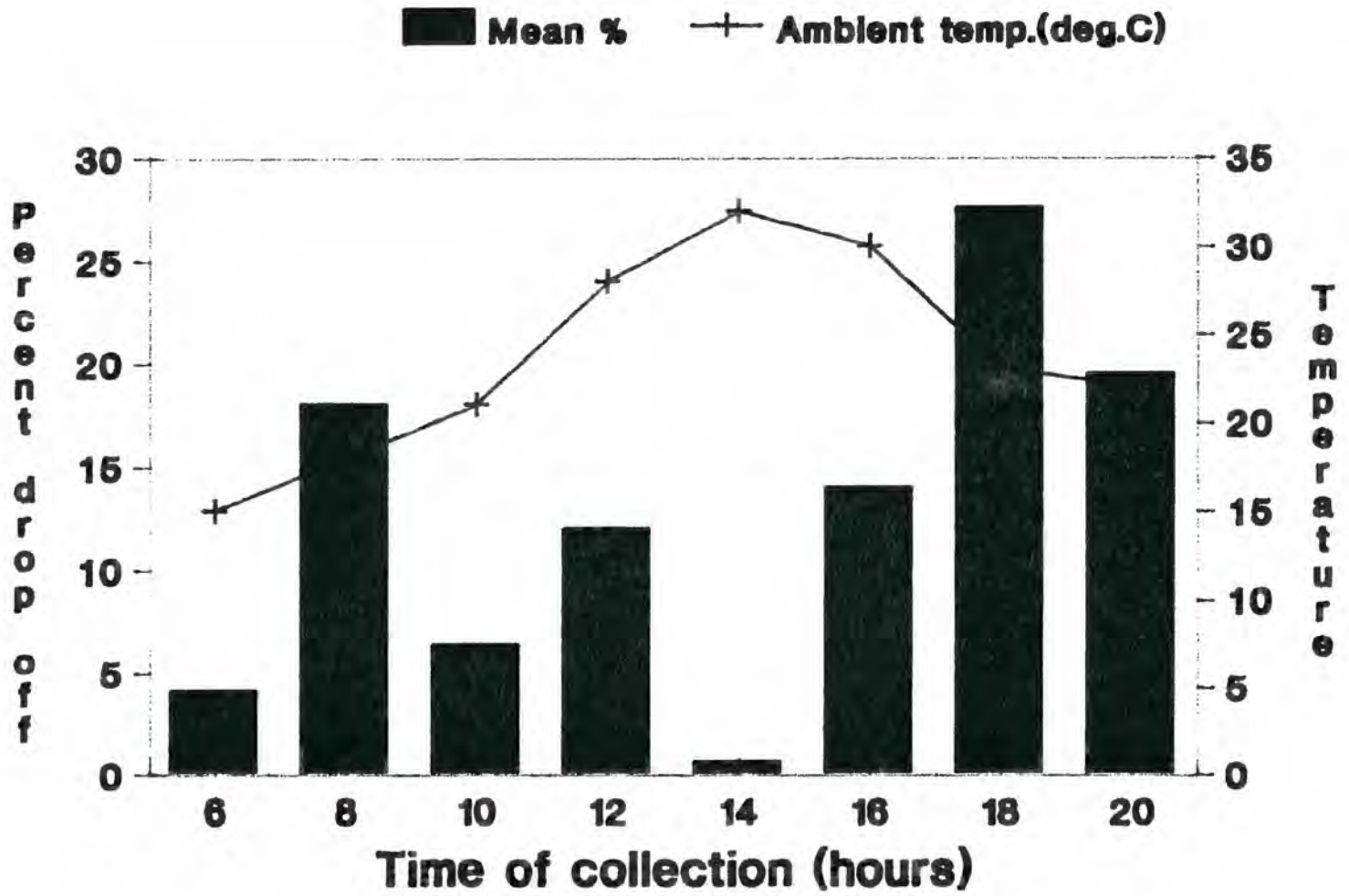

Figure 19 Distribution of drop -off by time of collection for R. appendiculatus engorged larvae from cattle.

TABLE 28

Percentage of 13,200 engorged larvae of R. appendiculatus dropping off according to time of collection

	Time of collection							
Animal	0600	0800	1000	1200	1400	1600	1800	2000
1	1.6	11.7	0.0	0.0	0.0	10.7	26.5	49.5
2	20.9	13.8	25.5	8.2	1.2	4.6	7.3	17.6
3	1.9	8.4	12.8	1.6	2.1	45.1	26.4	14.8
4	0.0	30.5	0.0	5.9	0.0	11.0	17.8	34.8
5	0.0	0.0	0.0	0.0	0.0	12.5	87.5	0.0
6	0.0	43.5	0.0	56.5	0.0	0.0	0.0	0.0
Mean	4.1	18.0	6.4	12.0	0.6	14.0	27.6	19.5
±SE	±3.4	±6.5	±4.4	±9.0	±0.4	±6.5	±12.7	±8.0

TABLE 29

Percentage of 13,200 engorged larvae of R. appendiculatus dropping by day, and their duration of feeding on six animals. They were put on the animals on day 1.

Day post-infestation	Animals 1	2	3	4	5	6	Mean±SE
4	100.0	63.6	74.6	29.3	56.7	0.0	54.0±14.3
5	0.0	0.0	22.0	67.9	13.3	100.0	33.9±16.7
6	0.0	36.4	3.4	2.8	0.0	0.0	7.1±5.9

TABLE 29

Percentage of 13,200 engorged larvae of R. appendiculatus dropping by day, and their duration of feeding on six animals. They were put on the animals on day 1.

Day post-infestation	Animals 1	2	3	4	5	6	Mean±SE
4	100.0	63.6	74.6	29.3	56.7	0.0	54.0±14.3
5	0.0	0.0	22.0	67.9	13.3	100.0	33.9±16.7
6	0.0	36.4	3.4	2.8	0.0	0.0	7.1±5.9

temperature could be important. Balashov (1954) and Kheisin and Lavrenenko (1956) independently reported that the activity of the host, and the feeding regime both influenced the drop-off rhythm of Ixodes ticks, and they were able to demonstrate this by reversing the feeding regime, which reversed the drop-off pattern. In the present study, animals started to graze at about 0800 hours, and so increased activity and movement could have contributed to the heavy drop-off.

When ticks were put on the animals with a 12-hour difference, the drop-off pattern started at the same morning hours (0800 -1000) but 24 hours later, indicating that there was a definite stimulus in the morning which caused massive drop-off. This means that the effect of activity and grazing could be important, as ticks which were put on the animals at 2000 hours waited until the morning of day 7 to start dropping. The same delay of drop-off was experienced by ticks fed on tick-sensitised animals, as they started to drop-off 24 hours later than those in the first infestation.

The duration of feeding of females was therefore affected by the presence of an immune response in the sensitised animals. Heavy infestations, the presence of oedema and other feeding lesions were found to prolong feeding Branagan (1969), Balashov (1972), Chiera (1986). The presence of the immune defence mechanism, however, did not affect the drop-off pattern of females. This confirms that the drop-off pattern is a process distinct from

attachment and feeding.

The time of maximum drop-off of nymphs reported by Minshull (1982) was 1600 to 1800 hours, which was the same time as found in this study. She, however, found a rhythm of drop-off for larvae, with maximum drop-off at 1000 to 1400 hours, while there was no definite rhythm for larvae in this study. Furthermore, time of maximum drop-off was 1600 to 1800 hours, followed by 1800 to 2000 hours. While the animals in the present study were kept in an open field all the time, the animals used by Minshull (1982) were kept in open stalls. The effect of extremes of temperatures, wind, shade, light intensity and humidity could therefore be different in the two studies, and might account for the differences.

In the present study, it appears that decreasing temperatures and light intensity may stimulate drop-off of nymphs and larvae. Minshull (1982) concluded that there were three oscillators governing drop-off pattern of the immatures. Two oscillators were in the tick and were light sensitive. One of them appeared to be set in the immature before attachment, so that pre-conditioning ticks to reversed light regime prior to attachment affected drop-off pattern of larvae, but she was not able to demonstrate the same in nymphs. Other workers have investigated this pre-attachment oscillator in other ticks but have not been able to demonstrate its presence (Hadani and Ziv, 1974; Rechav, 1978). Doube (1975) however showed the presence of this

oscillator in the argasid Ornithodoros gurneyi.

The second light-sensitive oscillator demonstrated by Minshull (1982) in the immatures was to be set after attachment, which was also demonstrated by George (1971) in Haemaphysalis leporispalustris, and by Hadani and Rechav (1969, 1970) in Hyalomma excavatum. The third oscillator, according to Minshull (1982), was set in the hosts and works irrespective of pre-conditioning of ticks to light-dark regimes, and was demonstrated under constant light and temperature. The implication from Minshull's work is that the immatures will have a rhythm of drop-off, using one or more of the three oscillators. The oscillator that is dominant depends on the conditions, and that the pre-conditioning to a light-dark regime is the strongest and overrides the other two. Assuming this theory is true, it is expected that ticks of the same species would have different patterns of dropping depending on the ratio of darkness to light, which varies seasonally, to which they are subjected prior to feeding. Kenya is a tropical country which is on the Equator, and periods of day and night are more or less equal (12 hours each) throughout the year. The results obtained here might therefore be different from those obtained in countries where days are shorter than nights or vice versa, as in temperate countries or in sub-tropical countries as in Zimbabwe where Minshull did her work. It is, however, unlikely that such differences occur because R. appendiculatus has its seasonality, and is not

active in winter when nights are longer than days. While Minshull (1982) demonstrated a rhythm in larvae drop-off, no such rhythm was found in this study, and the reasons for that are not clear.

In all three instars, the mean weight of dropping ticks was not affected by the hour of drop-off, which again shows that the dropping-off is a process which starts only after the necessary feeding is accomplished. The triggering mechanism for drop-off has no effect therefore on partially fed ticks. Factors such as the immune status have been found to affect weight of dropping ticks, and this was more pronounced in nymphs and larvae than in female ticks (Chiera 1986). In this study, females which fed on sensitised animals had slightly lower weights, which were significantly different from the weights of those from the first infestation, which shows that some immunity was already acquired by the time of second infestation, which was done four weeks later (this was the reason tick naive cattle had to be used for the first feed for comparison with the tick-sensitised ones).

The results of this study indicate that drop-off of most ticks could be allowed to happen while the cattle are in the boma by delaying them until 1000 hours, and bringing them in again at 1600 hours, causing them to drop in conditions unfavourable for their survival. The results in the present study also suggest that studies which involve counts of ticks on the animals should be done before 1000

hours, as most fully-engorged females will have dropped off later in the day. Alternatively, counts should only be based on standard ticks (those which are likely to drop within 24 hours). However, it should be noted that this study was done using a strain of R .appendiculatus which has been in the laboratory for many years, and it is possible that different results might be obtained from other field strains.

As the drop-off rhythm is also influenced by the onset of light and rise in temperature, it is possible that there are differences between drop-off patterns of ticks in different countries, and in different seasons. All the studies which have so far been done were conducted at one particular place and season, and there is therefore a need to investigate drop-off in contrasting seasons especially where sunrise takes place at different hours.

8 CHAPTER EIGHT

GENERAL DISCUSSION

All major pests are attacked by a variety of natural enemies to keep them at a much reduced level (Varley *et al.*, 1973). Natural enemies of ticks have largely been ignored when evaluating causes of mortality in the life cycle of ticks. The present study has however shown that predators play an important role in reducing numbers of engorged females and engorged nymphs of *R. appendiculatus*. It would be assumed also that there is substantial predation of eggs and engorged larvae although they were not investigated in this study.

The findings of this study are important in making a computer model for *R. appendiculatus*. A computer model is a guiding tool which could be used to predict numbers of organisms which will complete the life cycle, and can therefore be used to advise farmers about the most effective time for control of ticks. To make a model for *R. appendiculatus*, all the causes of mortality of each instar should be established, and their effects quantified in as natural situations as possible. However, the results would have to be representative of all the ecological zones in which *R. appendiculatus* is found. Kenya is divided into seven ecological zones (Pratt and Gwynne, 1977). Zone 1 is forest area, and so *R. appendicualtus* is not found there, while Zones 4-7 are arid and semi arid lands, and therefore are too dry

for the ticks. Zones 2 and 3, and a few isolated parts of Zone 4 are the natural areas where R. appendiculatus thrives. The Kabete area where the study was done is in Zone 2. In the short grass higher temperatures were recorded and this could therefore represent the conditions in Zone 3, in terms of grass cover and grass fauna found there. These results would therefore be useful for a computer model.

Even with the level of predation reported in this work, cattle continue to carry many ticks. This is because each female that survives is able to lay 3000 to 5700 eggs (Hoogstraal, 1956). Although other studies done on predation of ticks were done for shorter periods, higher predation rates were recorded (Wilkinson, 1970a; Butler et al., 1979).

Although there has not been much success in controlling ticks with parasitoids, there are a few succes stories using parasitoids against important pests (Hussey & Bravenboer, 1971; Parker, 1971; Varley et al., 1973).

The results obtained from the parasitoid work in this study are however only for the Trans-Mara area, and cannot be generalised. There could be parasitoids in other areas in Kenya, and a full study involving other species of ticks and other areas should be done to give a full picture of the parasitoids in Kenya. It is possible that parasitoids will in

future be found in Rusinga Island because there is now more cattle movement into the Island following the building of a causeway joining it with the mainland.

The use of chemical acaricides is largely the method used for tick control in Kenya. It is estimated that Kenya is currently using $ US 6-10 million annually to buy acaricides, and a further $ US 3-5 million for associated recurrent infrastructural costs. There is therefore a great need to look for other methods which can be incorporated in an integrated control package which should be chemical free. Of the seven types of tick predators identified in this work, the domestic chicken should obviously be more heavily utilised as a tick control agent, by keeping chickens routinely at cattle holding grounds, pens or bomas, where they will forage during the day. The results obtained from drop-off rhythm experiments in this study showed that over 50% of engorged females and engorged nymphs could be eaten by chickens, if cattle were delayed and probably fed in the boma until 1000 hours before going to the pasture, and were then brought in again before 1800 hours. The cattle would have to have additional food in the boma to make up for lost grazing time. In any case, ticks which drop in the boma stand a higher chance of mortality due to being scorched by heat in the hot season, and lack of good vegetation cover for any eggs which would be laid. It must, however, be pointed out that domestic chickens should not be used to eat ticks if the cattle are being sprayed with acaricides for tick control. Acaricidal

residues would accumulate in the chickens, and be passed on to humans via meat and eggs.

For the parasitic stages of ticks, oxpeckers were found to be important predators (Moreau, 1933; Van Someren, 1951; Olivier and Laurie, 1974; Stutterheim, 1976). However, they would only be useful if there was an acaricide-free control regime, as their numbers are reduced by acaricide poisoning.

In all the trials that have been done to control ticks by the use of the parasitoids, there was initial success, but the parasitoids were eventually insufficient to control the ticks (Larrouse et al, 1928; Cooley, 1928; Bishopp, 1934; Alveef, 1940; Smith and Cole 1943). This suggests that for parasitoids to be effective they would have to be boosted from culture frequently at carefully chosen times. It is therefore necessary to develop a good method for mass rearing of the parasitoids found in this study, if control in field is to be tried in future. The method used for infesting A. variegatum in the laboratory is only a first step which could be improved on in future if large numbers of parasitoids were needed. Rearing A. variegatum in the laboratory takes a lot of time, and so investigations should be done on rearing the ticks on other blood based media. However for Kenya, more study is required to locate possible areas where the parasitoid from Trans-Mara could be introduced. An isolated locality, such as an island, if found to favour the parasitoid, would be ideal.

However, parasitoids can only be used as a component of an integrated control package, rather than an absolute method of control on their own. Furthermore, no parasitoids of one-host ticks have been found, and so control of such ticks as B. decoloratus would require other methods.

The pathogens found in this study are not specific for ticks, and so their uncontrolled use might affect domestic animals and even human beings. Toxins of pathogens such as B. bassiana and B. thuringiensis have been extracted and are being used for other arthropods (Calberg, 1986; Ferron 1986). Studies should be done to try these toxins on ticks. It is possible that toxins of B. bassiana would be effective on R. appendiculatus as the live fungus was found effective in this study. If so, then they could be sprayed on cattle like other acaricides.

REFERENCES

Alfeef, N.I. (1940). The utilization of Hunterellus hookeri for the control of the tick Ixodes ricinus with reference to peculiarity of their metamorphosis under conditions of Leningrad. In: Pavlosky second conference on the parasitological problems, November 1940 Izvestiya Akademii Nauk SSSR, pp. 23-25. (In Russian).

Anderson, T.E., Roberts, D.W. and Soper, R.S. (1988). Use of Beauveria bassiana for suppression of Colorado potato beetle populations in New York State (Coleoptera: Chrysomelidae) Environmental Entomology 17, 140-145.

Anon, (1962). The water-tortoise Pelomedusa subrufa Lammergeyer 2, 69

Attwell, R.I.G. (1966). Ox-peckers and their association in Zambia.
Puku 4, 17-48.

Bailey, K.P. (1960). Notes on the rearing of Rhipicephalus appendiculatus and their infection with Theileria parva for experimental transmission. Bulletin of Epizootic Diseases of Africa 8, 34-43.

Balashov, Yu. S. (1954). Peculiarities of the daily rhythm of dropping of engorged female Ixodes persulcatus from cattle. Doklady Akademii SSSR 98, 317-319. (In Russian).

Balashov, Y.S.(1972). Bloodsucking ticks (Ixodidea)- Vectors of diseases of man and animals. Miscellaneous Publications of the Entomological Society of America. 8, 161-376. Translated from Russian.

Bishopp, F.C. (1934). Records of hymenopterous parasites of ticks in the United States. Proceedings of the Society of Entomology (Washington) 36, 87-88.

Bowman, J.L., Logan, T.M. and Hair J.A. (1986). Host suitability of Ixodiphagus texanus Howard on five species of hard ticks. Journal of Agricultural Entomology 3, 1-9.

Branagan, D.(1969). The maintenance of Theileria parva infections by means of the Ixodid tick Rhipicephalus appendiculatus. Tropical Animal Health and Production 1, 119-130

Branagan, D. (1973). Observations on the development and survival of the Ixodid tick Rhipicephalus appendiculatus Neumann, 1901, under quasi-natural conditions in Kenya. Tropical Animal Health and Production 5, 153-165.

Branagan, D. (1974). The feeding performance of the ixodid Rhipicephalus appendiculatus Neumann on rabbits, cattle and other hosts. Bulletin of Entomological Research 64, 387-400.

Brooke, R.S. (1963). Birds around Salisbury, then and now. South African Avifauna Series of the Percy Fitzpatric Institute of African Ornithology 9, 1-67.

Brown, R.S. (1970). An endemic disease among populations of Dermacentor andersoni Journal of Invertebrate Pathology 16, 142-143.

Burgdorfer, W. (1973). Isolation an characterization of symbiotes from the Rocky Mountain wood tick, Dermacentor andersoni Journal of Invertebrate Pathology 22, 424-434.

Burns, E.C. and Melachon, D. G. (1977). Effect of imported fire-ant invation on lone star tick populations. Journal of Invertebrate Pathology 14, 247-249.

Butler, J.F., Camina, M.L. and Perez, T.O. (1979). In: Recent advances in Acarology, Vol. 1, (Rodriguez, J.G. ed), pp. 469-472. New York Academic Press.

Calberg, G. (1986). Bacillus thuringiensis and microbial control of flies. MIRCEN Journal 2, 267-274.

Chiera, J.W. (1986). Adaptation of field strains of Rhipicephalus appendiculatus Neumann, to host resistance to tick infestation. PhD thesis, Nairobi University.

Clifford, M.C., Hoogstraal, H. , Radovsky, F.J. , Stiller, D. and Keirans, J.E. (1980). Ornithodoros amblus. Identity, marine bird and human hosts, virus infections and distribution in Peru. Journal of Parasitology 68, p.1052.

Cobb, S. (1942). Tick parasites of Cape Cod. Science 67, 351-353.

Cole, M.M. (1965). Biological control of ticks by the use of hymenopterous parasites. A review. WHO/EBL/43.65, pp. 1-12.

Cooley, R.A. (1928). Preliminary report on the tick parasite, Ixodiphagus caucurtei. Seventh Biennial Report, Montana State Board of Entomology. Pp. 17-31.

Cooley, R.A. (1930). Review of tick parasite work for 1929 and 1930, Eighth Biennial Report, Montana State Board of Entomology, pp. 16-25.

Cooley, R.A. and Kohls, G.M. (1928). Egg-laying of Ixodiphagus caucurtei in larval ticks. Science 67, p. 556.

Cooley, R.A. and Kohls, G.M. (1934). A summary of tick parasites. Proceedings of the Fifth Pacific Scientific Congress 35, 3375-3381

Cunningham, M.P. (1981). Biological control of ticks with particular reference to Rhipicephalus appendiculatus. In: Advances in the control of Theileriosis (Irvin, A.D., Cunningham, M.P. and Young, A.S. eds.), pp. 160-164. Nijhoff, The Hague.

Darling, P. unpublished report.

Davey, R.B., Osburn, R.I. and Castillo, C. (1983). Longevity and mating behaviour in males and parthenogenesis in females in hybridized Boophilus microplus ticks (Acari: Ixodidae).
Journal of Medical Entomology 20, 614-617.

Diego, J.R. , Villalba, G. , Abrey, R. and Castaneiras, A. (1983). Pheidole megacephala como depredador de Amblyomma cajennense en Cuba.
Revista de. Salud Animal 5, 437-440

Doube, B.M. (1975). Regulation of the circadian rhythm of detachment of engorged larvae and nymphs of the argasid kangaroo tick, Ornithodoros gurneyi.
Journal of Medical Entomology 12, 15-22.

Ferron, P. (1986). Pest control by the fungi Beauveria and Metarhizium. In: Microbial control of Pests and Plant diseases (Burges H.D. ed.), 1970-1980. Academic Press, London.

Fiedler, O.G.H. (1953). A new African tick parasite, Hunterellus theilerae. Ondersteepoort Journal of Veterinary Research 26, 61-63.

Geevarghese, G. (1977). A new species of Chalcid parasitizing Haemaphysalis bispinosa from Karnataka, India. Oriental Insects 11, 49-52.

George, J.E. (1971). Drop-off rhythms of engorged rabbit ticks, Haemaphysalis leporispalustris (Acari:Ixodidae). Journal Medical Entomology 8, 461-479.

Gillespie, A.T., Collins, M.D. and Atienza, A. (1986). Control of Nilaparvata lugens with entomogenous fungi.In Fundamental and Applied Aspects of Invertebrate Pathology (R.A. Samson, J.M. Vlak and D.Peters eds.) p. 244. Wageningen, The Netherlands.

Gladney, W.J., Ernst, S.E. and Grabbe, R.R. (1974). The aggregation response of the Gulf Coast ticks on cattle.
Annals of the Entomological Society of America 67, 750-752.

Goldberg, L.J. and Margalit, J. (1977).A bacterial spore demonstrating rapid larvicidal activity against Anopheles sergentii, Urantaenia unguiculata, Culex univittatus, Aedes aegypti and Culex pipiens.
Mosquito News 37, 355-358.

Graf, J.F. (1979). The biology of an encyrtid wasp parasitizing ticks in the Ivory Coast.
Recent Advances in Acarology 1, 463-468.

Graham, O.H., Price, H.A. and Trevino, J.L. (1972). Cross mating experiments with Boophilus annulatus and Boophilus microplus (Acarina:Ixodidae).
Journal of Medical Entomology 9, 531-537.

Grobler, J.H. (1980). Host selection and species preferences of the red-billed oxpecker, Buphagus erythrorhynchus in Kruger National Park.
Koedoe 23, 89-97.

Grobler, J.H. and Charsley G.W. (1978). Host preferences of the yellow-billed ox-pecker, Buphagus africanus in the Rhodes Matopos Natioanal Park, Rhodesia. South Africa Journal of Wildlife Research 8, 167-170.

Hadani, A. and Rechav, Y. (1969).Tick-host relationships I. The existence of a circadian rhythm of drop-off of engorged ticks from their hosts. Acta Tropica 26, 173-179.

Hadani, A. and Rechav, Y. (1970). Tick-host relationships II. Factors affecting the circadian rhythm of drop-off of engorged preimaginal stages of the tick Hyalomma excavatum from the gerbil, Meriones tristrami. Acta Tropica 27, 184-190.

Hadani, A. and Ziv, M. (1974). Tick-host relationships III. The effect of photoperiodic pre-condition on the circadian rhythm of drop-off of engorged pre-imaginal stages of the tick Hyalomma excavatum from the gerbil Meriones tristrami. Acta Tropica 31, 89-94.

Haile, D.G. and Mount, G.A. (1987). Computer simulation of population dynamics of the Lone Star tick Amblyomma ammericanum.
Journal of Medical Entomology 24, 356-369.

Hair, J.A. and Howell, D.F. (1970). Lone star ticks. Their biology and control in Ozark recreation areas.
Oklahoma Agricultural Experimental Bulletin 13, 47.

Harris, W.G. and Burns, E.C. (1977). Predation of the lone star tick by the imported fire ant.
Environmental Entomology 1, 362-365.

Hendry, D.A. and Rechav, Y. (1981). Acaricidal bacteria infecting a laboratory colony of the tick Boophilus decoloratus.
Journal of Invertebrate Pathology 38, 149-151.

Hewetson, R.W. (1972). Resistance of cattle to cattle tick Boophilus microplus. III. The development of resistance to experimental infestations by pure bred Sahiwal and Australian Illawara shorthorn cattle.
Australian Journal of Agricultural Research 122, 331-342.

Hewetson, R.W. (1979). Results of selection for cattle tick resistance in cattle. Proceedings of the 56th Annual Conference of Australian Veterinary Association, pp. 91-93.

Hoogstraal, H. (1956). Ticks of the Sudan. African Ixodoidea, Vol 1. United States Navy, Washington DC. pp.600-615.

Howard, L.O. (1907). A chalcidoid parasite of a tick. Entomological News 18, 375-378.

Howard, L.O. (1908). Another chalcidoid parasite of a tick. Canadian Entomology 40, 239-241.

Hunter, W.D. and Bishopp, F.C. (1911). The Rocky mountain spotted fever tick. USDA Entomological Bulletin No. 105.

Hussey, N.W. and Bravenboer, F.G. (1971). Control of pests in glass house culture by the introduction of natural enemies. In: Biological Control (Huffaker, C.B. ed.), pp. 195-216. Plenum Press, New York.

ICIPE Annual Report (1983). Eleventh Annual Report, p.115. The International Centre of Insect Physiology and Ecology. Nairobi.

ICIPE Annual Report (1984). Twelfth Annual Report, p.98.The International Centre of Insect Physiology and ecology. Nairobi.

Irvin, A.D., Chumo, R.S.C., Dobbelaere, D.A.E., Goddeeris, B., Katende, J., Mimani, T., Ocama, J.G.R. and Spooner, P.R. (1981). Preliminary studies of East Coast Fever in the Coast Province of Kenya. In: Advances in the Control of Theileriosis,(Irvin, A.D., Cunningham, M.P. and Young, A.S. eds.). pp. 75-79. Nijhoff, The Hague.

Johnston, L.A.Y., Kemp, D.H. and Pierson, R.D. (1986). Immunization of cattle against Boophilus microplus using extracts derived from adult female ticks : effects of induced immunity on tick populations. International Journal for Parasitology 16, 27-34.

Jones-Davies, W.J. (1972). Tick control and a history of tick resistance. Rhodesian Veterinary Journal 2, 53-59.

Kaaya, G.P. (1988). Glossina morsitans morsitans mortalities caused in adults by experimental infection with entomopathogenic fungi. Acta Tropica 46, 107-114.

Kaaya, G. P. and.Dirji, N. (1989). Mortality in adult tsetse, Glossina morsitans, caused by Entomophagus bacteria. Journal of Invertebrate Pathology, 54, 32-38.

Keating, M.I. (1983). Tick control by chemical acaricides in Kenya. A review, 1912-1981. Tropical Animal Health and Production 15, 1-6.

Kheisin, E.M. and Lavrenenko, L.E. (1956). Duration of blood-sucking and diurnal rhythm of nutrition and dropping of female Ixodes ricinus . Zoologiskie Zhurnal 35, 379-383. (In Russian).

Kitaoka, S. (1962). Physiological and ecological studies on some ticks. VIII Diurnal and nocturnal changes in feeding activity during the blood-sucking process of Haemaphysalis bispinosa. National Institute of Animal Health Quarterly 2, 106-111.

Knipling, E.F., Laven, H., Graig, G.B., Pal, R., Kilzmiller, J.B., Smith, C.N. and Brown, A.W.A. (1968). Genetic control of insects of public health importance.
Bulletin of World Health Organization 38, 421-438.

Krylova, V.N. (1977). Action of toxins of entomopathogenic fungi on blood sucking arthropods. In: Boilogicheskie Metody Bor'by Krovososuchchini Nasekomymi : Kleschami,(Romasheva, L.F. ed.), pp.74-77.

Larrouse, F., King, A.G. and Wolbash, S.B. (1928). The overwintering in Massachusetts of Ixodiphagus caucurtei.
Science 67, 351-353.

Lavreniuk, N.M. and Uzdenov, U.B. (1977). Action of a thermostable exotoxin of the Bacillus thuringiensis group, isolated from strains from blood sucking arthropods on Dermanyssus gallinae. In: Biologicheskie Metody Bor'by Krovososushchini Nasekomymi : Kleschchami, pp. 32-34 (Romasheva, L.F. ed.) (In Russian).

Legg, J. (1930). Some observations on the cattle tick Boophilus australis. Proceedings of the Royal Society of Queensland 41, 121-132.

Lewis, E.A. (1950) Conditions affecting the East Coast Fever parasite in ticks and cattle. East African Agricultural Journal 16, 65-77.

Lipa, J.J. (1971). Microbial control of mites and ticks. In: Microbial Control of insects and mites. (Burges, H.D. and Hussey, eds), pp.357-373. Academic Press, New York.

Mather, T. N., Piesman, J. and Spielman, A. (1987). Absence of spirochaetes (Borrelia burgdorferi) and piroplasms (Babesia microti) in deer ticks (Ixodes dammini) parasitized by chalcid wasps (Hunterellus hookeri). Medical and Veterinary Entomology 1, 3-8.

Maywald, G.F. (1987). Modelling tick populations. 2. Developmental phase. In: Ticks and tick borne diseases. ACGIAR Proceedings 17, 39-40.

McKilligan, N.G. (1984). The food and feeding ecology of the cattle egret Ardeola ibis when nesting in South Eastern Queensland. Australia Wildlife Research 11, 133-134.

Megaw, M.W.J. (1978). Virus-like particles pathogenic to salivary glands of the tick Boophilus microplus. Nature (London) 271, 483-484.

Milne, A. (1950). The ecology of the sheep tick Ixodes ricinus. Microhabitat ecology of the adult tick. Parasitology 40, 14-34.

Minshull, J.I. (1982). Drop-off rhythms of engorged Rhipicephalus appendiculatus (Acarina:Ixodidae). Journal of Parasitology 68, 484-489.

Mongi, A.O. (1980). Immunization of rabbits with antigens from the tick Rhipicephalus appendiculatus (Neumann) 1901. Ph.D Thesis, University of Nairobi.

Moreau, R.E. (1933). The food of the red-billed ox-pecker, Buphagus erythrorhynchus.
Bulletin of Entomological Research 24, 324-335.

Murphy, R.C. (1925). Bird Islands of Peru: the record of a sojourn on the West Coast,p.362. Putnam, New York and London.

Norton, G.A., Sutherst, R.W. and Maywald, G.F. (1983). A framework for integrating control methods against the cattle tick Boophilus microplus in Australia.
Journal of Applied Ecology 20, 489-502.

Norval, R.A.I. (1976). Lizards as opportunist tick predators
Rhodesia Veterinary Journal 7, 63.

Norval, R.A.I. and McCosker, P.J. (1983). Tick predation by a rainbow skink.
Zimbambwe Veterinary Journal 13, 53.

Olivier, R.C.D. and Laurie, W.C. (1974). Birds associating with elephants and hippopotamuses.
Auk 80, 196-197.

Page, N. and Oately, T.B. (1979). Indian mynas feeding on ticks.
Lammergeyer 27, 50.

Parker, F.D. (1971). Management of pest populations by manipulating densities of both hosts and parasites through periodic releases. In: Biological Control,(Huffaker, C.B. ed.), pp. 365-376. Plenum Press, New York.

Philip, C.B. (1931). Occurrence of a colony of the tick parasite Hunterellus hookeri in West Africa. Public Health Report (Washington) 46, 2168-2172.

Philip, C.B. (1954). A new locality record in Africa for the tick parasite Hunterellus hookeri. Journal of Parasitology 40, 234.

Poinar, G.O. Jr., van der Geest, L., Helle, W. and Wassink, H. (1977). Experiments with organisms from hosts other than Glossina. In: Tsetse: The future for Biological Methods in Integrated Control (Laird, M. ed.),pp. 88-92. IDRC, Ottawa.

Pratt, D. J. and Gwynne M.D. (1977). Rangeland Management and Ecology in East Africa, p.130. Hodder and Stoughton, London.

Rechav, Y. (1978). Drop-off rhythm of engorged larvae and nymphs of the bont tick, Amblyomma hebraeum (Acari:Ixodidae) and the factors that regulate them.
Journal of Medical Entomology 14, 667-687.

Rechav, Y., Parolis, H., Whitehead, G.B., and Knight, H.M. (1977). Evidence for an assembley pheromone(s) produced by males of the bont tick Amblyomma hebraeum (Acarina:Ixodidae).
Journal of Medical Entomology 14, 395-400.

Rechav, Y. and Whitehead, G.B. (1978). Field trials with pheromone-acaricide mixtures for control of Amblyomma hebraeum (Acarina:Ixodidae).
Journal Of Economic Entomology 71, 150-151.

Rechav, Y., and Whitehead, G.B. (1981). Can Amblyomma pheromones be used for control. In: Tick Biology and Control (Whitehead, G.B.and Gibson, J.D. eds.) pp.209-210. Proceedings of an International Conference held from 27-29 January 1981, Rhodes University, Grahamstown, South Africa.

Rehacek, J. (1965). Development of animal viruses and rickettsiae in ticks and mites.
Annual Review of Entomology 10, 1-24.

Riek, R.F. (1962). Studies on the reactions of animals to infection with ticks. VI. Resistance of cattle to infestation with the tick Boophilus microplus. Australian Journal of Agricultural Research 13, 532-551.

Roberts, J.A. (1968). Resistance of cattle to the tick Boophilus microplus (Canestrini). II. Stages of the life-cycle of the parasite against which resistance is manifest. Journal of Parasitology 54, 667-673.

Roberts, D.W. and Wright, S.P. (1986). Current status on the use of insect pathogens as biological agents in agriculture: Fungi In: Fundamental and Applied Aspects of Invertebrate Pathology,(R.A. samson, J.M. Vlak and D. Peters eds.), p.241. Wageningen,The Netherlands.

Rothschild, M. and Clay, J. (1952). Fleas, flukes and cuckoos, p.302. Collins, London.

Samsinakova, A. (1974). Entomogenous fungi associated with the tick Ixodes ricinus. Folia Parasitica (Praha) 21, 38-48.

Seifert, G.W. (1971). Variations betweeen and within breeds of cattle in resistance to field infestations of the cattle tick Boophilus microplus. Australian Journal of Agricultural Research 22, 159-168.

Seifert, G.W. (1984). Selection of beef cattle in Northern Australia for resistance to the cattle tick Boophilus microplus: research and application. Preventive Veterinary Medicine 2, 553-558.

Short, N.J. and Norval, R.A.I. (1982). Tick predation by shrews in Zimbambwe. Journal of Parasitology 68, 1052.

Sidorov, V.E. and Shcherborov, S.V. (1973). Mass epizootics among Alveonasus lahorensis Neumann ticks. Meditsinskaya Parazitologiya Parazitarnye Bolezni 42, 47-51.

Smith, C.N. and Cole, M.M. (1943). Studies of parasites of the American dog tick. Journal of Economic Entomology 36, 569-572.

Sonenshine, D.E., Silverstein, R.M. and Homsher, P.J. (1979). Female produced pheromones of Ixodidae. In: Recent Advances in Acarology, Vol.2.(Rodriquez, J.G. ed.),pp.281-290. Academic Press, New York.

Sonenshine, D.E. Silverstein, R.M. and Rechav, Y. (1982). Tick pheromone mechanisms. In: Physiology of ticks.(Obenchain, F.D., Galun, R.eds.).pp.439-468. Pergamon Press, Oxford.

Steinhaus, E.A. (1959). Serratia marcescens as an insect pathogen. Hilgardia 28, 351-380.

Steinhaus, E.A. and Marsh, G.A. (1962). Report of diagnosis of diseased insects, 1951-1961. Hilgardia 33, 349-390.

Stelfox, D. (1968). A note on magpies and Rocky Mountain sheep. Canadian Field Naturalist 82, 234.

Storey, G.K. and Gardner, W.A. (1987). Vertical movement of commercially formulated Beauveria bassiana conidia through four Georgia soil types. Environmental Entomology 16, 178-181.

Storey, G.K. and Gardner, W.A. (1988). Movement of an aqueous spray of Beauveria bassiana into the profile of four Georgia soils. Environmental Entomology 17, 135-139.

Stutterheim, C.J. (1976). The biology of the red-billed oxpecker, Buphagus erythrorhynchus in the Kruger National Park. MSc. Thesis, University of Pretoria.

Stutterheim, C.J.and Brooke, R.K.(1981). Past and present ecological distribution of the yellowbilled oxpecker in South Africa. South African Journal of Zoology 16, 44-49..

Stutterheim, C.J. and Stutterheim, I.M. (1980). Evidence of an increase in red-billed oxpecker population in the Kruger National park. South African Tydskr Dierk 15, 284.

Sutherst, R.W. and Dallwitz, M.J. (1979). Progress in the development of a population model for the cattle tick Boophilus microplus. Proceedings of the Fourth Congress of Acarology, Austria.p. 87.

Sutherst, R.W., Jones, R.J. and Schnitzerling, H.J. (1982). Tropical legumes of the genus Stylosanthes immobilize and kill cattle ticks. Nature (London) 295, 320-321.

Sutherst, R.W., Norton, G.A., Barlow, N.D.,Conway, G.R.,Birley, M. and Cummins, H.N. (1979). An analysis of management strategies for the cattle tick Boophilus microplus in Australia. Journal of Applied Ecology 16, 359-382.

Sutherst, R.W., Wharton, R.H. and Utech, K.B.W. (1978). Guide to studies on tick ecology. CSIRO, Australia, Division of Entomology, Technical paper No. 14.

Tachikawa, T. (1980). Discovery of a tick parasite, Hunterellus sagarensis Geevarghese from Japan, (Hymenoptera: Chalcidoidea-Encyrtidae). Transactions of Shikoku Entomological Society 15, 19-20.

Thomson, G.D., Osburn, R.L., Davey, R.B. and Price, M.A. (1981). The dynamics of hybrid sterility between Boophilus annulatus and Boophilus microplus through successive generations. Journal of Medical Entomology 18, 413-418.

Thomson, K.C. Roa, E.J. and Romero, N.T. (1978). Anti-tick grasses as the basis for developing practical tropical tick control packages. Tropical Animal Health and Production 10, 179-182.

Tukahirwa, E.M. (1976). The feeding behaviour of larvae, nymphs and adults of Rhipicephalus appendiculatus. Parasitology 72, 65-74.

Ushakova, G.V. (1962). On the findings of Hunterellus hookeri , parasite of ixodid ticks. Kazakhstan, Tr. Kazan Inst. Sool. Akad Nauk. SSR 16, 183-184 (In Russian).

Utech, K.B.W., Wharton, R.H. and Kerr, J.D. (1978). Resistance to Boophilus microplus in different breeds of cattle. Australian Journal of Agricultural Research 29, 885-895.

Van Someren, V.D. (1951). The red-billed ox-pecker and its relations to stock in kenya. The East African Agricultural Journal 17, 1-11.

Varley, G.C., Gradwell, G.R., Hassel, M.P. (1973). Insect population Ecology, an analytical approach. Blackwell Scientific Publications, p. 155.

Walker, J.B. (1974). The Ixodid ticks of Kenya. Commonwealth Institute of Entomology, London.

Weyer, F. (1973). Versuche zur Ubertragung von Wolbachia persica auf keiderlause. Zeitschrift fur Angewwandte Zoologie 60, 77-93 .

Wharton, R.H. (1979). Cattle tick in Australia. Recent research and prospects for control an eradication. Proceedings of the 56th Annual Conference of Australian Veterinary Association pp. 30-35.

Wharton, R.H. and Roulston, W.J. (1970). Resistance of ticks to chemicals. Annual Review of Entomology 15, 381-404.

Wharton, R.H., Utech, K.W.B. and Turner, H.G. (1970). Resistance to the cattle tick, Boophilus microplus in a herd of Australian Illawarra shorthorn cattle: Its assessment and heritability. Australian Journal of Agricultural Research 21, 163-181.

Thomson, K.C. Roa, E.J. and Romero, N.T. (1978). Anti-tick grasses as the basis for developing practical tropical tick control packages. Tropical Animal Health and Production 10, 179-182.

Tukahirwa, E.M. (1976). The feeding behaviour of larvae, nymphs and adults of Rhipicephalus appendiculatus. Parasitology 72, 65-74.

Ushakova, G.V. (1962). On the findings of Hunterellus hookeri , parasite of ixodid ticks. Kazakhstan, Tr. Kazan Inst. Sool. Akad Nauk. SSR 16, 183-184 (In Russian).

Utech, K.B.W., Wharton, R.H. and Kerr, J.D. (1978). Resistance to Boophilus microplus in different breeds of cattle. Australian Journal of Agricultural Research 29, 885-895.

Van Someren, V.D. (1951). The red-billed ox-pecker and its relations to stock in kenya. The East African Agricultural Journal 17, 1-11.

Varley, G.C., Gradwell, G.R., Hassel, M.P. (1973). Insect population Ecology, an analytical approach. Blackwell Scientific Publications, p. 155.

Walker, J.B. (1974). The Ixodid ticks of Kenya. Commonwealth Institute of Entomology, London.

Weyer, F. (1973). Versuche zur Ubertragung von Wolbachia persica auf keiderlause. Zeitschrift fur Angewwandte Zoologie 60, 77-93 .

Wharton, R.H. (1979). Cattle tick in Australia. Recent research and prospects for control an eradication. Proceedings of the 56th Annual Conference of Australian Veterinary Association pp. 30-35.

Wharton, R.H. and Roulston, W.J. (1970). Resistance of ticks to chemicals. Annual Review of Entomology 15, 381-404.

Wharton, R.H., Utech, K.W.B. and Turner, H.G. (1970). Resistance to the cattle tick, Boophilus microplus in a herd of Australian Illawarra shorthorn cattle: Its assessment and heritability. Australian Journal of Agricultural Research 21, 163-181.

Zimmerman, R.H., Garris, G.I. and Beaver, J. (1984). Potential of *Stylosanthes* plants as a component in an in integrated pest management approach to tick control. *Preventive Veterinary Medicine* **2**, 579-588.

APPENDIX 1

Percent mortality of engorged *R. appendiculatus* females tethered in long grass (40-60 cm) for 8 days

Month	N	Mortality: Predation	Mortality: Other causes
Nov. 87	21	52.4	0.0
Dec. 87	21	42.8	0.0
Jan. 88	21	33.3	4.8
Feb. 88	21	28.6	14.3
Mar. 88	21	47.6	19.0
Apr. 88	21	52.4	0.0
May 88	21	33.3	0.0
Jun. 88	21	66.7	4.8
Jul. 88	21	66.7	0.0
Aug. 88	21	28.6	0.0
Sep. 88	21	28.6	0.0
Oct. 88	21	47.6	4.8
Nov. 88	21	42.8	0.0
Dec. 88	21	33.3	19.0
Jan. 89	21	52.4	4.8
Feb. 89	21	42.8	14.3
Mar. 89	21	28.6	0.0
Apr. 89	21	38.1	0.0
Mean± SE	21	42.5±2.9	4.7±1.6

APPENDIX 2

Percent mortality of engorged *R. appendiculatus* females put out in metal cages in long grass (40-60 cm.) for 8 days

		Mortality	
Month	N	Predation	Other causes
Nov. 87	21	0.0	0.0
Dec. 87	21	9.5	0.0
Jan. 88	21	9.5	0.0
Feb. 88	21	0.0	4.8
Mar. 88	21	28.6	23.8
Apr. 88	21	0.0	4.8
May 88	21	19.0	0.0
Jun. 88	21	4.8	0.0
Jul. 88	21	4.8	0.0
Aug. 88	21	0.0	0.0
Sep. 88	21	0.0	23.8
Oct. 88	21	0.0	0.0
Nov. 88	21	14.2	4.8
Dec. 88	21	14.2	0.0
Jan. 89	21	0.0	0.0
Feb. 89	21	0.0	33.3
Mar. 89	21	14.2	0.0
Apr. 89	21	4.8	0.0
Mean±SE	21	6.9±2.0	5.3± 2.4

APPENDIX 3

Percent mortality of engorged R. appendiculatus females put out in nylon bags plus metal cages in long grass (40-60 cm) for 8 days

		Mortality	
Month	N	Predation	Other causes
Nov. 87	21	0.0	4.8
Dec. 87	21	4.8*	4.8
Jan. 88	21	0.0	4.8
Feb. 88	21	0.0	23.8
Mar. 88	21	0.0	9.5
Apr. 88	21	0.0	0.0
May 88	21	0.0	0.0
Jun. 88	21	0.0	0.0
Jul. 88	21	0.0	0.0
Aug. 88	21	0.0	0.0
Sep. 88	21	0.0	14.3
Oct. 88	21	0.0	0.0
Nov. 88	21	0.0	4.8
Dec. 88	21	0.0	0.0
Jan. 89	21	0.0	0.0
Feb. 89	21	0.0	0.0
Mar. 89	21	0.0	0.0
Apr. 89	21	0.0	0.0
Mean±SE	21	0.3±0.3	3.7±1.5

* Nylon bags not placed in metal cages

APPENDIX 4

Percent mortality of engorged R. appendiculatus females tethered in short grass (6 - 10 cm) for 8 days

		Mortality	
Month	N	Predation	Other causes
Nov. 87	21	38.1	0.0
Dec. 87	21	61.9	0.0
Jan. 88	21	33.3	0.0
Feb. 88	21	23.8	28.6
Mar. 88	21	33.3	28.6
Apr. 88	21	38.1	0.0
May 88	21	57.1	0.0
Jun. 88	21	23.8	0.0
Jul. 88	21	42.8	4.8
Aug. 88	21	33.3	0.0
Sep. 88	21	42.8	19.0
Oct. 88	21	38.1	4.8
Nov. 88	21	33.3	9.5
Dec. 88	21	28.5	0.0
Jan. 89	21	14.3	4.8
Feb. 89	21	47.1	0.0
Mar. 88	21	28.6	4.8
Apr. 88	21	42.8	4.8
Mean±SE	21	36.7±2.7	6.1±2.2

APPENDIX 5

Percent mortality of engorged R. appendiculatus females put out in metal cages in short grass (6-10 cm) for 8 days

		Mortality	
Month	N	Predation	Other causes
Nov. 87	21	0.0	4.8
Dec. 87	21	0.0	0.0
Jan. 88	21	9.5	0.0
Feb. 88	21	28.6	4.8
Mar. 88	21	28.6	4.8
Apr. 88	21	0.0	4.8
May 88	21	28.6	0.0
Jun. 88	21	4.8	0.0
Jul. 88	21	4.8	4.8
Aug. 88	21	0.0	0.0
Sep. 88	21	14.3	4.8
Oct. 88	21	0.0	0.0
Nov. 88	21	0.0	0.0
Dec. 88	21	4.8	4.8
Jan. 89	21	0.0	0.0
Feb. 89	21	0.0	0.0
Mar. 89	21	0.0	0.0
Apr.89	21	4.8	4.8
Mean±SE	21	7.2±2.5	2.1±0.6

APPENDIX 6

Percent mortality of engorged *R. appendiculatus* females put out in nylon bags in short grass (6-10 cm) for 8 days

		Mortality	
Month	N	Predation	Other causes
Nov. 87	21	19.0*	4.8
Dec. 87	21	0.0	4.8
Jan. 88	21	0.0	9.5
Feb. 88	21	0.0	23.8
Mar. 88	21	0.0	23.8
Apr. 88	21	0.0	0.0
May 88	21	0.0	0.0
Jun. 88	21	0.0	0.0
Jul. 88	21	0.0	23.8
Aug. 88	21	0.0	0.0
Sep. 88	21	0.0	14.3
Oct. 88	21	0.0	0.0
Nov. 88	21	0.0	4.8
Dec. 88	21	0.0	0.0
Jan. 89	21	0.0	0.0
Feb. 89	21	0.0	0.0
Mar. 89	21	0.0	0.0
Apr. 89	21	0.0	0.0
Mean±SE	21	1.1±1.1	6.0±2.1

* Nylon bags not placed in metal cages

APPENDIX 7

ANALYSIS OF VARIANCE

General Linear Models procedure data in Appendices 1-6, comparing the three treatments, for both predation and other causes of mortality:

Source of variation	DF	SS	MS	F value	P
Treatment	2	432810.9	216405.4	4194.9	0.0001

Duncan's Multiple Range Test:

	Grouping	Mean	N	Treatment
(a)Predators				
	A	39.8	756	Tethered
	B	7.0	756	Caged
	C	0.7	756	Nylon bags
(b) Other causes of death				
	A	2.9	756	Nylon bags
	A	3.7	756	Caged
	B	5.4	756	Tethered

Means with the same letter are not significantly different ($P > 0.05$).

APPENDIX 8

ANALYSIS OF VARIANCE

General Linear Models procedure for results shown in Appendices 1-6, comparing causes of mortality in long grass and short grass:

Source of variation	DF	SS	MS	F value	P
Grass	1	1046.1	1046	20.3	0.0001

Duncan's Multiple Range Test:

Grouping	Mean	N	Grass
(a)Predators			
A	16.5	1134	Long
B	14.7	1134	Short
b) Other causes of death			
A	4.5	1134	Long
B	6.6	1134	Short

Means with the same letter are not significantly different ($P > 0.05$).

APPENDIX 9

Percent mortality of engorged *R. appendiculatus* nymphs tethered in long grass (40-60 cm) for 8 days

		Mortality	
Month	N	Predation	Other causes
Nov. 87	28	71.4	0.0
Dec. 87	28	42.9	0.0
Jan. 88	28	25.0	42.9
Feb. 88	28	17.9	57.1
Mar. 88	28	71.4	7.1
Apr. 88	28	71.4	0.0
May 88	28	28.6	0.0
Jun. 88	28	28.6	3.6
Jul. 88	28	14.3	0.0
Aug. 88	28	25.0	0.0
Sep. 88	28	25.0	0.0
Oct. 88	28	10.7	0.0
Nov. 88	28	14.3	7.1
Dec. 88	28	14.3	0.0
Jan. 89	28	39.3	0.0
Feb. 89	28	35.7	21.4
Mar. 89	28	28.6	7.1
Apr. 89	28	53.6	0.0
Mean±SE	28	34.3±4.8	8.1±3.8

APPENDIX 10

Percent mortality of engorged *R. appendiculatus* nymphs put out in nylon bags in long grass (40-60 cm)

		Mortality	
Month	N	Predation	Other causes
Nov. 87	28	0.0	0.0
Dec. 87	28	7.1*	0.0
Jan. 88	28	0.0	10.7
Feb. 88	28	0.0	32.1
Mar. 88	28	0.0	17.9
Apr. 88	28	0.0.	0.0
May 88	28	0.0	14.3
Jun. 88	28	0.0	7.1
Jul. 89	28	0.0	0.0
Aug. 88	28	0.0	0.0
Sep. 88	28	0.0	7.1
Oct. 88	28	0.0	0.0
Nov. 88	28	0.0	3.6
Dec. 88	28	0.0	3.6
Jan. 89	28	0.0	0.0
Feb. 89	28	0.0	14.3
Mar. 89	28	0.0	10.7
Apr. 89	28	0.0	7.1
Mean±SE	28	0.4±0.4	7.1±2.0

* Nylon bags not placed in metal cages

APPENDIX 11

Percent mortality of engorged R. appendiculatus nymphs tethered in short grass (6-10 cm) for 8 days

		Mortality	
Month	N	Predation	Other causes
Nov. 87	28	53.6	0.0
Dec. 87	28	42.9	0.0
Jan. 88	28	60.7	35.7
Feb. 88	28	10.7	71.4
Mar. 88	28	64.3	21.4
Apr. 88	28	35.7	0.0
May 88	28	67.9	0.0
Jun. 88	28	28.6	7.1
Jul. 88	28	21.4	0.0
Aug. 88	28	28.6	0.0
Sep. 88	28	25.0	0.0
Oct. 88	28	46.4	7.1
Nov. 88	28	50.0	3.6
Dec. 88	28	35.7	0.0
Jan. 89	28	25.0	7.1
Feb. 89	28	35.7	21.4
Mar. 89	28	28.6	5.3
Apr. 89	28	32.1	0.0
Mean±SE	28	38.4±3.7	10.0±4.3

APPENDIX 12

Percent mortality of engorged R. appendiculatus nymphs put out in nylon bags in short grass (6-10 cm) for 8 days

		Mortality	
Month	N	Predation	Other causes
Nov. 87	28	0.0	0.0
Dec. 87	28	3.6*	0.0
Jan. 88	28	0.0	10.7
Feb. 88	28	0.0	46.4
Mar. 88	28	0.0	10.7
Apr. 88	28	0.0	0.0
May 88	28	0.0	0.0
Jun. 88	28	0.0	3.6
Jul. 88	28	0.0	0.0
Aug. 88	28	0.0	0.0
Sep. 88	28	0.0	0.0
Oct. 88	28	0.0	0.0
Nov. 88	28	0.0	3.6
Dec. 88	28	0.0	7.1
Jan. 89	28	0.0	0.0
Feb. 89	28	0.0	32.1
Mar. 89	28	0.0	7.1
Apr. 89	28	0.0	7.1
Mean±SE	28	0.2±0.2	7.1±3.0

* Nylon bags not placed in metal cages

APPENDIX 13

Percent mortality of engorged *R. appendiculatus* nymphs tethered for 8 days (long grass and short grass combined).

		Mortality	
Month	N	Predation	Other causes
Nov. 87	56	64.3	0.0
Dec. 87	56	42.9	0.0
Jan. 88	56	42.9	39.3
Feb. 88	56	14.2	64.3
Mar. 88	56	64.3	14.2
Apr. 88	56	53.6	0.0
May 88	56	42.9	0.0
Jun. 88	56	28.6	5.4
Jul. 88	56	17.9	0.0
Aug. 88	56	28.6	0.0
Sep. 88	56	25.0	0.0
Oct. 88	56	28.6	3.6
Nov. 88	56	32.1	7.1
Dec. 88	56	25.0	0.0
Jan. 89	56	32.1	14.2
Feb. 89	56	35.7	21.4
Mar. 89	56	28.6	0.0
Apr. 89	56	42.9	0.0
Mean±SE	56	36.1±3.3	9.4±4.1

APPENDIX 14

Percent mortality of engorged R. appendiculatus, nymphs put out in nylon bags for 8 days (long and short grass combined)

		Mortality	
Month	N	Predation	Other causes
Nov. 87	56	0.0	0.0
Dec. 87	56	5.4*	0.0
Jan. 88	56	0.0	12.5
Feb. 88	56	0.0	39.3
Mar. 88	56	0.0	14.2
Apr. 88	56	0.0	0.0
May 88	56	0.0	7.1
Jun. 88	56	0.0	5.4
Jul. 88	56	0.0	0.0
Aug. 88	56	0.0	0.0
Sep. 88	56	0.0	7.1
Oct. 88	56	0.0	0.0
Nov. 88	56	0.0	3.6
Dec. 88	56	0.0	5.4
Jan. 89	56	0.0	0.0
Feb. 89	56	0.0	23.2
Mar. 89	56	0.0	8.9
Apr. 89	56	0.0	7.1
Mean±SE	56	0.3±0.3	7.4±2.4

* Nylon bags not placed in metal cages

APPENDIX 15

ANALYSIS OF VARIANCE

General Linear Models procedure for Table 21:

Source of variation	DF	SS	MS	F value	P
Time	7	10030.4	1432.9	12.7	0.0001
Animals	8	340.0	42.5	0.38	0.9282

Duncan's Multiple Range Test:

Grouping		Mean	Time
	A	41.1	1000
	A	29.9	0800
	B	9.5	1400
	B	8.7	1200
C	B	6.1	1600
C	B	2.7	1800
C	B	1.4	2000
C		0.4	0600

Means with the same letter are not significantly different ($P>0.05$).

APPENDIX 16

ANALYSIS OF VARIANCE
General Linear Models procedure for Table 23:

Source of variation	DF	SS	MS	F value	P
Time	7	5798.0	828.2	15.2	0.0001
Animal	5	4.5	0.5	0.02	0.9999

Duncan's Multiple Range Test:

Grouping	Mean	Time
A	37.1	1000
B	22.7	0800
C B	13.9	1400
C D	10.7	1200
C D	6.5	1800
D	5.7	2000
D	3.5	1600
E	0.0	0600

Means with the same letter are not significantly different ($P>0.05$).

APPENDIX 17

ANALYSIS OF VARIANCE

General Linear Model procedure for table 24:

Source of variation	DF	SS	MS	F value	P
Animal	5	0.0005	0.00006	0.67	0.71
Infestation	1	0.003	0.003	37.1	0.0003

Duncan's Multiple Range Test:

(a)	Grouping	Mean weight	Infestation
	A	0.41	1
	B	0.38	2

(b)	Grouping	Mean numbers	Infestation
	A	141.8	1
	B	140.0	2

Means followed by the same letter are not significantly different ($P>0.05$).

APPENDIX 18

ANALYSIS OF VARIANCE

General Linear model procedure for Table 25:

Source of variation	DF	SS	MS	F value	P
Time	7	7775.2	1110.7	9.6	0.0001
Animals	5	128.1	25.6	0.2	0.95

Duncan's Multiple Range Test:

Grouping	Mean	Time
A	55.1	1800
B	11.1	1600
B	9.9	1000
B	7.0	1200
B	6.6	2000
B	5.1	0800
B	4.7	1400
B	0.4	0600

Means with the same letter are not significantly different ($P>0.05$).

APPENDIX 19

ANALYSIS OF VARIANCE

General Linear Model procedure for Table 28:

Source of variation	DF	SS	MS	F value	P
Time	7	3335.1	476.4	1.86	0.1066
Animal	5	550.0	110.0	0.43	0.82

Duncan's Multiple Range Test:

Grouping		Mean	Time
	A	27.6	1800
B	A	19.5	2000
B	A	18.0	0800
B	A	14.0	1600
B	A	12.0	1200
B	A	6.4	1000
B		4.1	0600
B		0.6	1400

Means with the same letter are not significantly different ($P>0.05$).