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Tick-Host Interactions in *Hyalomma* species

By

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Dedication

This thesis is dedicated to my loving mother **Makidiane Magano**, and to the memory of my late father **Letsoma-Tshukudu Magano**

"I will always remember you and love you"

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SUMMARY

Three experiments were performed to study the tick-host interactions in *Hyalomma* species. In experiment one a comparative study was made of the feeding patterns of immature stages of *Hyalomma truncatum* (the smooth bont-legged tick) and *Hyalomma marginatum rufipes* (the rough bont-legged tick) on the four-striped mouse (*Rhabdomys pumilio*), the single-striped mouse (*Lemniscomys rosalia*), guinea-pigs and rabbits. The larvae of *Hyalomma truncatum* developed through a three-host pattern on both *Rhabdomys pumilio* and *Lemniscomys rosalia*. On guinea-pigs this tick species followed a mixed two-host and three-host pattern, with the latter the preferred route, since more than 70% of the fully fed larvae dropped off. *H. truncatum* was a two-host tick on rabbits. The larvae of *H. marginatum rufipes* appeared not to prefer *R. pumilio* and *L. rosalia* as hosts. On guinea-pigs, the life cycle of this tick species showed a mixed two-host and three-host pattern with a bias towards the two-host life cycle, since approximately 58% of the fully fed larvae dropped off from their hosts as engorged nymphs. On rabbits, *H. marginatum rufipes* also was exclusively a two-host tick.

The mean engorgement weights and mean blood quantities ingested by nymphs of *H. truncatum* which developed through a three-host pattern on *R. pumilio* and *L. rosalia*, were significantly higher ($p<0.0001$) than those which developed through a two-host pattern on guinea-pigs and rabbits. For *H. marginatum*

rufipes, there were no significant differences ($p>0.05$) between engorgement weights of nymphs that developed through two-host and three-host patterns. However, there were significant differences ($p<0.0001$) in blood-meal quantities ingested by nymphs of this tick species following feeding on different hosts.

In experiment two the feeding performances of embedded and non-embedded nymphs of *H. truncatum* and *H. marginatum rufipes* on *R. pumilio*, *L. rosalia* and the white laboratory were examined. Infestation with the nymphs of *Hyalomma truncatum* was done on *Rhabdomys pumilio*, *Lemniscomys rosalia* and laboratory rat. About 72% and 81% of the nymphs which attached on *R. pumilio* (n=10) and *L. rosalia* (n=8) respectively, were embedded. The number of embedded nymphs on the white laboratory rat was low and accounted only for 9.1% of the total number (53) of nymphs that attached on five of these hosts. Similarly embedment was found to be a common type of attachment when *R. pumilio* and *L. rosalia* were infested with the nymphs of *H. marginatum rufipes*. There were no significant differences ($p>0.05$) in mean engorgement weights of embedded and non-embedded nymphs of *H. truncatum* and *H. marginatum rufipes*. Although no significant differences ($p>0.05$) occurred between the quantities of blood ingested by nymphs of both *Hyalomma* ticks following feeding on different hosts, the concentration of the blood meal was significantly less ($p<0.0001$) in nymphs which fed on *L. rosalia*.

Furthermore acquired resistance to ticks in *R. pumilio*, *L. rosalia* and white-laboratory rats, following repeated infestations of these hosts with nymphs of *H. truncatum*, was tested. Repeated infestations of *H. truncatum* nymphs, on *R. pumilio* and *L. rosalia*, yielded no significant increase ($p>0.05$) in β- or γ-globulins. The tick engorgement weight appeared not to be affected even in post primary infestations. However, a significant increase in γ-globulins ($p<0.05$) was observed in the white laboratory rats during the secondary and tertiary infestations. This increase coincided with a significant decline ($p<0.01$) in the mean engorgement weight during the secondary infestation on these hosts. On *R. pumilio* and *L. rosalia* embedment persisted as the predominant form of attachment throughout the three consecutive infestations. However, on the white laboratory rat, embedment appeared to be a rare feature and was only observed during the primary infestation.

In experiment three I examined the patterns of CO_2 release in nymphs and females of *H. truncatum* using a flow-through respiratory system (chapter four). All measurements for free-living phases were done at 23±1°C. For on-host ticks (i.e. embedded and non-embedded) the mean temperature of the host skin was 36.4±0.6°C. Ventilation patterns were identified and the nature of the discontinuous gas exchange cycle (DGC) examined for engorged nymphs and both non-engorged and engorged females of *H. truncatum.* Embedded and non-embedded nymphs (on-host) ventilated continuously. This study is the first to report on observations of DGC in engorged nymphs and females of *H.*

truncatum. No significant ($p>0.05$) differences were found between individual metabolic rates (ml h^{-1}) of embedded and non-embedded nymphs. A similar result was found when comparing the mass specific metabolic rates (ml g^{-1} h^{-1}) in these two groups of ticks. However, the mass specific metabolic rates of embedded and non–embedded nymphs were significantly higher ($p<0.05$) than those of engorged nymphs that had dropped off from the hosts. The mean individual metabolic rate (ml h^{-1}) in engorged females was significantly higher ($p<0.0001$) than that in non-engorged ones. However the mass specific metabolic rate (ml g^{-1} h^{-1}) in these two groups of females did not differ significantly. The durations of open and inter-burst phases in non-engorged females were significantly longer ($p<0.0001$) than those of engorged females. Distinct closed and flutter phases were identified in two of the non-engorged females and ten engorged females.

In sum, data obtained in this study demonstrates among others, the capacity of *Hyalomma truncatum* and *Hyalomma marginatum rufipes* to develop through two-host pattern, mixed two-host and three host patterns and three-host pattern. Furthermore, I report for the first time on observations of embedded *H. truncatum* and *H. marginatum* nymphs on *L. rosalia.* Also for the first time, I report on the occurrence of DGCs in engorged nymphs and females of *H. truncatum*. This study also provides a report on the successful attempt to measure the respirometry of ticks that are attached to their hosts.

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CHAPTER ONE

General biology of tick-host interaction

1.1 Classification and life cycle

Although ticks are obligatory haematophagous ectoparasites of reptiles (Norval 1975; Petney and Horak 1988; Walker 1991; Fielden *et al.* 1992; Rechav and Fielden 1995), birds, and mammals (Howel *et al.* 1978; Hoogstraal 1979; Rechav 1979; Horak and MacIvor 1987), they are non-permanent feeders. Two main taxonomic groups are recognized; the Argasidae (soft-ticks), which are fast feeders and thus spend a few hours feeding to full engorgement, and the Ixodidae (hard-ticks), which are slow feeders spending days to few weeks ingesting blood from their hosts.

The tick life-cycle consists of four stages (the egg, hexapod larva, octopod nymphs, and adult male or female), and includes the alternation of parasitic, blood feeding, and free-living phases. Ixodid ticks have only one nymphal stage whereas argasids may have as many as five (Schmidt and Roberts 1989). Ixodid ticks are often described as one-host, two-host or three-host ticks, depending on the number of hosts required for development from an unfed larval form to an engorged adult form. In one-host ticks, feeding and moulting through all instars occur on the same host, as, for example, in *Boophilus* (Service 1980; Kettle 1984). In two-host ticks, larval and nymphal feeding, and moulting between

these two immature stages, take place on the same individual host. The adults attach to another host. *Rhipicephalus evertsi evertsi* and some species in the genus *Hyalomma* belong to this group (Service 1980; Kettle 1984). In three-host ticks, the larvae feed to engorgement and drop off the host prior to moulting. Nymphs feed on a second host and adults on a third host. Most ixodid ticks, including those that belong to the genera *Amblyomma*, *Ixodes*, and *Haemaphysalis*, are three-host ticks (Howel *et al.* 1978; Hoogstraal 1979).

1.2 Feeding and digestion in Ixodid ticks

Waladde and Rice (1982) consider feeding in ticks a complex of behavioural processes that starts with hunger and ends with satiation. According to these authors, this complex of behavioural processes can be divided into the following sequence of events:

(1) Appetence (Locomotory hunting for a host or seeking of one from a vantage point)

(2) Engagement (Physical contact with and adherence to the host's pelage)

(3) Exploration (Searching on the host for an attachment site)

(4) Penetration (Insertion of the mouthparts into the host's integument)

(5) Attachment (Establishment of a feeding site)

(6) Ingestion (Uptake of host body fluids)

(7) Engorgement (Uptake of a full meal)

(8) Detachment (Withdrawal of the mouthparts from the host's integument)

(9) Disengagement (Leaving the host)

The structure and function of the mouthparts in ixodid ticks have been described by Londt and Arthur (1975), Balashov (1983), Kemp *et al.* (1982), Bergman (1996) and Service (1996). Once a suitable feeding site has been selected, the skin of the host is penetrated by the outward cutting movements of the chelicerae (Kemp *et al.* 1982). Both the hypostome and chelicerae penetrate the host during feeding (Bergman 1996; Service 1996). Balashov (1972) observed that the insertion of the hypostome was accompanied by rocking movements and by secretion of cement around the mouthparts. The idea that the salivary glands of several tick species secrete a cement which secures the mouthparts to the host is supported by many authors (Moorhouse and Tatchell 1966; Chinery 1973; Meredith and Kaufman 1973; Sauer 1977; Bennington 1978; Kemp *et al.* 1982; Shapiro *et al.* 1989). However, some tick species of the genus *Ixodes* do not secrete cement but secure themselves by a deeper penetration of their mouthparts into the host's skin (Kemp *et al.* 1982). Furthermore, some tick species of the genera *Ixodes* and *Hyalomma* do not only attach to the host with their mouthparts but may also become somewhat enveloped by the host skin, a phenomenon known as tick embedment (Lebeda 1962; Tovornik 1984; Els *et al.* 1988; Els 1992). Reports of this phenomenon pertain largely to ticks feeding on their natural hosts. An extreme example of embedment was reported by Lebeda (1962) who observed a living tick of the genus *Ixodes* attached to the lymph node of a fox, *Vulpes vulpes.* The mechanism of survival of these ticks (*Ixodes* and *Hyalomma*) within the host tissue has not yet been appropriately explained. Important aspects to note are the failure of embedded adult *Ixodes* ticks to feed

to full engorgement (Lebeda 1962), in contrast to embedded nymphs of *Hyalomma* which feed to full engorgement (Els *et al.* 1988; Els 1992). Els *et al.* (1988) and Els (1992) observed that most of the nymphs of *Hyalomma truncatum* that were fully embedded in the skins of *Tatera brantsii* and *Rhabdomys pumilio* had a narrow canal or pore leading to the external environment enabling them to remain alive for a long period.

Ixodid ticks usually feed once in an instar, a process which takes from several days to several weeks, and during which time large quantities of blood are ingested and the fully engorged tick can weigh over 100 times that of the unfed tick (Arthur 1962; Kemp *et al.* 1982). While blood is the principal constituent of the tick's meal, females and immature stages of some ixodid ticks also ingest significant quantities of non-blood tissue (Snow 1970). Digestion of the blood-meal in ixodid ticks is primarily an intracellular process taking place in the mid-gut epithelial cells and it begins soon after the onset of the ingestion process (Balashov 1972; Grandjean and Aeschlimann 1973). The feeding and digesting processes in ixodid ticks occur in three phases. These are the preparatory, the growth and the expansion phases (Balashov 1972; Araman 1979). In the preparatory phase, the rate of feeding and digestion is very low and as a result there is little or no change in body weight. During the growth phase, feeding and digestion are very intensive. The nutrients gained during this phase are used to build the cuticle in preparation for the enormous expansion of the body during the third phase (Araman 1979). The third phase, the expansion phase, is short and

lasts for a day. In this phase, there is a decline in the digestion rate while the tick ingests huge amounts of blood.

During the process of feeding, ixodid ticks intermittently ejaculate salivary secretions into the host (Tatchell 1967; Balashov 1972; Kaufman and Phillips 1973; Meredith and Kaufman 1973; Binnington 1978; Brown 1988). This process, in addition to concentrating the blood-meal, also serves to regulate the ionic composition of the haemocoel. As the blood-meal is ingested, excess ions and water are moved across the gut epithelium into the haemocoel and secreted back into the host via the salivary glands (Tatchell 1967; Kaufman and Phillips 1973; Meredith and Kaufman 1973). This process forms the first step in the digestion of blood by ixodid ticks (Akov 1982).

1.3 Pharmacology of saliva in ixodid ticks

Details of tick salivary gland morphology and physiology are reviewed in Sauer *et al.* (1996). In order to feed successfully on their vertebrate hosts, ticks must have the capacity to overcome or neutralize the haemostatic responses induced in the host as a result of tick infestation. Many authors (e.g. Ribeiro *et al.* 1985; Fivaz 1989; Waxman *et al.* 1990; Ramachandra and Wikel 1992; Gordon and Allen 1991; Champagne 1994; Joubert *et al.* 1995; Champagne and Valenzuela 1996; Bowman *et al.* 1997) agree that the salivary secretions of some ticks have a variety of factors including anticoagulants, vasodilators, platelet aggregation inhibitors and immunosupressants that allow such ticks to feed to repletion even

on immunologically competent hosts. These factors do not only facilitate tick feeding but may also favour the establishment of pathogens in the host (Wikel 1999). Investigations on the role of ticks in Thogoto virus (Jones *et al.* 1992) and encephalitis virus (Labuda *et al.* 1993) transmission, indicated that tick salivary gland extracts enhance transmission of these viruses.

1.4 Host resistance to tick infestation

The salivary secretions of ticks, secreted into the hosts during feeding, have antigenic properties and thus induce host resistance to tick infestation. Resistance acquired by hosts to tick infestation is a well documented phenomenon and has been demonstrated in cattle (Rechav 1987; Kostrzewski 1989; Essuman *et al.* 1991), and laboratory animals such as guinea-pigs and rabbits (Heller-Haupt *et al.* 1981; Njau and Nyindo 1987; Rechav and Dauth 1987; Dipeolu 1990; Rechav *et al.* 1991; Magano 1993; Rechav *et al.* 1994). These hosts have been shown to acquire resistance as a consequence of exposure to the feeding ticks (Willadsen 1980; Wikel and Whelen 1986; Rechav and Dauth 1987; Brown 1988; Rechav 1992; Magano 1993; Rechav *et al.* 1994) or as a result of immunization to antigens originating from salivary gland and gut extracts as well as homogenates of ticks (Allen and Humpreys 1979; Brown and Askenase 1984; Brown 1987; Heller-Haupt *et al.* 1987, 1989; Ribeiro 1989; Varma *et al.*1990; Rechav *et al.* 1992; Tembo 1996). This resistance has an immunological basis including humoral, cellular and complement components (Wikel and Allen 1977; Allen *et al.* 1979; Brossard and Girardin 1979; Wikel *et al.*

1986; Willadsen and Kemp 1988; Willadsen and McKenna 1991; Neitz *et al.* 1993) and is expressed by (i) prolonged feeding periods (ii) reduced number and weight of engorged ticks (iii) and decrease of oviposition and failure in egg hatching.

1.5 Factors affecting host's resistance to tick infestation

The interaction between ticks and their hosts is known to be influenced by several factors which have been reviewed by Rechav (1992) and Els (1992). These factors include:

(i) Quality of nutrition

Gladney *et al.* (1973) established that protein deficiency in the diet of cattle reduces the level of resistance of cattle to ticks. This finding is further supported by observations of Dicker and Sutherst (1981), Sutherst *et al.* (1983) and Rechav (1987) that resistance of cattle to ticks was significantly low during autumn and winter when the nutritional value of grass has deteriorated.

(ii) Sex of host

Els (1992) observed that females of *Mastomys natalensis*, *Aethomys chrysophilus*, and *Lemniscomys rosalia* carried more larvae than males of these rodents. According to Wharton *et al.* (1970) and Rechav (1992) this difference could also be a result of differences in hormonal levels in the two sexes.

(iii) Age

Conflicting views exist with regard to the role played by host age on tick infestation. Surthest *et al.* (1979) observed that young calves carry fewer ticks

than adult cows, while Els (1992) observed that sub-adult rodents often carried more ticks (immature stages) than adult rodents.

(iv) Pregnancy

Utech *et al.* (1978) established that pregnant cows were significantly more sensitive to tick infestation than non-pregnant ones, but only carried larger tick loads during the late stages of pregnancy.

(v) Lactation

The role played by lactation in the host on resistance to tick infestation needs further clarification. Observations by Wharton *et al.* (1970) and Utech *et al.* (1978) that lactating European cattle carried more ticks than non-lactating ones, could not be substantiated by Johnston and Hydock (1971) and Rechav (1991) on zebu or crossbred cattle. Furthermore, Els (1992) observed that non-lactating *Mastomys natalensis* carried significantly more tick larvae when compared to lactating females.

1.6 Mechanism of host resistance to tick infestation

Although there is a large body of literature which deals with the immunological responses of hosts to tick infestations (reviewed by Wikel 1996), little of this work focuses on the way in which these responses interfere with the feeding of ticks. However, a few studies have shed some light on this problem. From the study by Allen *et al.* (1979) and the most recent review by Wikel (1996), it appears that during primary infestations, epidermal Langerhans cells in the areas of the epidermis immediately surrounding the attached ticks trap the salivary antigens

of the tick. The antigen-laden cells are believed to migrate to draining lymph nodes and to present antigens to appropriate lymphocytes. Nithiuthai and Allen (1985) further suggested that after trapping the salivary antigens, the epidermal Langerhans cells act as foci for antigen concentration and antigen-antibody reactions which lead to the formation of epidermal vesicles beneath the mouth parts of the attached ticks. Subsequently, basophil and eosinophil granulocytes as well as mast cells accumulate within these vesicles as a result of chemotactic factors released on complement-activation.

Several authors, for example, Allen (1973), Brown (1982) and Brown *et al.* (1982) have shown that basophils, and to a lesser extent eosinophils, make up a large proportion of the cells infiltrating the dermis and epidermal vesicles in guinea-pigs resistant to ticks. Brown *et al.* (1982) showed that depletion of basophils in resistant animals after administration of antibasophil serum, inhibited the basophil reactions in the subsequent tick challenge and also blocked the expression of resistance. A similar response was observed by these authors when anti-eosinophil serum was administered, resulting in blocking the eosinophil reactions which resulted in partial loss of resistance.

In their *in vitro* study, Paine *et al.* (1983) showed that cell mediators such as histamine, released from degranulating basophils at attachment sites, have direct effects on attached ticks. When histamine and serotonin were added to the feeding medium at concentrations less than 10 μM, salivation and blood sucking

tended to cease, and several ticks were induced to detach. The exact way through which antibodies interfere with tick feeding is also not clearly known. Allen (1979) suggested that ticks ingest specific antibodies while feeding on resistant animals and that such antibodies could react deleteriously with tick tissues. This view was supported by Kemp *et al.* (1989) who showed that ticks which were fed on cattle vaccinated against *Boophilus microplus,* with antigens derived from partially fed female ticks, had lysed gut cells. A more recent study by Fivas *et al.* (1991) on *R. zambeziensis* fed on resistant rabbits confirmed this report.

Although additional information exists on the interaction between ticks and hosts, most of these studies used laboratory hosts such as guinea-pigs and rabbits. Knowledge concerning the natural tick-host association is still lacking in many respects. According to Randolph (1979), Willadsen (1980) and Ribeiro (1987, 1989), interactions between ticks and their natural hosts are often characterised by inefficient or non-existant host resistance, since during the process of co-evolution with their hosts, ticks evolved means of avoiding host reactions. This view is supported by Fielden *et al.* (1992) who found that tortoises and guinea-fowls, which are the natural hosts of immature stages of *Amblyomma hebraeum* and *A. marmoreum*, failed to express resistance to the larvae of these tick species following repeated infestations. On the contrary, following the same treatment, guinea-pigs (non-natural hosts) expressed resistance to the larvae of these two *Amblyomma* ticks.

1.7 Gaseous exchange in ixodid ticks

Gaseous exchange in nymphal and adult ixodid ticks takes place through the spiracles, a pair of which occurs in each individual posterior to the fourth pair of legs (Balashov 1983; Walker 1991). Details of the spiracular structure in ticks are provided in Rudolph and Knulle (1979), Pugh *et al.* (1988, 1990), Schol *et al.* (1995) and Pugh (1997). The slit-like spiracular aperture leads to a large atrial cavity, which in turn joins the main tracheal system (Pugh *et al.* 1990, Pugh 1997). The larvae of ixodid ticks are atracheate (Rudolph and Knulle 1979) and therefore lack spiracles.

One of the striking features of gaseous exchange in some tracheate arthropods is the occurrence of discontinuous gas exchange cycles (DGC). Knowledge on discontinuous cycles in CO_2 release in insects has been present for many years (Schneiderman 1953; Punt *et al.* 1957; Schneidenman 1960). Discontinuous gas exchange cycles in insects are compatible with the widely accepted passive ventilation model (Lighton 1987, 1996). This model describes a ventilatory cycle consisting of three phases. In the first phase, the spiracles are closed (C phase), thus preventing any gas exchange or respiratory water loss. This stage is followed by the flutter phase (F phase) during which slight opening of the spiracles, on an intermittent basis, allows a slow ingress of O_2 but little egress of CO_2 or water vapour. The last phase of the ventilatory cycle, the burst or open phase (B or O phase), occurs when the spiracles open widely resulting in rapid release of CO_2 and water vapour to the outside. The mechanism through which

the spiracles are opened and closed is less understood in ticks. However, like in insects, evidence exists which suggest the existence of a CO_2-sensitive control mechanism in the tick spiracle (Hefnawy 1970; Rudolph and Knulle 1979).

A study by Rudolph and Knulle (1979) which demonstrated intermittent bouts of rapid mass loss in females of *A. variegatum* and also that tick spiracles, like those of insects, open at high CO_2 concentrations, provided inferential evidence on the occurrence of discontinuous CO_2 release in ticks. This feature was confirmed by Lighton *et al.* (1993) who demonstrated discontinuous CO_2 release in the unfed nymphs and female adults of the African tortoise tick, *A. marmoreum*. The engorged nymphs and adults of this tick species showed continuous ventilation. Similarly, Fielden *et al.* (1994) also demonstrated the occurrence of discontinuous CO_2 release in unfed females of *A. hebraeum*.

While discontinuous ventilation in tracheate arthropods is generally viewed as being an adaptive mechanism for minimizing respiratory transpiration, reports exists on ventilatory patterns and transpiration in lubber grasshopers (*Romalea guttata* and *Taeniopoda eques*) which contradict this view (Hadley and Quinlan 1993; Quinlan and Hadley 1993). It was found that in these grasshoper species, respiratory transpiration contributes very little to total water loss, even during the burst phase. Furthermore, it was observed that in dehydrated individuals, discontinuous gas exchange was replaced by continuous random release of CO_2. The opposite of this result would have been expected since dehydrated

animals face a greater need to conserve water. The problem of water loss via the tracheal system in the course of respiratory gas exchange appears to be restricted to the free-living ticks. Feeding ticks have a considerable surplus of water because the ingested blood is hyposmotic to their own body fluids (Rudolph and Knulle 1979).

1.8 Ticks and hosts examined in this study

H. truncatum and *H. marginatum rufipes* are indigenous to South Africa. Both of these tick species are widely distributed in the country and almost share a common distribution in the southern sub-region of Africa (see Howel *et al.* 1978; Hoogstraal 1979; Horak and MacIvor 1987; Walker 1991).

Apart from causing direct damage on the hosts by ingesting large volumes of host blood, these two tick species are known to transmit toxins and/or pathogens of veterinary and medical importance. In South Africa, *H. truncatum* and *H. marginatum rufipes* are known to transmit the Crimean-Congo Haemorrhagic Fever (CCHF) virus (Howel *et al.* 1978; Hoogstraal 1979; Gear *et al.* 1981; Rechav 1986; Rechav *et al.* 1987) to man. The adults of some strains of *H. truncatum* transmit the toxin causing sweating sickness in cattle (Howel *et al.* 1978; Hoogstraal 1979).

Hoogstraal (1979) described *H. marginatum rufipes* as a two-host tick and *H. truncatum* as three-host tick while Howel *et al.* (1978), Horak and MacIvor (1987)

described both species as two-host ticks. The host range appears to be similar for adults of both tick species and includes large herbivores such as cattle, horses, and wild animals such as rhino, zebra, giraffe, buffalo and various antelope species (Howel *et al.* 1978; Hoogstraal 1979; Horak and MacIvor 1987; Walker 1991). However, the immature stages of these two tick species appear to differ with regard to host preference. In their survey Horak and MacIvor (1987) only found the immature stages of *H. marginatum rupes* and *H. marginatum turanicum* on the birds they examined. They also established that some mice and rats are only capable of hosting immatures of *H. truncatum*. However, both tick species also appear to have a strong predilection for the Cape hare, *Lepus capensis* and the scrub hare, *Lepus saxatilis* (Howel *et al.* 1978; Horak and MacIvor 1987; Walker 1991).

Although survey studies conducted by Horak and MacIvor (1987) only implicated *Rhabdomys pumilio* (four-striped mouse) as a common host for immatures of *H. truncatum*, in this study I explored the possibility that this host, and the closely related species *Lemniscomys rosalia* (single-striped mouse), may both act as hosts for immatures of both *H. truncatum* and *H. marginatum rufipes*. This was considered in view of the coincidence of the presence of both rodents in the northern parts of Southern Africa (De Graaf 1981). *R. pumilio* is widely distributed throughout the southern sub-region of Africa compared to *L. rosalia,* since the latter species is absent in the Eastern, Western and Northern Cape provinces and occur sparsely in Namibia and Botswana.

Guinea-pigs, rabbits and laboratory rats were also used as hosts for ticks in this study. These animals provide excellent host models in experiments designed to study ticks hence many authors have used them in studies involving ticks (e.g. Allen 1973; Bowessidjaou *et al.* 1976; Geevarghese and Dhanda 1982; Rechav and Dauth 1987; Heller-Haupt *et al.* 1989; Varma *et al.* 1990; Rechav *et al.* 1994 and Rechav and Fielden 1997). However, it is important to assess the reliability of results obtained using such non-natural hosts, hence their inclusion here. Furthermore, their small size enables the researcher to keep them under controlled laboratory conditions during experimentation, thus reducing artifacts which may reduce the reliability of data.

1.9 Aims and objectives of this study

The practical aim of research on tick-host interaction is to develop control measures against ticks and the pathogens they transmit to humans and animals. Many authors (e.g. Wharton 1976; Sutherst and Comis 1979; Solomons 1983; Wikel and Whelen 1986; Heller-Haupt *et al.* 1989; Kemp *et al.* 1989; Logan *et al.* 1989; Fielden *et al.* 1992; Tembo and Rechav 1992; Rechav *et al.* 1992; Magano 1993; Rechav *et al.* 1994) have acknowledged that basic information which includes host preference, life-cycle and feeding behaviour of ticks, is critical to the development of tick control measures.

From the preceding review it is clear that there are a number of important issues regarding tick-host interactions that remain poorly understood. These include, the

life-cycle or feeding pattern of *H. truncatum*, the role played by small rodents in the development of *H. marginatum rufipes*, the occurrence or non occurrence of embedment during infestations with nymphs of *H. truncatum* and *H. marginatum rufipes* on natural and non-natural hosts, the feeding performance of the nymphs of *H. truncatum* and *H. marginatum rufipes* on different hosts, the effect of repeated infestations on the occurrence of nymph embedment, and the respirometry requirements of on-host and off-host ticks. If an understanding of these issues is to be achieved, considerable additional work is required. The goal of this thesis is to examine these issues using the interactions between *H. truncatum* and *H. marginatum rufipes* ticks and their natural and non-natural hosts. Firstly, these ticks were chosen because *Hyalomma* ticks are one of the two genera known to embed in the skins of their hosts. Thus together with their hosts, they provide a suitable model for studying tick embedment. Secondly, the role played by these tick species in the transmission of diseases to humans and animals, necessitates further investigation on aspects of their biology. The specific objectives of this study are:

(i) To study the feeding patterns of immature stages of *H. truncatum* and *H. marginatum rufipes* on field mice (*R. pumilio* and *L. rosalia*), guinea-pigs and rabbits

(ii) To study the feeding performance of *H. truncatum* and *H. marginatum rufipes* nymphs which become embedded in the host skin, and compare the results with those of nymphs which feed with only the mouth-parts inserted into the integument of the host (non-embedment)

(iii) To determine whether repeated infestation of hosts (white laboratory rats, *R. pumilio* and *L. rosalia*) with the nymphs of *H. truncatum* influences the type of attachment (embedment or non-embedment) of nymphs to their hosts

(iv) To study the respiratory activities of on-host (both embedded and non-embedded nymphs) and off-host (non-engorged females and engorged nymphs and females) *H. truncatum* ticks

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CHAPTER TWO

The feeding patterns of immature stages of *Hyalomma truncatum* and *Hyalomma marginatum rufipes* on different hosts

2.1 Introduction

Ixodid ticks are described as being one-host, two-host or three-host depending on the number of hosts required for development from the unfed larval form to the engorged adult form (Howel *et al.* 1978). In all these developmental patterns, each of the three instars takes a single blood-meal (Kemp *et al.* 1982). The capacity of *Hyalomma* ticks to undergo two-host and three-host life patterns was demonstrated by Geevarghese and Dhanda (1982), and more recently by Rechav and Fielden (1997). These authors showed that *H. anatolicum anatolicum*, *H. dromedarii*, *H. marginatum isaaci* and *H. truncatum* are capable of pursuing two-host, mixed two-host and three-host, and three-host life cycles depending on the host type they feed on. Several authors (e.g. Rechav *et al.* 1977; Hoogstraal 1979, Rechav and Fielden 1997) consider a two-host pattern of development derived, and argue that it is beneficial to the tick compared to a three-host pattern of development. These benefits stem from the elimination of the off-host stage between the larval and nymphal stages, resulting in a shorter life cycle and improved survivorship.

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In South Africa, *H. truncatum* and *H. marginatum rufipes* have been described by some authors (Howel *et al.* 1978; Horak and MacIvor 1987; Rechav *et al.* 1987) as two-host ticks. On the other hand Hoogstraal (1979), refers only to *H. marginatum rufipes* as a two-host tick and considers *H. truncatum* a three-host tick. In contrast Rechav and Fielden (1997) showed that *H. truncatum* behaves as a three-host tick on gerbils and guinea-pigs and as a two-host tick on rabbits. Given the economic importance of these species (Howel *et al.* 1978; Walker 1991), and the role they play in the transmission of Crimean-Congo Haemorrhagic Fever virus to humans (Hoogstraal 1979; Swanepoel *et al.* 1983), it is clear that these apparently contradictory results must be resolved, especially if effective control strategies for these species are to be developed and implemented. Such knowledge is also critical for the refinement of predictive models of tick population dynamics (Randolph and Rogers 1997), which are of particular importance in the development of effective control strategies.

In the present study I investigated the feeding patterns of immature stages of *H. truncatum* and *H. marginatum rufipes* on different hosts by determining the proportions of fed larvae to fed nymphs following the release of specified numbers of larvae on hosts. To assess the feeding performance of ticks developing through the two-host or three-host patterns, and thus to gain additional insight into the relationships between these ticks and the different hosts employed, the mean engorgement weights and mean blood-meal quantities were also determined.

2.2 Material and methods

2.2.1 *Ticks, hosts and infestation*

Three weeks old larvae and nymphs of *H. truncatum* and *H. marginatum rufipes* used in this study, were obtained from laboratory colonies, maintained on guinea-pigs, in the Department of Biology at the Medical University of Southern Africa. These ticks were maintained in glass humidity chambers at 25 ± 1°C, 75 ± 5% RH, and L:D 14:10h. The study was undertaken from April to September 1998.

Laboratory bred indigenous hosts, *Rhabdomys pumilio* and *Lemnyscomys rosalia,* used in this study were three to four months old and weighed 40 – 47 g and 45 – 55 g respectively. Two commonly used non-natural hosts were included in this study, i.e. Dunkin/Hartley guinea-pigs, which were four months old and weighed 400 - 600g, and four to five months old Himalayan rabbits that weighed 2 - 4kg. These animals are regularly used as host models in experiments designed to study ticks (e.g Heller-Haupt *et al.* 1989; Varma *et al.* 1990; Rechav *et al.* 1994 and Rechav and Fielden 1997). However, it is important to assess the reliability of results obtained using such non-natural hosts, hence their inclusion here. All animals used in this study were tick-naive and kept in laboratory holding facilities, at room temperature (20 - 25°C) and natural light/dark regime, in the Animal Production Unit at the Medical University of Southern Africa.

The animals were housed individually during experimentation and were fed pellets and water *ad libitum*. *R. pumilio*, *L. rosalia* and the guinea-pigs were housed in plastic cages (34cm x 34cm x 22cm) with screened metal floors. Rabbits were housed in similar, though larger metal cages (56cm x 56cm x 38cm). For *R. pumilio* and *L. rosalia*, each cage was suspended over a plastic tray, using large vials. This prevented rodents from treading on or eating the ticks which dropped off. The edges of the plastic trays were coated with vaseline to prevent dropping ticks from crawling away.

Tick loads used for infestation on different hosts are indicated in Table 2-1. For *R. pumilio* and *L. rosalia* infestation was carried out by placing the host in a cotton bag containing ticks. To facilitate attachment the hosts were shaved on their backs prior to infestation. The cotton bags were closed to allow time for ticks to attach before the rodents freed themselves by chewing through the cotton bags. Thus, time for attachment was approximately 1hour.

For guinea pigs and rabbits, infestation was carried out by fixing cotton bags using adhesive glue on the shaved backs of these hosts. Ticks were released into these bags 24 hrs after the bags were fully secured on the animals and the glue had dried. The larvae which were still hanging onto the cotton bag on the second day following infestation were removed. For each host, the number of ticks dropping off as engorged larvae and nymphs was determined. In addition, the number of newly emerged nymphs which were still looking for attachment

sites following moulting on the host was noted. The engorgement weight of nymphs was measured to 0.1mg on an electric balance (Mettler AE 100, Mettler Instruments, Greifensee, Switzerland).

2.2.2 *Colorimetric estimation of blood-meal quantities*

The blood content of fed nymphs was converted to pyridine haemochromogen and measured spectrophotometrically by a method developed by Sutton and Arthur (1962) (modified by Snow 1970). This method was successfully employed by these authors to estimate blood meal quantities ingested by various tick species fed on dogs and guinea-pigs (see also Kitaoka and Fujisaki 1975). In brief, each engorged nymph was homogenized in 0.1N NaOH (2 ml). Subsequently 0.5 g of sodium dithionate ($Na_2S_2O_4$) and 0.4 ml of pyridine were added. The mixture was then centrifuged (Mixtasel ce95 S Spania) at 2 000 rpm for 10 min. The absorbance of the clear supernatant was read with a Jenway 6300 spectrophotometer at 525 nm. Separate standard curves were prepared for each of the host types. The absorbance values obtained for fed ticks were compared with those of the blood of hosts on which they had fed. Ticks from different animals (2 - 4 individuals) for each host type were used for the estimates of blood-meal quantities. The accuracy of this method depends on the fact that during feeding in ixodid ticks, it is only excess plasma water that is salivated back into the host (Kaufman and Phillips 1973). The haemoglobin content of host blood is unaffected by the elimination of excess plasma water.

Table 2-1. The number of larvae and nymphs of *H. truncatum* and *H. marginatum rufipes* used for infestation on different hosts (sample size in parentheses; ND = not done).

Larval Infestation			Nymphal Infestation		
Hosts	**Number of ticks per host**		**Hosts**	**Number of ticks per host**	
	H. truncatum	*H. m. rufipes*		*H. truncatum*	*H. m. rufipes*
R. pumilio (n=2)	200	200	*R. pumilio* (n = 10)	40	40
L. rosalia (n = 2)	200	200	*L. rosalia* (n = 10)	40	40
Guinea-pigs (n=4)	1300	460	Guinea-pigs (n=4)	ND	ND
	1000	1000			
	700	1000			
	200	500			
Rabbits (n=2)	160	800	Rabbits (n=2)	ND	ND
	100	1300			

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2.3 Results

2.3.1 *Feeding period and life pattern*

H. truncatum: All of the attached larvae of *H. truncatum* feeding on *R. pumilio* and *L. rosalia* developed through the three-host pattern (Table 2-2). The feeding period of this tick species on both *R. pumilio* and *L. rosalia* was 4 – 7 days for larvae, and 7 - 11days for nymphs. The premoult period between the two immature stages ranged from 5 - 11 days. On guinea-pigs, this tick species showed a mixed two-host and three-host pattern (Table 2-2). Approximately 70% of the attached larvae, which successfully fed on guinea-pigs dropped off as engorged larvae (indicating that they mainly developed through the three-host pattern), while the remaining larvae moulted into nymphs while still attached to the host. The feeding period for these larvae ranged from 5 - 9 days following infestation. Empty shells remained loosely fixed on the host while the newly emerged nymphs reattached on the same individual host. While it was not practical to establish the exact duration of the moulting process, some of the newly emerged nymphs were seen crawling on the inside of cotton bags from 9 - 13 days following infestation (Fig. 2-1). The drop-off range of engorged nymphs extended from 15 - 21 days following infestation. On rabbits, *H. truncatum* showed a two-host pattern of development exclusively (Table 2-2). Some of the newly emerged nymphs of this tick species on rabbits were seen crawling the inside of cotton bags from 6 - 8 days following infestation (Fig. 2-2).

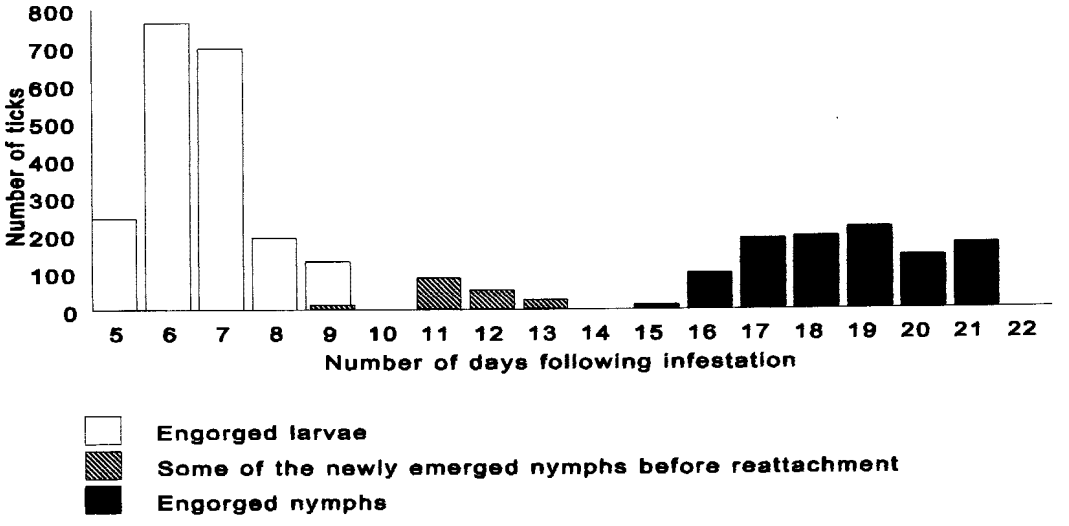

Fig. 2-1: Development of immature stages of *H. truncatum* on guinea-pigs through two and three-host life patterns

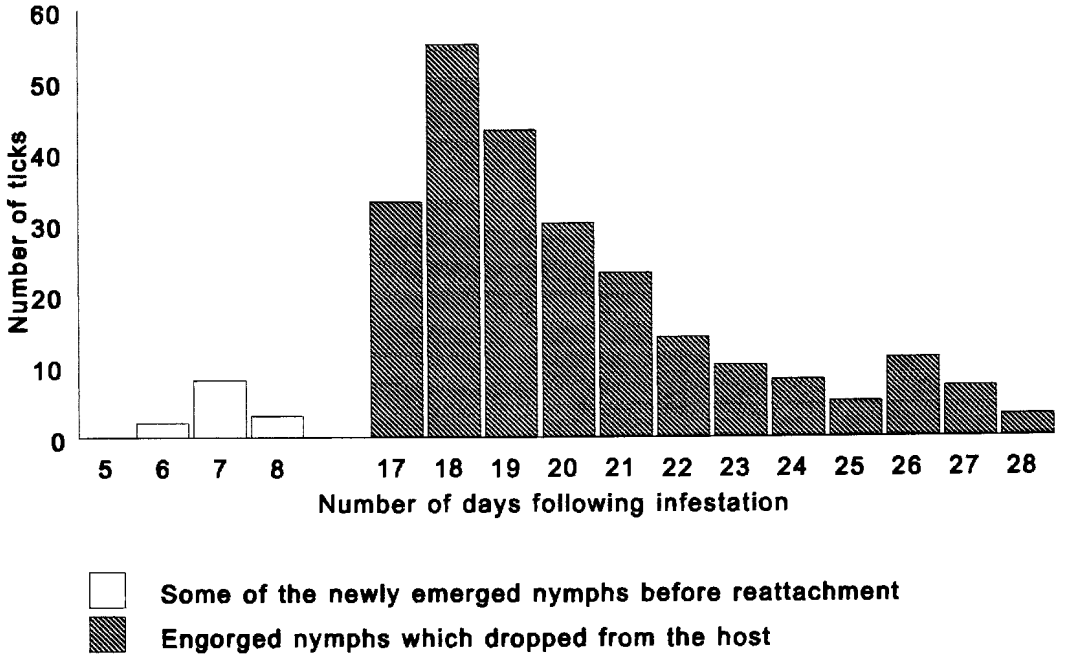

Fig. 2-2: Development of immature stages of *H. truncatum* on rabbits through a two-host life pattern

H. marginatum rufipes: This species attached poorly on *R. pumilio* and *L. rosalia*. Almost all of the ticks that dropped off from these hosts were engorged larvae (typical of three-host pattern) (Table 2-2). Flat nymphs of this tick species attached successfully on both *R. pumilio* and *L. rosalia* taking 7 - 11 days to reach full engorgement. On guinea-pigs this tick also showed a mixed two-host and three-host patterns (Table 2-2). In contrast with *H. truncatum*, most larvae of *H. marginatum rufipes* (about 58% of those that attached) dropped off as fully fed nymphs (a two-host pattern of development). Some of the newly emerged nymphs were seen crawling inside the cotton bags from 11 - 15 days following infestation. The drop-off period of engorged nymphs on guinea-pigs ranged from 16 - 21 days following infestation (Fig. 2-3). Similar results to those observed with *H. truncatum* were found for *H. marginatum rufipes* on rabbits (Table 2-2). The drop-off period of engorged nymphs of this tick species on rabbits ranged from 17 - 29 days following infestation (Fig. 2-4).

2.3.2 *Engorgement weight, blood-meal quantity and moulting success of nymphs*

H. truncatum: Mean engorgement weights of *H. truncatum* nymphs fed on the field mice (*R. pumilio* and *L. rosalia*) following a three-host development were significantly higher ($p<0.0001$) than those which fed on guinea-pigs and rabbits following a two-host development. Similarly, blood-meal quantities ingested by nymphs emerging through the three-host development pattern on the field mice (*R. pumilio* and *L. rosalia*) were significantly higher ($p<0.0001$) than those which emerged through a two-host development pattern on guinea-pigs and rabbits

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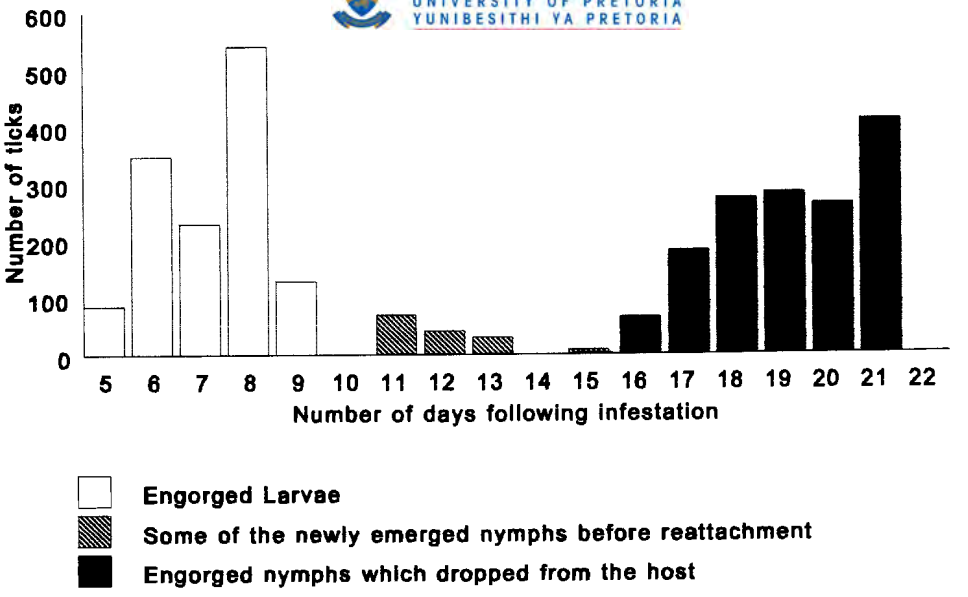

Fig. 2-3: Development of immature stages of *H. marginatum rufipes* on guinea-pigs through two and three-host life patterns

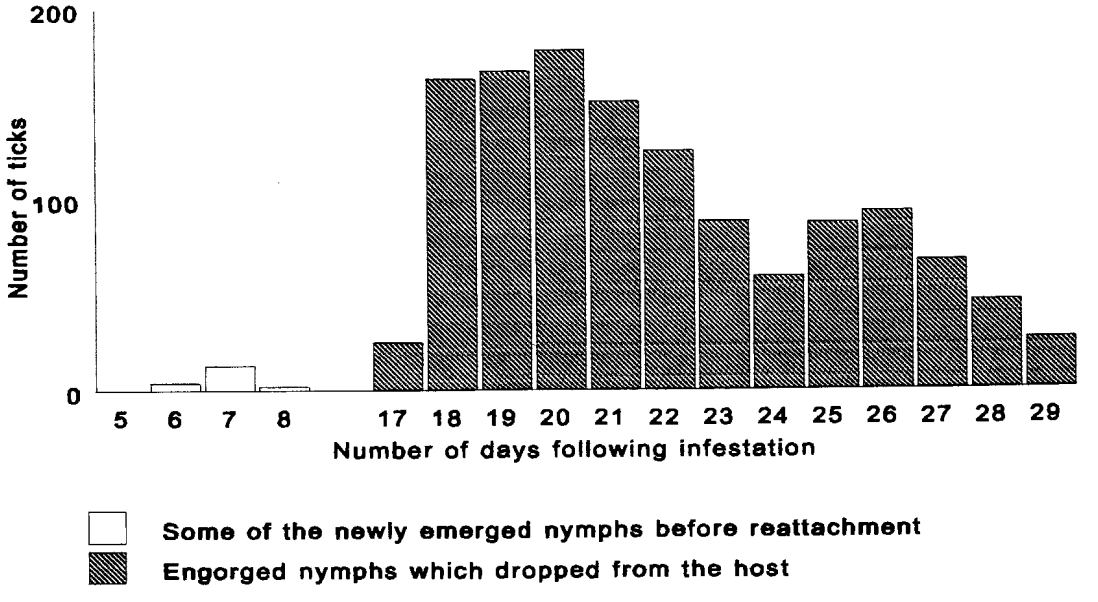

Fig. 2-4: Development of immature stages of *H. marginatum rufipes* on rabbits through a two-host life pattern

Table 2-2: The numbers of *H. truncatum* and *H. marginatum rufipes* that fed on *R. pumilio*, *L. rosalia*, guinea-pigs, and rabbits.

Tick	**Hosts**									
	R. pumilio		*L. rosalia*		Guinea-pigs				Rabbit	
H. truncatum	1	2	1	2	1	2	3	4	1	2
Apprx. No. of larvae released on host	200	200	200	200	1300	1000	700	200	160	100
Apprx. No. of larvae attached	27	30	55	22	1235	991	616	190	150	92
No. of dropped fed larvae	27	30	55	22	848	613	395	160	0	0
No. of dropped engorged nymphs	0	0	0	0	387	378	221	30	150	92
H. marginatum rufipes										
Apprx. No. of larvae released on host	200	200	200	200	460	1000	1000	500	800	1300
Apprx. No. of larvae attached	3	1	0	11	445	973	929	456	754	1253
No. of dropped fed larvae	2	1	0	10	344	457	338	191	3	0
No. of dropped engorged nymphs	1	0	0	1	101	516	591	265	751	1253

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(Table 2-3). The moulting success of engorged nymphs of *H. truncatum* fed on *R. pumilio*, guinea-pigs and rabbits are presented in Table 2-5. The number of *H. truncatum* nymphs which moulted successfully into adults was lower for those nymphs that fed on rabbits following a two-host development, than for those that developed on the field mice and guinea-pigs.

H. marginatum rufipes: In the case of *H. marginatum rufipes*, the mean engorgement weights of nymphs fed on the field mice (*R. pumilio* and *L. rosalia*) following a three-host development were similar ($p>0.05$) to those of nymphs fed on guinea-pigs and rabbits following a two-host development pattern. However, a significant difference ($p<0.0001$) was observed between the blood-meal quantities ingested by nymphs emerging through a two-host pattern and those emerging through a three-host development pattern. The lowest blood-meal quantities ingested by nymphs, were recorded for those that followed a two-host development pattern while feeding on rabbits (Table 2-4). The moulting success of engorged nymphs of *H. marginatum rufipes* fed on *R. pumilio*, guinea-pigs and rabbits are presented in Table 2-5. Moulting success was high in both nymphs that developed through a two-host and a three-host life patterns.

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Table 2-3: Mean (± S.D.) engorgement weight (mg) and mean volume (µl) of blood ingested by *H. truncatum* nymphs on different hosts. Identical superscripts denote no significant differences based on the analysis of variance.

Parameter	Host				Statistic
	R.pumilio	*L. rosalia*	Guinea-pig	Rabbit	
Feeding pattern	3-Host	3-Host	2-Host	2-Host	
Engorgement weight	25.96±6.44[a]	35.00±7.81[b]	22.53±5.06[c]	22.47±6.19[c]	F=46.053 df = 3, 212 p<0.0001
n	47	58	29	82	
Blood volume	42.99±19.27[a]	42.25±17.72[a]	28.32± 7.68[b]	23.06 ± 8.77[c]	F = 16.998 df=3,164 P<0.0001
n	46	58	29	35	

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Table 2-4: Mean (± S.D.) engorgement weight (mg) and volume (µl) of blood ingested by *H. marginatum rufipes* nymphs on different hosts. Identical superscripts denote no significant differences based on the analysis of variance.

Parameter	Host				Statistic
	R. pumilio	*L. rosalia*	Guinea-pig	Rabbit	
Feeding pattern	3-Host	3-Host	2-Host	2-Host	
Engorgement weight	22.19± 5.77[a]	24.23 ± 6.06[a]	23.65± 11.88[a]	20.95 ± 6.71[a]	F=1.554 df = 3, 215 p>0.05
n	25	37	89	68	
Blood volume	39.39± 14.11[a]	50.98± 10.01[b]	36.16± 15.52[a]	16.01 ± 8.38[c]	F = 45.594 df=3,110 p<0.0001
n	26	29	25	34	

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Table 2-5: Moulting success of *H. truncatum* and *H. marginatum rufipes*nymphs following two and three-host feeding patterns on different hosts.

Tick	Host		
H. truncatum	*R. pumilio*	Guinea-pig	Rabbit
Feeding pattern	3-Host	2-Host	2-Host
No. incubated	92	113	66
Moulting success	89/92 (96.7%)	113/113 (100%)	48/66 (72.7%)
Sex ratio (♂:♀)	55:34	77:36	24:24
H. marginatum rufipes			
Feeding pattern	3-Host	2-Host	2-Host
No. incubated	104	129	158
Moultiung success	103/104 (99.9%)	129/129 (100%)	149/158 (94.3%)
Sex ratio (♂:♀)	65:38	81:48	98:51

2.4 Discussion

The observations made on the feeding patterns of *H. truncatum* and *H. marginatum rufipes* in this study compare favourably with those made by Geevarghese and Dhanda (1982) and Rechav and Fielden (1997) on *H. anatolicum anatolicum, H. dromedarii, H. marginatum isaaci* and *H. truncatum*. These authors demonstrated that the feeding patterns of these tick species depended on the host type. Likewise, in the current study it is apparent that *H. truncatum* is a three-host tick when feeding on the field mice *R. pumilio* and *L. rosalia*, a mixed two-host and three-host tick on guinea-pigs, and an exclusively two-host tick on rabbits. Given the fact that the field mice are regarded as the natural hosts for immatures of *H. truncatum* (Horak and MacIvor 1987; Rechav *et al.* 1987) it is reasonable to suggest that this tick species could be more of a three-host tick in the natural environment.

At least two subspecies of the genus *Hyalomma* do not conform to a three-host development pattern on small rodents. The larvae of *H. marginatum asaaci* do not attach well to white mice (Geevarhese and Dhanda 1982), and a similar low attachment success for the larvae of *H. marginatum rufipes* on *R. pumilio* and *L. rosalia* was found here (Table 2-2). The poor attachment success of *H. marginatum rufipes* larvae on *R. pumilio* and *L. rosalia* observed in this study further supports the observations from the survey studies made by Horak and MacIvor (1987) and Howel *et al.* (1978). These authors could not find the immatures of this tick species on small rodents. The poor attachment of *H.*

marginatum rufipes on two of the studied rodent species (*R. pumilio* and *L. rosalia*), coupled with the result that 58% and 100% of the attached larvae on guinea-pigs and rabbits respectively developed through a two-host pattern, suggests that this tick species has shifted more towards a two-host life pattern, than *H. truncatum* which had 30% and 100% of the attached larvae on guinea-pigs and rabbits, respectively developing through the two-host pattern.

The significantly higher mean engorgement weights and ingested blood volumes recorded on nymphs of *H. truncatum* which fed on the field mice (through the three-host pattern), indicate that these ticks performed better with regard to feeding than those that developed through a two-host pattern on guinea-pigs and rabbits. There are two possible explanations for this finding. Firstly, the comparison order taken here is between ticks feeding on their natural hosts and those feeding on non-natural hosts. Natural tick-host associations are often typified by inefficient or non-existent anti-tick resistance, since during the process of co-evolution with their hosts, ticks evolved means of avoiding or evading host reactions (Willadsen 1980; Ribeiro 1987, 1989). Secondly, because of a prolonged contact period between ticks and their hosts in a two-host development, nymphs developing through this pattern run a risk of feeding on hosts that have already developed some resistance to tick infestation (Clarke *et al.* 1989). Trager (1939), Allen (1973) and Boese (1974) established that the host's immune response to tick infestation starts 7-14 days after primary infestation. Given the prolonged contact periods (16 - 28 days for *H. truncatum*

and 17 - 30 days for *H. marginatum rufipes*) between ticks and hosts following a two-host development in the present study, it is reasonable to expect emerging nymphs to have fed on hosts that were already immunologically hostile. Nonetheless, these conclusions should be interpreted cautiously, given the range of animals and specimens used.

Ixodid ticks are known to have tendencies to ingest non-blood tissue and also to concentrate the blood-meal during the process of feeding. These tendencies have been shown to vary according to tick species and host type (Snow 1970; Kaufman and Phillips 1972; Kitaoka and Fujisaki 1975; Koch and Sauer 1984). This probably explains the results obtained from infestations of hosts with the nymphs of *H. marginatum rufipes* in this study. While no significant differences occurred between mean engorgement weights of the nymphs of this tick species following feeding on different hosts, the mean blood-meal quantities ingested were significantly different.

The survivorship of nymphs of *H. truncatum* which developed on rabbits through a two-host pattern, was lower than those of nymphs that fed on other hosts. Furthermore, with the exception of *H. truncatum* nymphs that developed on rabbits, that yielded equal proportions of males and females, all other groups yielded more males than females. Rechav *et al.* (1977) and Knight *et al.* (1978) obtained similar results with *Rhipicephalus evertsi evertsi* and *H. marginatum rufipes* and consider these ticks two-host species. However, Fielden *et al.* (1992)

established that more females resulted from nymphal moulting of *Amblyomma marmoreum* that is considered to be a three-host tick. It appears from the results obtained in this study, that the life development pattern in both *H. truncatum* and *H. marginatum rufipes* has no influence on the sex ratio of emerging adults. Once again these results should be treated with some caution because of relatively small sample sizes.

In conclusion, it appears that the life patterns followed by *H. truncatum* and *H. marginatum rufipes* depend on the host type. Therefore it is essential that any categorization or description of these two southern African *Hyalomma* ticks along the lines of life patterns, must consider the type of host on which development is taking place. In addition, models for the investigation of immunological aspects of tick-host interactions, and for the investigation of the effects of hosts on life patterns, should use natural, rather than non-natural hosts, or should at least acknowledge the pronounced difference in tick response found between these two groups. Furthermore, while both tick species demonstrate the capacity to develop through the two-host life pattern, *H. marginatum rufipes* appears to have proceeded more towards this pattern of development than *H. truncatum*. This provides some support for the idea that a two-host life pattern may be the derived form.

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CHAPTER THREE

The feeding performance of embedded and non-embedded nymphs of *Hyalomma truncatum* and *Hyalomma marginatum rufipes*

3.1 Introduction

Although ticks are typically haematophagous ectoparasites, reports on ticks occurring in subcuticular regions or deeper layers of the host's skin exist (Lebeda 1962; Tovornik 1984; Els *et al.* 1988; Els 1992). Such cases, where a tick becomes embedded in the host's skin, are thought to be rare and appear to be restricted to ticks of the genera *Ixodes* and *Hyalomma* when these ticks feed on their natural hosts. Schulze (1921) cited by Lebeda (1962) described the occurrence of the larvae and nymphs of *Ixodes canisuga* in the deeper layers of a horse's skin, while Lebeda (1962) and Tovornik (1984) discovered females of *I. ricinus* in the deeper layers of the skin of foxes (*Vulpes vulpes*). More recently, Els *et al.* (1988) and Els (1992) reported that nymphs of *H. truncatum* were embedded in the skins of their natural hosts, *Rhabdomys pumilio* and *Tatera brantsii*.

The importance of embedment of a tick in the skin of its host is still not clear. Previously, embedment of ticks in the host's skin during infestation was perceived as a form of resistance mounted by the host against ticks (Lebeda 1962; Torvonik 1984). This view needs further investigation since Els *et al.* (1988) and Els (1992) observed that the embedded nymphs of *H. truncatum* that

fed on their natural hosts (*R. pumilio* and *T. brantsii*), fed to full engorgement as did those that fed on these hosts without embedding. Thus, the present study examined the feeding performance of embedded and non-embedded nymphs of *H. truncatum* and *H. marginatum rufipes* on natural (*R. pumilio* and *Lemniscomys rosalia*) and non-natural (white laboratory rat) hosts by comparing the feeding periods, engorgement weights and the volumes of blood ingested by ticks in these two groups. To investigate the association between tick embedment and host resistance to tick infestation, I monitored the occurrence of embedment in post-primary infestations. The host's resistance to tick infestation was assessed by comparing the concentrations of serum proteins, in particular, beta (β) and gamma (γ) globulins prior to and after each infestation (see Clark *et al.* 1989; Rechav *et al.* 1989; 1994). The basis of this approach in assessing host resistance is the understanding that antibody globulins produced as a result of entry into the body by a foreign antigen, are present in the globulin portion of the plasma (Roitt 1988). These antibody globulins have the same electrophoretic mobility as beta or gamma globulins. Thus an increase in beta or gamma levels following tick infestation could result from production of antibody globulins.

3.2 Materials and methods

3.2.1 *Ticks*

Three weeks old nymphs of *H. truncatum* and *H. marginatum rufipes* used in this study, were obtained from laboratory colonies maintained in the Department of Biology at the Medical University of Southern Africa (MEDUNSA). These ticks

were maintained in glass humidity chambers at 25 ± 1^0 C, 75 ± 5% RH and normal light/dark regime (L:D, 14:10 h). The study was undertaken from May to December 1998.

3.2.2 *Hosts*

Laboratory bred natural hosts, *R. pumilio* and *L. rosalia,* used in this study were three to four months old and weighed 40 – 47 g and 45 – 55 g respectively. Four months old white laboratory rats of the strain Sprague Dawley weighing 268-305 g served as non-natural hosts for the ticks. These animals were housed individually during experimentation in plastic cages (34 cm x 34 cm x 22 cm) with screened metal floors. Each cage was suspended over a plastic tray, using large vials. This prevented rodents from treading on or eating the ticks that had dropped off. The edges of the plastic trays were coated with vaseline to prevent dropping ticks from crawling away. All animals used in this study were tick-naïve and kept in laboratory holding facilities at room temperature (20 – 25^{0}C) and normal light/dark (L:D, 14:10 h) regime in the Animal Production Unit at the Medical University of Southern Africa. The animals were fed pellets and water *ad libitum*.

3.2.3 *Infestation*

Feeding Performance: Two groups comprising of ten and five individuals of *R. pumilio* each, were formed. The first group was subjected to infestations with *H. truncatum* nymphs and the second group with *H. marginatum rufipes* nymphs. A

similar treatment was carried out on the two groups of *L. rosalia* comprising of eight and nine individuals each. A single group of five white laboratory rat individuals was subjected to infestations with *H. truncatum* nymphs. For *R. pumilio* and *L. rosalia* infestation was carried out by placing each host in a cotton bag (100 mm x 120 mm) containing ±40 nymphs. Larger cotton bags (140 mm x 220 mm) were used to infest laboratory rats with ± 50 nymphs per individual host. To facilitate attachment the hosts were shaved on their backs prior to infestation. The cotton bags were closed with staples to allow sufficient time for ticks to attach before the rodents freed themselves by chewing through the cotton bags. Thus time for attachment was approximately 1 hour. The hosts were monitored daily to observe engorging ticks. Embedded nymphs were easily identified in days 4 and 5 following infestation. Nymphs which did not embed were distinguished from those which embedded using an indelible marker. The engorgement weight of nymphs which dropped off the hosts was measured on an electronic balance sensitive to 0.1 mg (Mettler AE 100, Mettler Instruments, Greifensee, Switzerland).

Host Resistance: In each host type, *R. pumilio*, *L. rosalia* and the white laboratory rat, the animals were divided into the treatment and control groups of at least four individuals each. The treatment group was subjected to three consecutive infestations with the nymphs of *H. truncatum*. Infestation was carried by placing each host in a cotton bag containing about 40 nymphs of *H. truncatum*. Seven to ten days tick free intervals were allowed between

successive infestations. The control animals were treated the same as those in the treatment group save infestation with ticks. The tick parameter used to assess resistance was the average weight of engorged nymphs at drop off (see Heller-Haupt *et al.* 1981; Rechav *et al.* 1989; 1994 for rationale).

3.2.4 *Scanning electron micrographs*

A tick infested host (*L. rosalia*) was killed by suffocation using ether. Sections of the host's skin with attached ticks were fixed in 70% ethanol. After ultrasonic cleaning, they were dehydrated through graded ethanols, critical point dried, sputtercoated with carbon and gold, and thereafter viewed in a Leica Stereoscan 420 scanning electron microscope (SEM) at 5 to 10kV.

3.2.5 *Colorimetric estimation of blood-meal quantities*

The blood content of fed nymphs was converted to pyridine haemochromogen and measured spectrophotometrically by a method developed by Sutton and Arthur (1962) (modified by Snow 1970). In brief, each engorged nymph was homogenized in 0.1N NaOH (2 ml). Subsequently 0.5 g of sodium dithionate ($Na_2S_2O_4$) and 0.4 ml of pyridine were added. The mixture was then centrifuged (Mixtasel ce95 S Spania) at 2 000 rpm for 10 min. The absorbance of the clear supernatant was read with a Jenway 6300 spectrophotometer at 525 nm. Separate standard curves were prepared for each of the host types. The absorbance values obtained for fed ticks were compared with those of the blood of hosts on which they had fed. The concentration factor (CF) for each tick was

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calculated using the fo

CF = 1.058 x blood volume equivalent (µl) ÷ Engorgement Weight (mg) (assuming that the specific gravity of the host blood is 1.058) (Snow 1970).

The accuracy of this method depends on the fact that during feeding in ixodid ticks, only excess plasma water is secreted back into the host through salivation (Kaufman and Phillips 1973). The haemoglobin and its breakdown products are unaffected by this process.

3.2.6 *Blood samples*[1]

To assess the host's response to tick infestation serum protein analysis of host blood was carried. Prior to blood collection, each rodent was anaesthetized by injecting 0.3 ml (for *R. pumilio* and *L. rosalia*) and 0.7 ml (for the white laboratory rat) of sagatal (Pentobarbitone Sodium 6% m/v, Animal Health SA, Halfway House, South Africa) intraperitoneally. About 0.35 ml of blood was drawn from the heart by cardiac puncture and was collected into Microtainer Serum Seperator tubes (Becton Dickson, New Jersey, USA). Blood samples were taken a day before the primary infestation and a day after the last drop off of ticks in each of the three consecutive infestations. To reduce damage to the heart, small needles (26G) fitted to 1 ml disposable syringes were used to draw blood from each host.

[1]All procedures used for collection of blood sample were approved by the Animal Ethics Committee of MEDUNSA

3.2.7 *Serum protein electrophoresis (SPE)*

The procedure carried was described by Rechav *et al.* (1989). In brief, about 2 µl of serum obtained from clotted blood samples was applied on cellulose acetate membranes (Helena laboratories, Beaumont, Texas, U.S.A). Electrophoresis was conducted for 30 minutes in a barbital/sodium barbital buffer with a pH of 8.6 (Helena Laboratories) and staining was done in Paragon red stain solution for 15 minutes. The membranes were de-stained in 10% acetic acid before being scanned with an Appraise Densitometer (Beckman Instruments, Fullerton, Califonia) and were separated into the following fractions: albumin, alpha 1 (α_1), alpha 2 (α_2), beta (β) and gamma (γ) globulins (Fig. 3-1). Total protein levels were quantitated with an Astra 8 automated analyzer (Beckman Instruments) using the Biuret reaction method.

3.3 Results

3.3.1 *Proportion and feeding periods of embedded and non-embedded ticks*

The difference between tick embedment and non-embedment can be clearly seen in the scanning electron micrographs (Figs. 3-2 a, b, c). The nymphs of *H. truncatum* and *H. marginatum rufipes* that fed on *R. pumilio* and *L. rosalia* showed a predilection for site, attaching mostly around the neck and posterior parts of the head, extending to the anterior dorsal region of the trunk.

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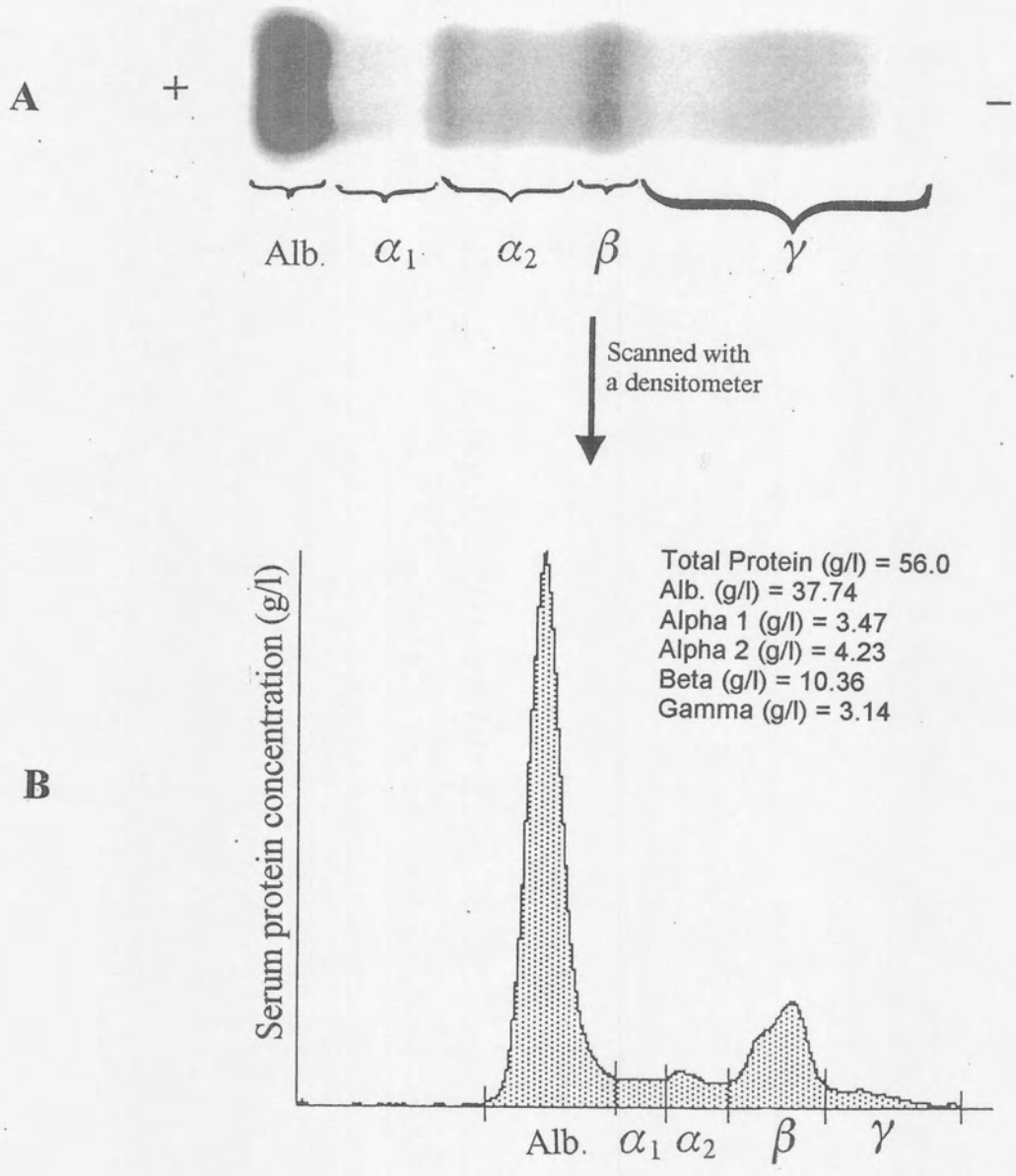

Fig. 3-1: Electrophoresis of *R. pumilio* serum on cellulose acetate membrane showing Alb. (Albumin), α_1(alpha 1), α_2 (alpha 2), β (beta) and γ (gamma) globulin bands (A). Quantitated electrophoresis profile of *R. pumilio* serum (B).

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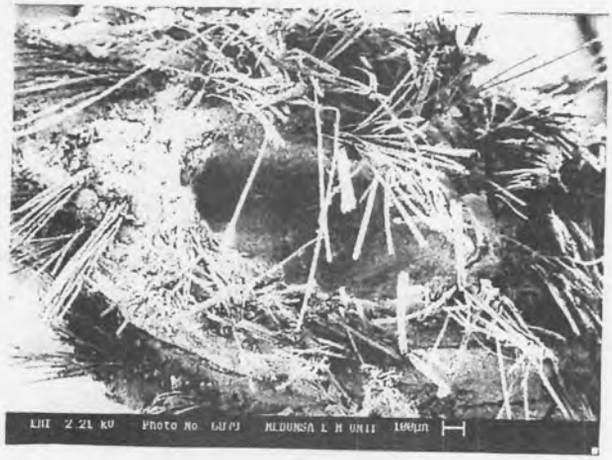
(a) An engorging nymph of *H. truncatum* embedded in the skin of *L. rosalia*→



(b) A pit in which a nymph of *H. truncatum* ← was embedded in the skin of *L. rosalia*

(c) An engorging nymph of *H. truncatum* with only the capitulum inserted in the skin of *L. rosalia*→

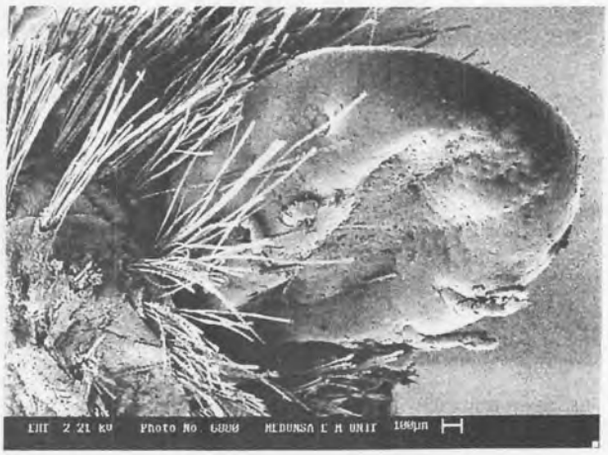


Fig. 3-2: Scanning electron-microscope pictures showing (a) embedded and (c) non embedded nymphs of *H. truncatum* in the skin of *L. rosalia* and an empty pit (b) left by a detached nymph

Although the larvae of *H. marginatum rufipes* failed to attach on *R. pumulio* and *L. rosalia* (see chapter 2), the nymphs of this tick species attached successfully on these hosts. A high proportion of the nymphs of both *H. truncatum* and *H. marginatum rufipes* ticks that attached to the hosts *R. pumilio* and *L. rosalia* were embedded in these hosts' skins (Figs. 3-3 & 3-4). Only 9.1% of the nymphs of *H. truncatum* that fed on the laboratory rat were embedded (Fig. 3-5). The mean feeding period of *H. truncatum* nymphs which fed on *R. pumilio* was 8.6±1.0 days (range: 6-10 days, n=45). This mean feeding period for embedded nymphs was significantly longer ($t=3.2$, $p<0.01$) than the 7.7±1.3 days (range: 6-9, n=27) of non-embedded nymphs. On *L. rosalia*, the mean feeding periods of embedded and non-embedded nymphs of *H. truncatum* were not significantly different (mean number of days in embedded and non embedded nymphs was approximately 8.5 days, $t=0.086$, $P>0.05$). The mean feeding period of embedded *H. marginatum rufipes* nymphs fed on *R. pumilio* was significantly longer (9.8±1.7 dayst=2.3, $p<0.05$) than the 8.6 ±1.2 days of non-embedded ones. A significant difference ($t=4.0$, $p<0.001$) occurred between the longer mean feeding period (10.2± 0.8 days, range 9-12 days, n=49) of embedded nymphs of *H. marginatum rufipes* nymphs fed on *L. rosalia* compared to that of non-embedded ones (9.7± 0.5 days, range: 8-10, n=19). A similar significant difference ($t=3.4$, $p<0.01$) was found between the longer feeding periods (9.8± 0.8 days, range 9-11) of embedded *H. truncatum* nymphs fed on the white laboratory rat than that of non-embedded ones (8.4± 1.1 days, range: 6-10).

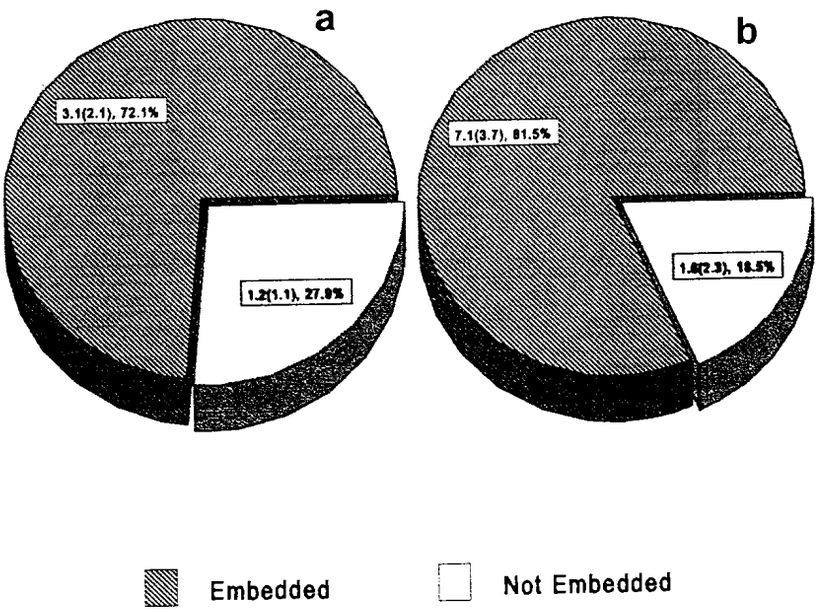


Fig. 3-3: Mean (±S.D.) proportion of embedded and non-embedded nymphs of *H. truncatum* fed on (a) *R. pumilio* (n=10, t = 2.5, p<0.05) and (b) *L. rosalia* (n=8, t = 3.59, p < 0.01)

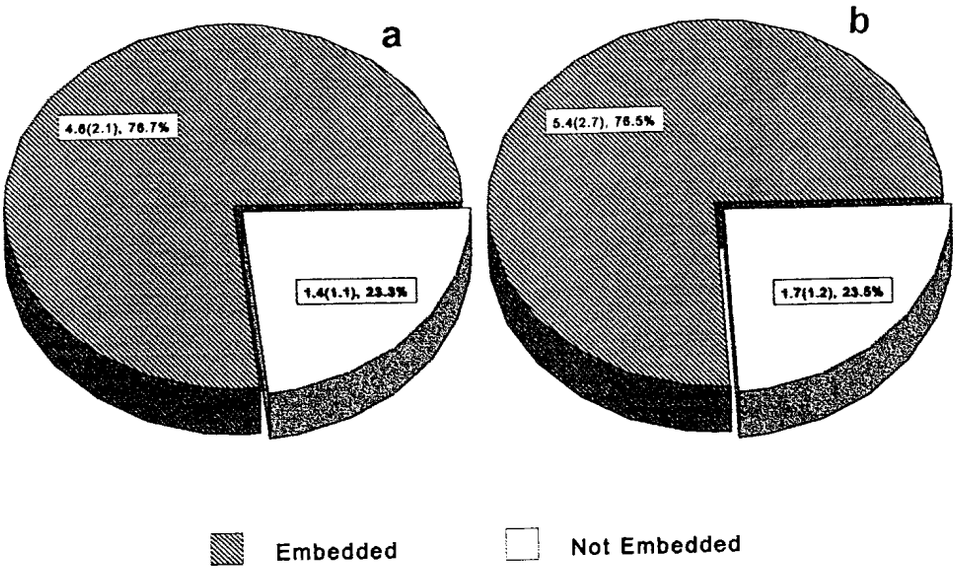


Fig. 3-4: Mean (±S.D.) proportion of embedded and non-embedded nymphs of *H. marginatum rufipes* fed on (a) *R. pumilio* (n=5, t = 3.14, p < 0.05) and (b) *L. rosalia* (n=9, t = 3.77, p<0.01)

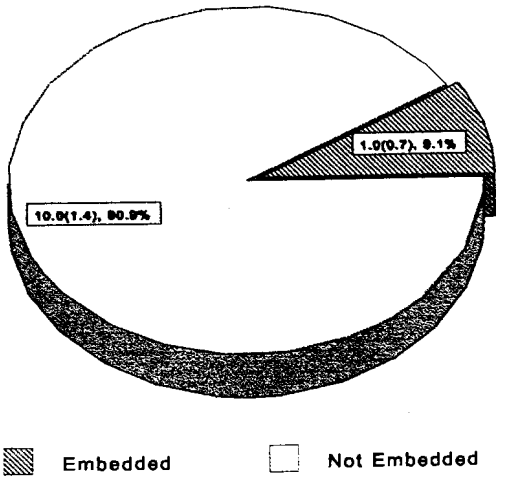


Fig. 3-5: Mean (±S.D.) proportion of embedded and non-embedded nymphs of *H. truncatum* fed on the white laboratory rat (n=5, t=10.58, p<0.0001)

3.3.2 *Engorgement weight*

The mean engorgement weights of nymphs which embedded and of those which did not embed were not significantly different (Table 3-1, p>0.05) in both *H. truncatum* and *H. marginatum rufipes*. However, the mean engorgement weights of the nymphs of *H. truncatum* that fed on *L. rosalia* were significantly higher (p<0.0001) than those of the nymphs which fed on other hosts (Table 3-1). In *H. marginatum rufipes*, no significant differences (Table 3-2, p>0.05) were recorded between mean engorgement weights of nymphs that fed on *R. pumilio* and *L. rosalia*.

The mean weights of engorged ticks following repeated infestation of hosts with the nymphs of *H. truncatum* are given in Table 3-3. The embedment of the nymphs of *H. truncatum* in the skins of *R. pumilio* and *L. rosalia* occurred in each of the three consecutive infestations and was the most prevalent form of attachment in each infestation. However, on the laboratory rat, embedment of *truncatum* was only observed during the primary infestation and appeared to be the least common type of attachment (Table 3-3). Repeated infestations of the hosts with the nymphs of *H. truncatum* resulted in a significant ($p<0.01$) decline in mean engorgement weights only in those nymphs that fed on the laboratory rat. The decline consecutive infestations and was the most prevalent form of attachment in each infestation. However, on the laboratory rat, embedment of the nymphs of *H. truncatum* was only observed during the primary infestation and appeared to be the least common type of attachment (Table 3-3). Repeated infestations of the hosts with the nymphs of *H. truncatum* resulted in a significant ($p<0.01$) decline in mean engorgement weights only in those nymphs that fed on the laboratory rat. The decline in the mean engorgement weight was the lowest in the secondary infestation (Table 3-3). The mean engorgement weights of embedded and non-embedded nymphs of *H. truncatum* that fed on each of the three host types (*R. pumilio, L. rosalia* and the white laboratory rat) were not significantly different ($p>0.05$) in each of the three infestations (Table 3-3).

3.3.3 *Blood volume ingested by nymphs*

There was no significant difference ($p>0.05$) between mean blood volumes of

nymphs of *H. truncatum* that fed on different hosts (Table 3-1). However, for *H. marginatum rufipes*, a significant difference ($p<0.05$) occurred between the mean blood volumes of nymphs that fed on *R. pumilio* and those that fed on *L. rosalia*. The blood-meal was significantly less concentrated ($p<0.0001$) in nymphs of *H. truncatum* that fed on *L. rosalia* when compared with those that fed on *R. pumilio* and the laboratory rat (Table 3-1). For *H. marginatum rufipes*, the ingested blood-meal was significantly more concentrated in nymphs that fed on *L. rosalia* than in those that fed on *R. pumilio* (Table 3-2).

In both *H. truncatum* and *H. marginatum rufipes*, ticks of similar engorgement weights showed variation in blood volume ingested, although there was a significant positive relationship between the engorgement weight and blood intake in both tick species, on each of their hosts (Figs. 3-6 – 3-10).

Table 3-1: Mean engorgement weight (mg) ± S.D., mean blood volume equivalent (µl) ± S.D. and mean concentration factor ± S.D. of nymphs of *H. truncatum* fed on various hosts

Host:	*R. pumilio*		*L. rosalia*		Lab rat	Statistics
Attachment type:	Emb	NEmb	Emb	NEmb	NEmb	
Mean engorgement weight (mg)	26.57±7.11[a]	24.26 ±4.83[a]	34.65±7.46[b]	35.66±9.46[b]	24.93±6.46[a]	F=16.16 DF=4;145 P<0.0001
n	31	17	38	20	44	
Mean blood volume (µl)	43.02±19.57	42.92±19.33	39.83±15.09	46.84±21.55	44.02±12.1	F=0.64 DF=4;143 P>0.05
n	29	17	38	20	44	
Mean concentration factor	1.73 ±0.64[a]	1.95±0.76[a]	1.24± 0.41[b]	1.36±0.44[b]	1.91±0.38[a]	F=12.42 DF=4;143 P<0.0001
n	29	17	38	20	44	

1. Same superscript in a row indicates no significant difference (Anova, t-test)
2. Emb = Embedded ; NEmb = Not embedded ; S.D. = Standard deviation

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Table 3-2: Mean engorgement weight (mg) ± S.D., mean blood volume equivalent (µl) ± S.D. and mean concentration factor± S.D. of *H. marginatum rufipes* fed on *R. pumilio* and *L. rosalia*

Host:	*R. pumilio*		*L. rosalia*		Statistics
Attachment type:	Emb	NEmb	Emb	NEmb	
Mean engorgement weight (mg)	22.34±6.12	21.57± 5.22	25.47±4.05	25.57±2.10	F=1.826 DF=3;56 P>0.05
n	23	9	17	11	
Mean blood volume (µl)	40.79±4.36[a]	39.36±13.24[a]	52.99±11.42[b]	48.65±9.35[b]	F=4.009 DF=3;56 P<0.05
n	23	9	17	11	
Mean concentration factor	1.90±0.33[a]	1.89±0.29[a]	2.21±0.40[b]	2.10±0.44[b]	F=2.810 DF=3;56 P<0.05
n	23	9	17	11	

1. Same superscript in row indicates no significant difference (Anova, t-test)
2. Emb=Embedment, NEmb=not embedded

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Table 3-3: Mean weight (mg) ± S.D. of engorged nymphs of *H. truncatum* that fed on various hosts during primary, secondary and tertiary infestations

HOST	INFESTATION			STATISTICS
	Primary	*Secondary*	*Tertiary*	
R. pumilio				
Embedded	22.58± 4.52[a]	25.27± 7.60[b]	27.50±7.57[b]	F=3.56;DF=2;56;P<0.05
n	34	11	14	
Non-Embedded	22.85±4.83	26.60± 8.75	24.32±6.61	F=1.27;DF=2;42;P>0.05
n	26	9	10	
	P>0.05	P>0.05	P>0.05	
L. rosalia				
Embedded	34.66±7.16	35.17±8.88	29.98±7.10	F=2.73;DF=2;82;P>0.05
n	38	30	17	
Non-Embedded	35.66±9.08	32.55±10.07	29.38±7.06	F=1.59;DF=2;36;P>0.05
n	20	10	9	
	P>0.05	P>0.05	P>0.05	
Lab rat				
Embedded	24.86± 5.13	Nemb	Nemb	
n	5			
Non-Embedded	24.93± 6.46[a]	21.64 ±4.80[b]	22.42± 2.92[a]	F=5.69;DF=2;14;P<0.01
n	64	56	19	
	P>0.05			

Same superscript in a row indicates no significant difference (Anova, t- test). Nemb=no embedment

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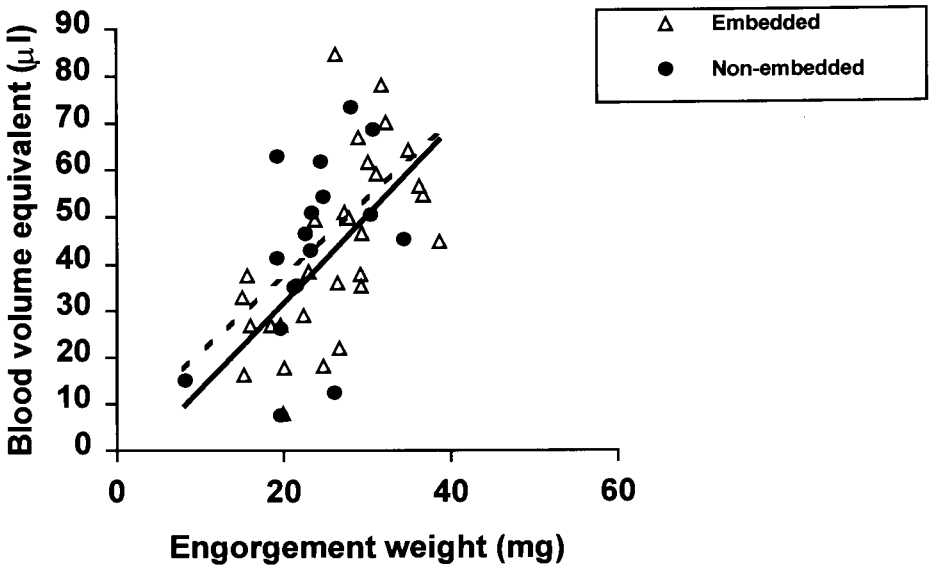

Fig. 3-6: Association between weight of engorgement (mg) and the volume of blood (µl) ingested by embedded (solid line: Y= 1.857X – 5.8437, R^2 = 0.409, $F_{(1;27)}$=18.692, P<0.001) and non-embedded (dashed line: Y=1.668X + 4.042, R^2 = 0.262, $F_{(1;15)}$=5.336, P<0.05) nymphs of *H. truncatum* fed on *R. pumilio*

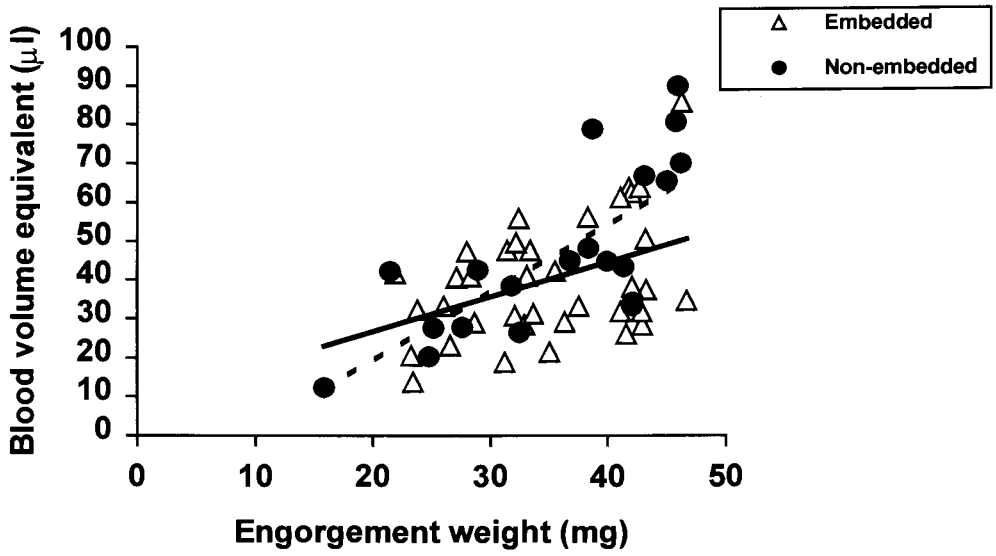

Fig 3-7: Association between weight of engorgement (mg) and the volume of blood (µl) ingested by embedded (solid line: Y=0.894X + 8.84, R^2=0.18, $F_{(1;36)}$=7.903, P<0.01) and non-embedded (dashed line: Y = 1.768X-16.223, R^2=0.556, $F_{(1;18)}$=22.501, P<0.01) nymphs of *H. truncatum* fed on *L. rosalia*

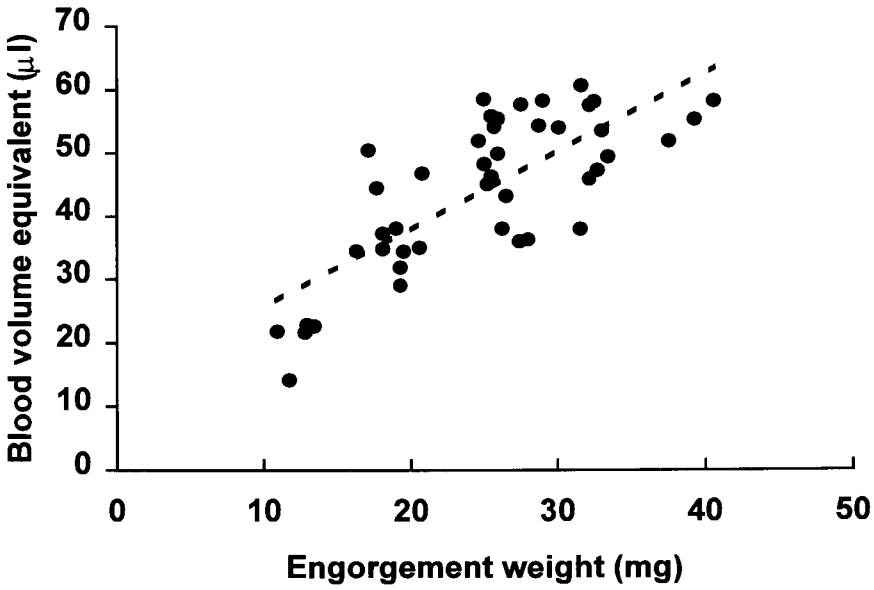

Fig. 3-8: Association between weight of engorgement (mg) and the volume of blood (µl) ingested by non-embedded (Y= 1.241X + 13.104, R^2 = 0.583, $F_{(1;42)}$=58.701, P<0.0001) nymphs of *H. truncatum* fed on the laboratory rat (data on embedded nymphs was not included due to small sample size)

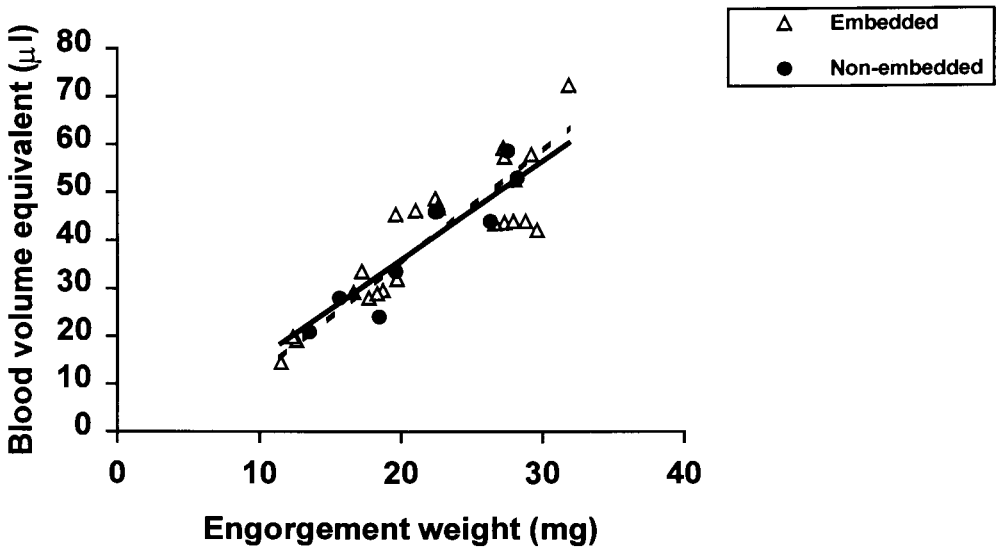

Fig. 3-9: Association between weight of engorgement (mg) and volume of blood (µl) ingested by embedded (solid line: Y = 2.053X – 5.075, R^2 = 0.766, $F_{(1;21)}$=68.969, P<0.0001) and non-embedded (dashed line: Y = 2.364X – 11.635, R^2 = 0.869, $F_{(1;8)}$= 46.601, P<0.001) nymphs of *H. marginatum rufipes* fed on *R. pumilio*

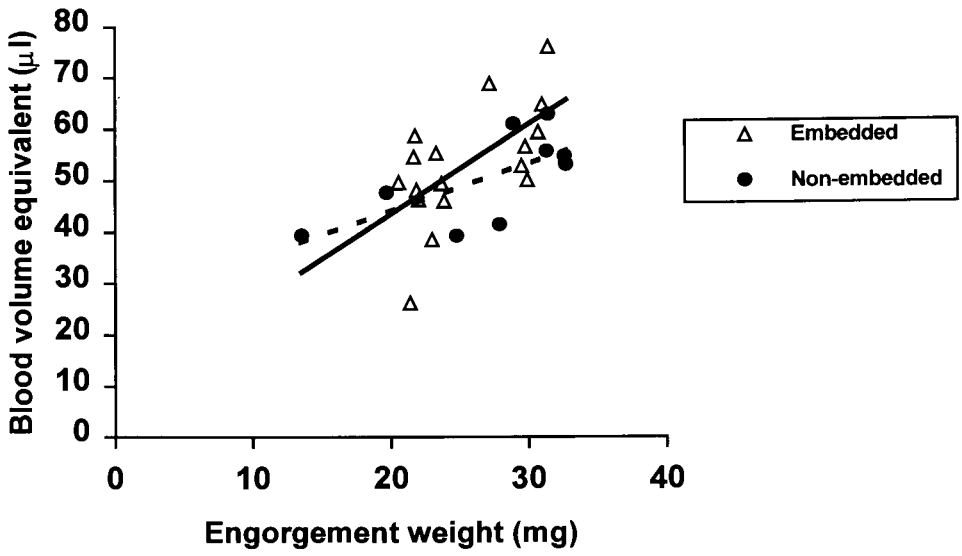

Fig. 3-10: Association between weight of engorgement (mg) and volume of blood (µl) ingested by embedded (solid line: Y= 1.729X + 8.957, R^2 = 0.375, $F_{(1;15)}$=9.01, P<0.01) and non-embedded (dashed line: Y = 1.035X + 22.177, R^2 = 0.595 $F_{(1;9)}$=13.201, P<0.01) nymphs of *H. marginatum rufipes* on *L. rosalia*

3.3.4 *Beta globulins*

Although the levels of beta globulins in *R. pumilio* and *L. rosalia* declined following the tertiary infestation, the decline was only significant (p<0.05) in *L. rosalia* (Table 3-4). Furthermore, beta globulin levels in *L. rosalia* of the treatment group were significantly higher (P<0.05) than those in the blood of the control animals after the primary infestation (Table 3-4). The level of beta globulins in the blood of the laboratory rat remained similar (P>0.05) in both the treatment and control animals throughout the duration of the three infestations.

However, a statistical comparison between the treatment and the control groups of the laboratory rat showed that the levels of beta globulins in the former group were significantly higher ($p<0.05$) after the tertiary infestation (Table 3-4).

3.3.5 *Gamma globulins*

The gamma globulin levels in the blood of *R. pumilio* did not change significantly ($p>0.05$) throughout the three consecutive infestations (Table 3-5). However, significant ($p<0.05$) decline in the gamma globulin levels was recorded in the blood of the treatment group of *L. rosalia* after the tertiary infestation (Table 3-5). Furthermore, the gamma globulin levels in the treatment group of *L. rosalia* were significantly higher than those in animals of the control group after the primary and secondary infestations (Table 3-5).

Also, the level of gamma globulins in the control animals of *L. rosalia* recorded prior to the primary infestation, was significantly ($p<0.05$) higher than those recorded after each of the three infestations. In the laboratory rat, the gamma globulin levels increased significantly ($p<0.05$) following repeated infestations, reaching the highest levels after the second infestation (Table 3-5). Furthermore, the mean gamma globulin levels of animals in the treatment group were significantly ($p<0.05$) higher than those of animals in the control group after each of the three consecutive infestations.

Table 3-4: Mean beta (β) globulin levels (g/l) ± S.D. in the blood of various hosts prior to the first infestation and after each of the primary, secondary and tertiary infestations with the nymphs of *H. truncatum*

HOST	INFESTATION				STATISTICS
	Zero	*Primary*	*Secondary*	*Tertiary*	
R. pumilio					
Treatment	8.21±0.18	7.49± 1.06	8.19±1.55	6.87±0.81	F=1.92;DF=3;19;P>0.05
n	6	5	6	5	
Control	8.21± 0.18	11.59± 3.48	8.67±2.55	8.69± 0.37	F=2.53;DF=3;14;P>0.05
n	6	4	4	4	
	P>0.05	P>0.05	P>0.05	P>0.05	
L. rosalia					
Treatment	9.55± 1.67[a]	10.76± 0.92[a]	10.44 ±1.61[a]	7.97±1.69[b]	F=3.59;DF=3;20;P<0.05
n	8	6	5	5	
Control	9.55±1.67	9.30 ±1.12	9.02 ±1.04	8.79±0.85	F=0.32;DF=3;16;P>0.05
n	8	4	4	4	
	P>0.05	P<0.05	P>0.05	P>0.05	
Lab. Rat					
Treatment	11.23± 1.29	10.52± 1.20	11.24 ±0.93	12.43± 0.96	F=2.09;DF=3;15;P>0.05
n	4	5	5	5	
Control	11.23±1.29	10.06± 0.79	10.34 ±0.69	10.11± 0.74	F=3.19;DF=3;15;P>0.05
n	4	5	5	5	
	P>0.05	P>0.05	P>0.05	P<0.05	

Same superscript in a row indicates no significant difference (Anova, t-test). Nemb=no embedment

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Table 3-5: Mean gamma (γ) globulin levels (g/l) ± S.D. in the blood of various hosts prior to the primary infestation and after each of the primary, secondary and tertiary infestations with the nymphs of *H. truncatum*

HOST	INFESTATION				STATISTICS
	Zero	Primary	Secondary	Tertiary	
R. pumilio					
Treatment	4.54 ±1.12	3.32± 0.92	5.27± 2.21	3.33± 0.32	F=2.59;DF=3;18; P>0.05
n	6	5	5	5	
Control	4.54 ±1.12	4.46± 2.14	3.17± 2.41	4.46± 1.81	F=0.57;DF=3;16;P>0.05
n	6	4	4	4	
	P>0.05	P>0.05	P>0.05	P>0.05	
L. rosalia					
Treatment	7.98 ±3.41[a]	6.48± 0.49[a]	7.35 ±0.97[a]	4.56 ±0.86[b]	F=10.82;DF=3;19;P<0.05
n	8	6	5	5	
Control	7.98 ±3.41[a]	4.21± 1.13[b]	4.05± 0.32[b]	3.95± 0.80[b]	F=14.83;DF=3;15;P<0.05
n	8	4	4	4	
	P>0.05	P<0.05	P<0.05	P>0.05	
Lab. Rat					
Treatment	2.91± 0.81[a]	4.11 ±1.43[a]	6.85± 2.26[b]	5.12 ±1.62[b]	F=4.68;DF=3;15;P<0.05
n	4	5	5	5	
Control	2.91± 0.81	2.61± 0.46	2.54 ±0.51	2.95 ±0.60	F=1.34;DF=3;15;P>0.05
n	4	5	5	5	
	P>0.05	P<0.05	P<0.05	P<0.05	

Same superscript in a row indicates no significant difference (Anova, t-test). Nemb=no embedment

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3.4 Discussion

3.4.1 *The occurrence of embedment on R. pumilio, L. rosalia and laboratory rat*

Predilection for attachment sites on *R. pumilio* and *L. rosalia* demonstrated by nymphs of *H. truncatum* in this study substantiates observations made by Els *et al.* (1988) and Els (1992). From their field study, these authors observed that the predominant occurrence of the nymphs of this tick species is around the neck and posterior parts of the head in the rodents *R. pumilio* and *T. brantsii*. *H. marginatum rufipes* nymphs showed similar preference for attachment sites on these hosts.

Observations of the occurrence of embedment of *H. truncatum* nymphs on *L. rosalia*, coupled with the view that tick embedment is found in certain tick species only when they feed on their natural hosts (Lebeda 1962; Torvonik 1984; Els *et al.* 1988 and Els 1992) suggests that *L. rosalia* is a natural host of this tick species. Poor evidence of nymphal embedment on white laboratory rats, further supports the view that this phenomenon is mostly an occurrence of certain ticks when they feed on their natural hosts. In the present study the mean feeding periods of embedded nymphs of *H. truncatum* fed on *R. pumilio*, *L. rosalia* and the white laboratory rat and that of *H. marginatum rufipes* nymphs fed *on R. pumilio* were significantly longer than those of non-embedded ones. These results support those of Els *et al.* (1988) and Els (1992) who also established that the nymphs of *H. truncatum* spent prolonged periods on their hosts *R. pumilio* and *T. brantsii* compared to non-embedded ones. According to these

authors the prolonged contact association between ticks and their hosts, which results from embedment, may contribute towards tick dispersal since the host can cover wider distances during such prolonged contact periods.

3.4.2 *Blood volume and engorgement weight*

The results of this study also show that a positive relationship exists between engorgement weight and the volume of blood ingested by ticks. However, there is considerable variance in the relationship indicating that blood volume alone does not contribute to engorgement weight. Other factors, the most important of which is the ingestion of non-blood tissue, could play a role in this regard. It is known that ticks often ingest non-blood tissue together with their blood-meal during feeding on their hosts (Sutton and Arthur 1962; Snow 1970; Kitaoka and Fujisaki 1975; Koch and Sauer 1984). Such non-blood tissue ingested together with the blood-meal, undoubtedly contributes towards the total engorgement weight of ticks. The mean engorgement weights of nymphs of *H. truncatum* which fed on *L. rosalia* in this study were significantly higher ($p<0.0001$) than those of nymphs which fed on other hosts, yet the blood volume of those nymphs were statistically similar to those of nymphs which fed on other hosts. This result indicates that the nymphs which fed on *L. rosalia* ingested considerable non-blood tissue compared to those which fed on other hosts.

Lack of significant differences between the engorgement weights of embedded and non-embedded nymphs observed in this study, indicates that the feeding

performance of embedded nymphs was in no way impaired compared to that of non-embedded nymphs. This result supports the findings by Els *et al.* (1988) and Els (1992) who established similar results on *H. truncatum* nymphs feeding on *R. pumilio* and *T. brantsii*. Lebeda (1962) and Tovornik (1984) previously argued that embedded ticks cannot feed to full engorgement as a result of the restriction imposed on them by the host skin. Clearly, this view is not supported by the results obtained in this study and those of Els *et al.* (1988) and Els (1992) on the engorgement weights of embedded *Hyalomma* ticks. However, it is important to note that Lebeda (1962) and Tovornik (1984) studied ticks of the genus *Ixodes* and thus the different result may be due to the specifics of the tick-host association.

Ticks concentrate their blood-meal by intermittently salivating excess water into the host during feeding (Kaufman and Phillips 1973). In their studies with females of *Dermacentor andersoni*, Kaufman and Phillips (1973) established that 74% of water loss in this tick takes place through the salivary glands. Rectal excretions accounted for 20-25% of water loss when water loss through the integument was extremely low (3%). In their *in vitro* study Willadsen *et al.* (1984) observed that females of *B. microplus* showed more ability to concentrate host blood when fed bovine blood. The ability to concentrate host blood in this tick species was less evident when the tick was fed blood of laboratory hosts (rat, guinea-pig and rabbit). In this study, although both *H. truncatum* and *H. marginatum rufipes* nymphs showed the ability to concentrate host blood, blood

was less concentrated in *H. truncatum* nymphs that fed on *L. rosalia* and more concentrated in *H. marginatum rufipes* nymphs that fed on the same host. This result suggest that blood-meal concentration not only depends on the tick species but also on the host type. The cover imposed by the host skin on ticks during embedment appears not to have influenced the concentration of blood meal since no significant differences occurred between the concentration factors of embedded and non-embedded nymphs.

3.4.3 *Embedment and host resistance*

The results of this study also show that embedment of the nymphs of *H. truncatum* in the skins of *R. pumilio* and *L. rosalia* (natural hosts) occurred throughout the three successive infestations and in each infestation appeared to be the most prevalent type of attachment. This finding, coupled with the observation that on the laboratory rats (non-natural hosts) embedment of *H. truncatum* nymphs was the less prevalent type of attachment and was only restricted to the primary infestation further substantiates the view by Lebeda (1962), Els *et al.* (1988) and Els (1992) that this phenomenon is a common feature of the interaction between ticks and their natural hosts.

Many authors (Wikel 1982; Heller-Haupt *et al.* 1981; Rechav *et al.* 1989; Fivas *et al.* 1991) regard a decline in feeding performance by ticks, expressed by reduced engorgement weights, as the most accurate criterion for assessing host resistance. The results of the present study, show that the mean engorgement

weight of the nymphs of *H. truncatum* that fed on *R. pumilio* and *L. rosalia* did not decline following the three successive infestations. However, the mean engorgement weights of *H. truncatum* nymphs that fed on the laboratory rat declined significantly ($p<0.01$). The decline in engorgement weight when ticks feed repeatedly on non-natural hosts is a well known phenomenon (Clark *et al.* 1989; Rechav *et al.*1989; Rechav *et al.* 1994). The ability of *H. truncatum* nymphs to feed to full engorgement on *R. pumilio* and *L. rosalia* observed in this study could be attributed to the natural tick host association which probably exists between this tick species and *R. pumilio* and *L. rosalia*. Willadsen (1980) and Ribiero (1987, 1989) reported that inefficient or non-existent anti-tick resistance often characterizes natural tick-host associations since during the process of co-evolution with their hosts, ticks evolved means of avoiding host reactions. Since the laboratory rats are not the natural hosts for the nymphs of *H. truncatum*, the salivary secretions of this tick probably stimulated both humoral and cellular immunity in the host resulting in observed significant decline in engorgement weights. Furthermore, the feeding performance of embedded nymphs was not impaired even during post primary infestations since no significant differences between mean engorgement weights of embedded and non-embedded nymphs were recorded. This further suggests that the immunological response of the hosts to both embedded and non-embedded nymphs was similar.

Lack of significant increases in β- and γ-globulins in blood sera of *R. pumilio* and

L. rosalia following repeated infestations with nymphs of *H. truncatum*, suggest that these hosts did not develop resistance to ticks. This result, coupled with the fact that embedment occurred during post-primary infestations on *R. pumilio* and *L. rosalia*, suggest that embedment is not a direct manifestation of host resistance. There is increasing evidence that some vertebrate hosts do not acquire immunity against some tick species that commonly parasitise them (Ribeiro *et al.* 1985; Champagne 1994; Fivaz 1989; Bowman *et al.* 1997; Wikel 1999). This is probably due to the presence of some factors in the saliva of such ticks which are immunosuppressive (Bowman *et al.* 1997; Wikel and Bergman 1997; Wikel 1999). However, γ-globulins in the blood serum of the white laboratory rat increased significantly ($P<0.01$) following repeated infestations. Since the laboratory rats are non-natural hosts for *H. truncatum*, salivary antigens of this tick species are likely to induce production of antibodies.

Knowledge on the immunosuppressive capabilities of *H. truncatum* is still lacking. However, Joubert *et al.* (1995) characterized an anticoagulant factor in the saliva of the females of *H. truncatum*. The occurrence of immunosuppressive factors such as prostaglandin E_2 (PGE_2) has been demonstrated in other tick species, for example *Ixodes dammini* (Ribeiro *et al.* 1985) and *Amblyomma americanum* (Bowman *et al.* 1995). In the light of *H. truncatum* nymphs having failed to stimulate resistance in *R. pumilio* and *L. rosalia*, it is reasonable to suspect the occurrence of immunosuppressive factors in the saliva of these ticks. Immunosuppressive factors in tick saliva do not only facilitate tick feeding,

but also favour the transmission of pathogens from ticks to hosts (Jones *et al.* 1989, 1990, 1992; Bowman *et al.* 1997; Wikel and Bergman 1997; Wikel 1999). In the light of this, and also considering the fact that *H. truncatum* plays a significant role in the transmission of the Crimean-Congo Hemorrhagic Fever (CCHF) virus to man and animals (Swanepoel *et al.* 1983; Logan *et al.* 1989; Shepherd *et al.* 1989; Rechav 1991; Scott *et al.* 1993), it becomes clear that further research aimed at identifying immunosuppressive factors in the saliva of *H. truncatum* is necessary.

3.5 References

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CHAPTER FOUR

Gas exchange in *Hyalomma truncatum* ticks

4.1 Introduction

Gas exchange has been well studied in insect arthropods (see review by Lighton 1996). In contrast, this subject has not received as much attention in ticks despite the medical and veterinary importance of these ectoparasites. Earlier work on gas exchange in ticks (Maklygin and Alexeyev 1960 cited by Fielden *et al.* 1994 and Belozerov 1966) was restricted to only the measurement of the respiratory metabolism. However, recent work by Lighton *et al.* (1993), Fielden *et al.* (1994, 1999) which respectively entailed measurements of CO_2 release in *Amblyomma marmoreum*, *A. hebraeum* and *Dermacentor variabilis* ticks, using a computerized flow-through respiratory system, has shed some light on the ventilatory patterns of ticks. Lighton *et al.* (1993) and Fielden *et al.* (1994) demonstrated that unfed nymphs and females of *A. marmoreum* and *A. hebraeum*, like insects, have the capacity to ventilate discontinuously. Discontinuous ventilation is compatible with the passive suction ventilation model (Kestler 1985; Slama 1988) which describes a ventilation cycle consisting of three phases:

(i) the Closed phase: during which the spiracles are closed with little or no external gas exchange

(ii) the Flutter phase: during which the spiracles open intermittently allowing a slow ingress of O_2, but little egress of CO_2

(iii) the Open phase during which the spiracles open completely and CO_2 escapes rapidly by diffusion

Evidence that discontinuous gas exchange contributes towards the reduction of respiratory water loss was provided by Rudolph and Knulle (1979). These authors demonstrated that water loss in females of *A. variegatum* increased seventeen-fold in 0% RH as well as in 93% RH when spiracles were opened. This increase in water-loss disappeared when the spiracles were blocked with paraffin wax. However, the contribution to water saving of discontinuous gas exchange cycles has been questioned in insects (Lighton 1996,1998) and ticks (Fielden *et al.* 1999).

So far, discontinuous gas exchange cycles (DGC) in ticks have been demonstrated only in unfed ticks and appeared not to be a feature of engorged ticks (Lighton *et al.* 1993; Fielden *et al.*1994; Rechav and Fielden 1995 and Fielden *et al.* 1999). The assertion made to explain this observation is that engorged ticks do not face water loss problems since the vertebrate host blood is known to be hyposmotic to tick body fluids (Kaufman and Sauer 1982). This view should be carefully considered since ticks concentrate their blood-meal by secreting excess water from the blood-meal back into the host during the feeding process (see Kaufman and Phillips 1973; Meredith and Kaufman 1973; Binnington 1978). This implies that the water content of host-blood in the tick body is low compared to that in the host itself. As a result, water may still remain a limited resource even in engorged ticks that have dropped off from the host, necessitating these ticks to use water loss

regulating mechanisms. Recently, Fielden *et al.* (1999) established that feeding *D. variabilis* females that have been forcibly removed from their hosts demonstrated DGCs in days 1-6 following attachment. During the last and final stages of feeding (days 7-9) discontinuous CO_2 release became essentially continuous. Based on these findings, it would be interesting to know the respiratory patterns of engorging (feeding) ticks that are still attached to their hosts.

Els *et al.* (1988) and Els (1992) established that some nymphs of *H. truncatum* embed in the skins of their hosts *R. pumilio* and *T. brantsii* during attachment. These authors (including data presented in Chapter 3 of this thesis) established that such nymphs are not compromised with regard to development on their hosts when compared to non-embedded ones. However, earlier reports on embedment (Lebeda 1962; Tovornik 1984) indicated that embedded females of *Ixodes ricinus* failed to feed to full engorgement when compared to non-embedded ones. To further investigate the phenomenon of tick embedment, CO_2 emission rates in embedded and non-embedded nymphs of *H. truncatum* were measured using the flow-through respiratory system. Also in this study, patterns of CO_2 release and CO_2 emission rates were investigated in non–engorged females, engorged nymphs and engorged females of *H. truncatum*.

Besides being widely distributed in the southern part of Africa (Horak and MacIvor 1987; Walker 1991), *H. truncatum* is drought resistant and inhabits dry areas in this region (Howel *et al.*1978; Matthysse and Colbo 1987). The

immature stages of *H. truncatum* prefer small mammals like rodents as hosts, while adults prefer large ungulates (Rechav *et al.* 1987; Walker 1991; Horak and MacIvor 1987). Apart from transmitting toxins, causing sweating sickness in cattle (Neitz 1959), this tick species is also well known in South Africa for its role in the transmission of Crimean-Congo haemorrhagic fever virus (Hoogstraal 1979; Swanepoel *et al.* 1983).

4.2 Materials and methods

4.2.1 *Ticks*

Three weeks old nymphs and adults (male and female) of *H. truncatum* used in this study were obtained from laboratory colonies maintained in the Department of Biology at the Medical University of Southern Africa. These ticks were maintained in glass humidity chambers at 25±1°C, 75±5% RH and natural day/night regime (L:D; 14:10h). The study was undertaken from September 1997 to March 2000.

4.2.2 *Infestation*

Nymphs were fed on three to four months old tick-naïve white laboratory rats, while adults were fed on four months old laboratory bred Dunkin/Hartly guinea-pigs. Infestation on white laboratory rats was carried out by placing the host in a cotton bag (140 mm x 220 mm) containing ±50 ticks. To facilitate attachment the hosts were shaved on their backs prior to infestation. The cotton bags were closed with staples to allow sufficient time for ticks to attach before the rodents freed themselves by chewing through the cotton bags.

Thus, the time for attachment was approximately 1hour. The procedure followed for infesting guinea-pigs was described by Rechav and Fielden (1995). Since mating is a necessary stimulus for female ixodid ticks to complete their blood-meal (Akov 1991), six pairs (male and female) of adult *H. truncatum* were released on each of the two guinea-pigs used as hosts. Only those ticks which fed to full engorgement were used in this study.

4.2.3 *Respirometry*

Measurements of the rate of CO_2 emission in ticks were made using a flow-through respirometry system (Sable Systems, Henderson, Nevada, USA) of which details have been described by Lighton *et al.* (1993), Fielden *et al.* (1994), Rechav and Fielden (1995) and Chown and Holter (2000). In brief, air from a cylinder was scrubbed of water and CO_2 by a Soda-Lime, Drierite columns and was passed through a respirometer chamber (held at 23 ± 1^0C) at 50 ml min^{-1} for engorged nymphs, nymphs that were feeding on the hosts and non-engorged females. In keeping with body size, the flow rate was increased to 100 ml min^{-1} for engorged female ticks. Flow rates were regulated by a Mass Flow Control Unit. A 2.5 ml cuvette was used as respirometer chamber for measurement of CO_2 emission rates of engorged nymphs and unfed females. A bigger cuvette (10 ml) was used for measuring CO_2 emission rates in engorged females. For measurements of CO_2 emission rates in ticks attached to the hosts, i.e. embedded and non-embedded nymphs, a Y-shaped connector tube was used as a respirometer chamber. One arm of the Y-shaped connector tube was connected to the incoming air current tube, the

second to the ex-current tube leading to the CO_2 analyzer and the third was cut off so as to enclose the tick on its host (Fig. 4-1). This arrangement necessitated that the respirometer chamber be held firmly on the host for the entire recording period. Prestik (Bostik Genkem, England) was used to make the contact between the body of the animal and respirometer chamber air-tight. This was tested on a blank cuvette and found to show no leaks.

For each embedded or non-embedded tick measured, CO_2 emission rates of the skin in the region of attachment were measured after removing the tick. The dry CO_2 free air-stream constituted the baseline reading for all measurements. Baseline measurements were taken before the beginning and the end of each recording using a computerized baselining system.

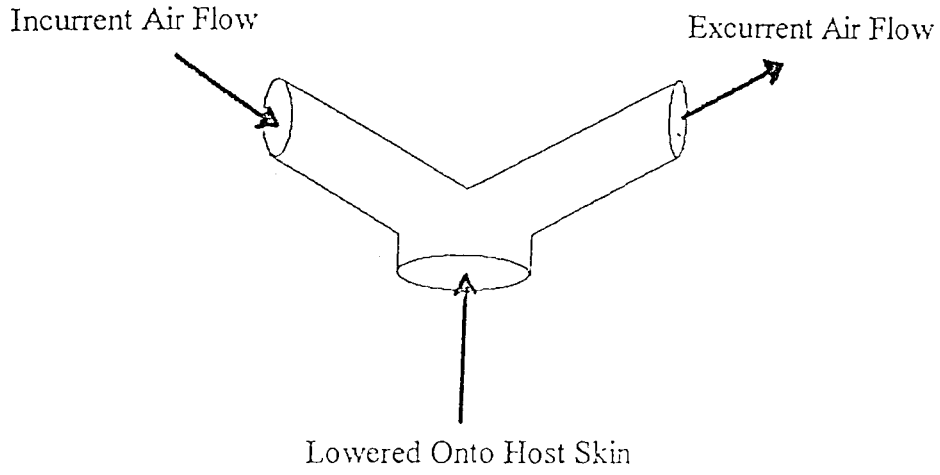

Fig. 4-1: Diagram of a Y-shaped connector tube used for measuring CO_2 emission rates of on-host ticks

For measurement of the off-host stages (i.e. engorged nymphs, unfed and engorged females) I weighed each tick to 0.1 mg on an electric balance

(Sartorius, Zeiss, Germany) and placed it in the respirometer chamber where it equilibrated to 23±1^{0}C for approximately an hour before starting the recording. Skin temperature was measured for embedded and non-embedded nymphs. The tick was kept in the dark by covering the respirometer chamber with black plastic prior to and during the recording. In the case of on-host ticks, tick weight was measured at the end of the recording period before saving data. Measurements of CO_2 emission in off-host ticks extended from four to nine hours. To reduce stress on the hosts, the recording period was limited to one hour for on-host ticks. Measurements were taken from days 1 - 3 following drop off in off-host ticks and on days four and five following infestation in on-host ticks. Computer records of CO_2 production were corrected for standard temperature and pressure using Datacan V (Sable Systems, Henderson, Nevada, U.S.A).

4.2.4 *Data presentation*

Measurements are presented as mean ± standard error. Analysis of variance (Anova) and t-test were used to determine statistical significance between measurements of various tick groups. DGC phase data were analyzed only for those individuals where at least three or more DGCs were recorded.

4.3 Results

4.3.1 *Gas exchange in H. truncatum nymphs*

Gas exchange was continuous for both embedded and non-embedded nymphs of *H. truncatum* (Fig. 4-2). No significant ($p>0.05$) difference was found between mean CO_2 emission rates in embedded and non-embedded

(Table 4-1). However, the mass specific CO_2 emission rates (ml g^{-1} h^{-1}) of embedded and non-embedded nymphs were significantly higher (Table 4-1, $p<0.05$) than that of fully engorged nymphs that dropped off from the hosts. Fully engorged nymphs that had dropped off from the host showed discontinuous gas exchange (Fig. 4-3). The mean durations of the burst and inter-burst phases accounted for about 36.26% and 63.74% of the DGC duration respectively. Only four of the eight engorged nymphs measured, showed a clear distinction between the closed and flutter phases of the inter-burst phase (Table 4-1). Extended discontinuous gas-exchange with the mean duration of 658.2±150.6 s was observed in three of the eight nymphs which showed discontinuous gas exchange.

4.3.2 *Gas .exchange in H. truncatum females*

The results for both non-engorged and engorged females of *H. truncatum* are summarized in Table 4-2. Individual CO_2 emission rates (ml h^{-1}) were significantly higher ($p<0.0001$) in engorged ticks compared to non-engorged (unfed) ones. However, the mass specific CO_2 emission rates (ml g^{-1} h^{-1}) in these two groups were not significantly different. Three gas exchange patterns; irregular elevated gas exchange, regular discontinuous gas exchange and extended discontinuous gas exchange were observed in both non-engorged and engorged females of *H. truncatum* (Figs. 4-4 & 4-5).

The nature of discontinuous CO_2 release in both non-engorged and engorged females was also characterized by a short but clearly defined burst of CO_2

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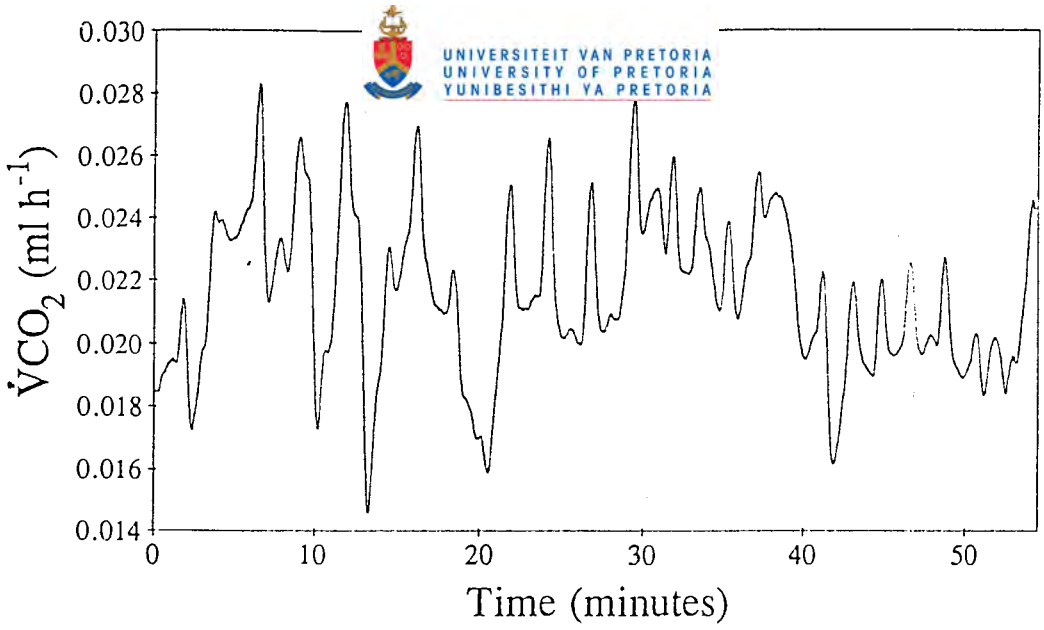

Fig. 4-2: Typical continuous CO_2 emission recording of embedded *Hyalomma truncatum* nymph

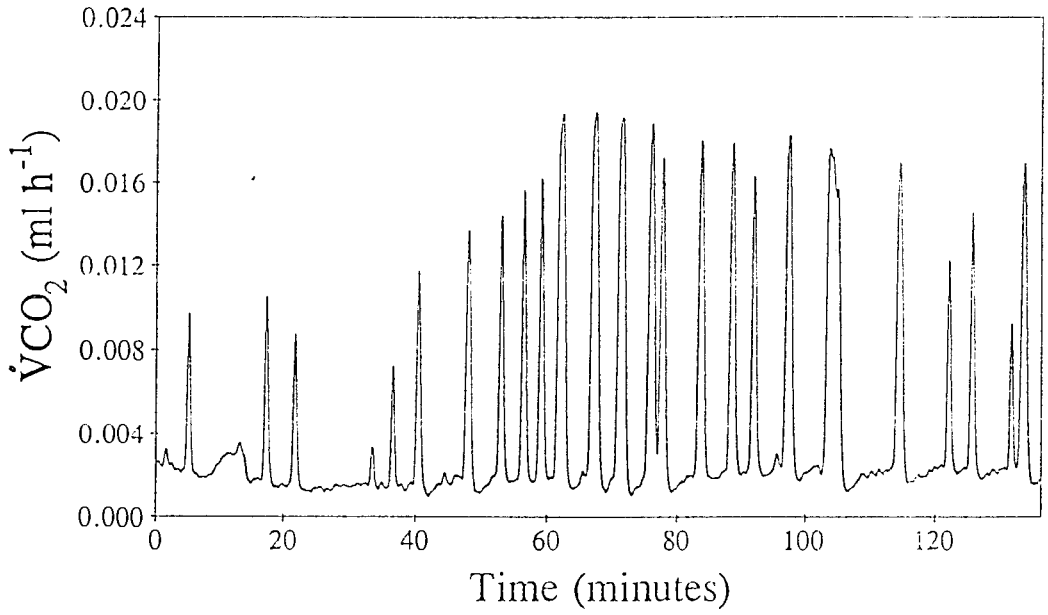

Fig. 4-3: Typical discontinuous CO_2 emission recording of engorged *Hyalomma truncatum* nymph

Table 4-1: Rate of CO_2 emission (Vco_2), burst CO_2 volume (O-Vco_2), rate of burst CO_2 emission (O-Vco_2), burst length (s), inter-burst length (s) and DGC frequency (DGCF) of embedded, non-embedded and fully engorged nymphs of *Hyalomma truncatum*. The host skin temperature was 36.4±0.6^{0}C

	Non-Embedded	Embedded	Engorged	Statistics
Tick weight (mg)	7.38±1.86^a	3.75±1.04^b	23.8±1.137^c	F=42.77, DF=2;30 P<0.0001
N	14	8	8	
Vco_2 (ml h^{-1})	0.0057±0.000942	0.0046±0.00223	0.004088±0.000836	F=0.57, DF=2;30 P>0.05
Vco_2 (ml g^{-1} h^{-1})	1.4436±0.353^a	1.2639±0.7235^a	0.172±0.00079^b	F=3.37, DF=2;29 P<0.05
O-Vco_2(ml h^{-1})	*******	*******	0.02818±0.02299	
O-Vco_2 (ml)	*******	*******	0.000946±0.000703	
O-length (s)	*******	*******	114.192±6.281	
Inter-Burst (s)				
Closed	*******	*******	50.858±5.548 (n=4)	
Flutter	*******	*******	140.045±27.374 (n=4)	
DGCF (mHz)	*******	*******	4.435±0.462	

N= number of nymphs. Each individual showed between 4 to 50 DGCs
******* = No DGC; Same superscript indicates no significant difference

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emission followed by an inter-burst phase. The mean frequency of the DGC in non-engorged females was significantly lower ($P<0.0001$) than that of engorged females (Table 4-2). A clear distinction between the closed and flutter phases was made in two of the fifteen non-engorged females and in all of the twelve engorged females examined in this study. The mean duration of the burst-phase was significantly higher ($p<0.0001$) in non-engorged females when compared to that of engorged females (Table 4-2). The duration of the inter-burst phase was much longer in non-engorged females (forming 77% of the DGC) compared to that of the open-phase. In engorged females the length of the inter-burst phase in relation to that of the open phase formed 55% of the DGC duration. Compared to non-engorged females, the duration of the inter-burst phase in engorged females was significantly short ($P<0.0001$). Expressed as a percentage of the duration of the inter-burst phase, the closed phase accounted for about 15% of the total inter-burst duration in the two non-engorged females that showed a clear distinction between the closed and flutter phases and those females that were engorged.

Extended discontinuous ventilation occurred in five of the fifteen non-engorged females and five of the ten engorged females examined in this study (Figs. 4-4 & 4-5). During this phase, the individual CO_2 emission rate (ml h^{-1}) in engorged females were significantly higher ($p<0.001$) than those of non-engorged females (Table 4-3). However, the mass specific CO_2 emission rates in these two groups did not differ significantly. Furthermore, extended discontinuous ventilation was much more prolonged ($P<0.05$) in non-engorged females than in females that were engorged.

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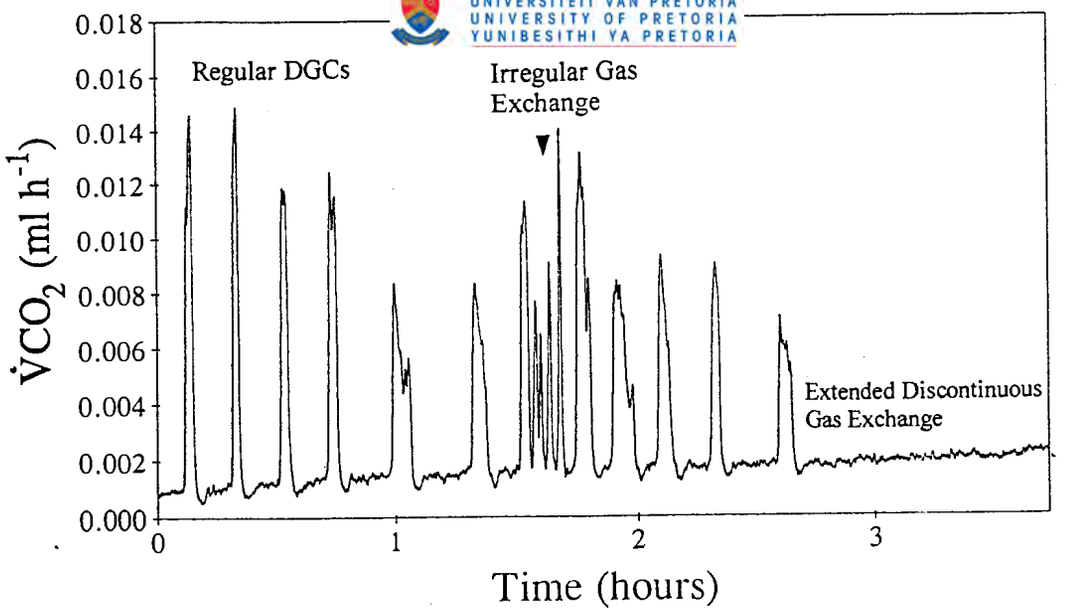

Fig. 4-4: Discontinuous CO_2 emission recording of non-engorged *Hyalomma truncatum* female showing regular DGCs, irregular CO_2 emission and extended discontinuous gas exchange

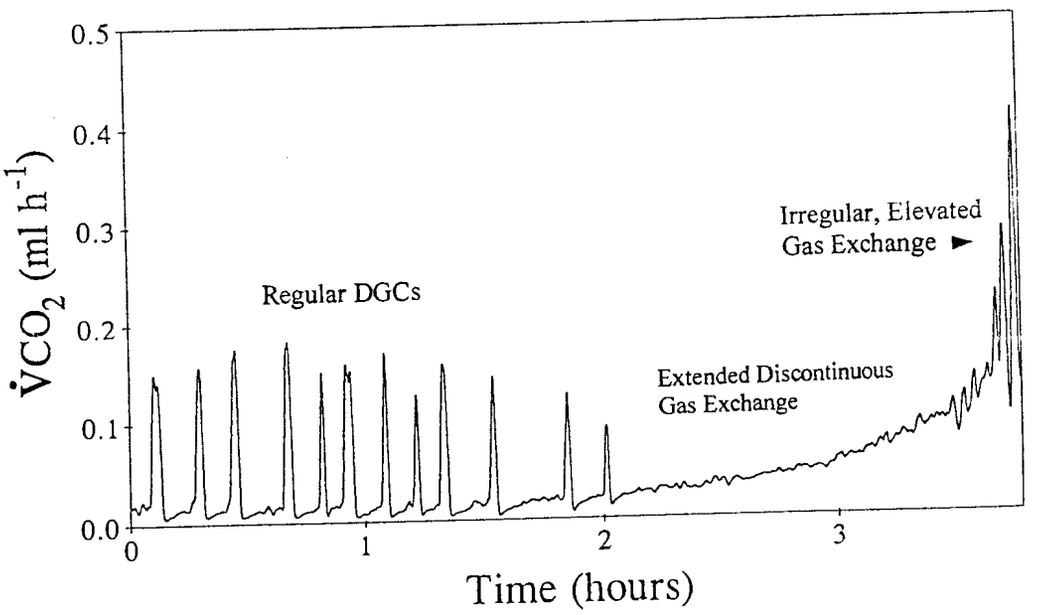

Fig. 4-5: Typical discontinuous CO_2 emission recording of engorged *Hyalomma truncatum* female showing regular DGCs, extended discontinuous gas exchange and irregular elevated gas exchange

Table 4-2: Rate of CO_2 emission (Vco_2), burst CO_2 volume ($O\text{-}Vco_2$), rate of Open CO_2 emission ($O\text{-}Vco_2$), burst length (s), inter-burst length (s) and DGC frequency (DGCF) of unfed and fed females of *Hyalomma truncatum*

	Non-Engorged	Engorged	Statistics
Tick weight (g)	0.00901±0.00064	0.5445±0.0184	P<0.0001
N	15	10	
Vco_2 (ml h^{-1})	0.00086±0.000015	0.06012±0.00519	P<0.0001
Vco_2 (ml g^{-1} h^{-1})	0.0911±0.00754	0.1111±0.01024	P>0.05
$O\text{-}Vco_2$(ml h^{-1})	0.00211±0.00014	0.0767±0.00173	P<0.0001
$O\text{-}Vco_2$ (ml)	0.000235±8.09E-06	0.00379±0.00228	P<0.0001
O-length (s)	444.188±19.182	168.651±6.0638	P<0.0001
Inter-Burst length (s)	1479.178±126.816	201.46±0.1557	P<0.0001
Closed	89.85±9.254[a]	32.17±1.4026	P<0.0001
Flutter	502.35±39.99[a]	172.69±8.62	P<0.0001
DGCF (mHz)	0.515±0.115	2.634±0.238	P<0.0001

N= number of females. Each individual showed between 4 to 50 DGCs
[a] Means calculated from 2 females with 6 DGCs each

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Table 4-3: Rate of CO_2 emission during extended discontinuous ventilation in females of *H. truncatum*

	Non-engorged	Engorged	Statistics
Tick weight (g)	0.010952±0.001886	0.5145±0.0104	P<0.0001
N	5	5	
Vco_2 (ml h^{-1})	0.000687± 0.000322	0.0401±0.00525	P<0.001
Vco_2 (ml g^{-1} h^{-1})	0.0615±0.0198	0.0782±0.01051	P>0.05
Duration (min.)	166.152±76.6239*	83.728±31.4958	P<0.05

N=number of females showing extended discontinuous ventilation
*=two extended discontinuous ventilation measurements could not be completed during the recording

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4.4 Discussion

4.4.1 *CO_2 Emission rate*

In this study embedded and non-embedded nymphs did not differ significantly with regard to CO_2 emission rates. This result further supports the view by Els *et al.* (1988) that embedded *H. truncatum* nymphs are in no way impaired with regard to feeding and development compared to non-embedded ones. Feeding ticks, more so those that are embedded in the host's skin, are in intimate contact with their hosts, and thus their metabolic rate could be influenced by the high temperature of their mammalian hosts. This is clearly one reason for the embedded and non-embedded feeding nymphs having significantly higher ($p<0.05$) mass specific CO_2 emission rates than engorged nymphs that had dropped off from the hosts in this study. Nonetheless, the large contribution of the mass of the ingested blood to the overall tick mass also means that the specific CO_2 emission rate will appear much lower in the engorged nymph than is, in effect, likely to be the case.

Although significant differences occurred between individual CO_2 emission rates (ml h^{-1}) of unfed and engorged females, mass specific CO_2 emission rates (ml g^{-1} h^{-1}) in these two groups were not significantly different. This is because most of the increase in weight in engorged ticks is contributed by host blood which is not part of the tick's active metabolic tissue.

4.4.2 *Ventilatory patterns in H. truncatum ticks*

Fielden *et al.* (1999) reported that the ventilation patterns of feeding *D. variabilis* females show discontinuous CO_2 release in the first days (1-6) of feeding and gradually progress to continuous CO_2 release towards the end (days 7-9) of the feeding period. Although this study has highlighted important information with regard to the respirometry of feeding ticks, it should however, be considered that these authors measured CO_2 emission rates in ticks whose feeding was interrupted when they were removed from their hosts. Tick feeding is also influenced by factors that are host based. This was clearly demonstrated in the *in vitro* study by Akov (1982), who demonstrated that the frequency of sucking phases in *B. microlpus* was increased by the addition of ATP (adenosine tri-phosphate) or GSH (growth stimulating hormone) at concentrations of 10^{-3} M. Off-host ticks are obviously not exposed to such factors thus the feeding and respiratory behaviour of ticks that have been removed from their hosts may not necessarily represent those of on-host ticks.

It is important to emphasise that in the present study measurements of CO_2 release in feeding ticks were done while ticks were attached to their hosts. All feeding nymphs (both embedded and non-embedded ones) of *H. truncatum* showed continuous CO_2 release in days 4-5 following infestation. Observations made in this study on the occurrence of the three ventilation patterns (i.e. irregular elevated gas exchange, regular DGCs and extended discontinuous gas exchange) in females of *H. truncatum* confirm the findings of Fielden *et al.* (1994) who studied the ventilation patterns of non-engorged

A. hebraeum ticks. However, Lighton *et al.* (1993) and Rechav and Fielden (1995) reported that engorged ticks of *A. marmoreum* and *Rhipicephalus evertsi evertsi* do not ventilate discontinuously. In contrast, the current study demonstrated the occurrence of discontinuous ventilation in engorged nymphs and females of *H. truncatum*.

While the reason for the difference between the results on *H. truncatum* and those on *A. marmoreum* (Lighton *et al.*, 1993) and *R. evertsi evertsi* (Rechav and Fielden 1995) remain unclear, it is important to note that discontinuous ventilation in ticks may be a phenomenon which is species specific and possibly manifest itself much more strongly in ticks that inhabit dry areas. In this regard it should be noted that, unlike *A. marmoreum* and *R. evertsi evertsi* which occur in humid habitats (Howel *et al.* 1978, Rechav 1982), *H. truncatum* is a dry-land tick (Howell *et al.* 1978, Matthysse and Colbo 1987). Therefore, the ability of this tick to ventilate discontinuously even when engorged, could be an adaptation for existence in dry habitats.

4.4.3 *The nature of discontinuous ventilation in non-engorged and engorged H. truncatum ticks*

The closed and flutter phases during inter-burst are clearly distinct in insect arthropods (Lighton 1996). In ticks, Lighton *et al.* (1993) and Fielden *et al.* (1994) could not distinguish between these two phases in the inter-bursts of *A. marmoreum* and *A. hebraeum*. The results of the present study, however, provide clear evidence of the existence of distinct closed and flutter phases in non-engorged females and engorged nymphs and females of *H. truncatum*

ticks. However, it is still unclear why this feature was not observed in all non-engorged *H. truncatum* females and engorged nymphs. In accounting for the lack of clear distinction between the closed and flutter phases in *A. marmoreum*, Lighton *et al.* (1993) cited the possibility of diffusive uptake of O_2 through the cuticle between burst phases as a basis for non-active respiration for prolonged periods during inter-bursts.

Discontinuous ventilation is thought to play a role in reducing respiratory water loss by restricting diffusive loss of CO_2 and water vapour to discrete cyclic events (Rudolph and Knulle 1979). Knulle and Rudolph (1991) view the need to conserve water to be more critical in host-seeking (non-engorged) ticks than those that are engorged. In the present study it is clear that the need to conserve water (indicated by the short burst duration relative to that of the inter-burst) was relaxed in engorged female ticks as compared to non-engorged ones. Lighton (1990) points out that in insect species subjected to severe water limitations, the burst phase should be short relative to the inter-burst phase. In the present study the engorged female *H. truncatum* ticks demonstrated an open phase that formed about 43% of the duration of the DGC, when in non-engorged females it formed 23% of the DGC length. In view of this observation it is evident that the need to conserve water through discontinuous gas exchange was relaxed in engorged ticks as compared to non-engorged ones. Individual and mass specific CO_2 emission rates were reduced during extended discontinuous gas exchange in both non-engorged and engorged females. This pattern of gas exchange could be a further

strategy by *H. truncatum* ticks to reduce respiratory water loss through reduced metabolic rates.

However, engorged nymphs showed a different result, with long inter-burst phases relative to burst phases. This feature according to Lighton (1990), signifies stringent respiratory water loss control. The reason for engorged nymphs to apply stringent control over respiratory water loss than engorged females could be attributed to the difference in surface to volume ratio between the two stages. Small animals have larger surface area to volume ratio than larger animals (Dorit *et al.* 1991). As a result of this, evaporative water loss through body surface is a greater problem for small animals than for large ones. Thus water stress is more problematic in nymphs than in adult ticks.

In conclusion, data obtained in this study clearly demonstrated the need of both engorged and non-engorged *H. truncatum* to conserve water through discontinuous ventilation. In this tick species discontinuous gas exchange is not only limited to non-engorged phases. Engorged *H. truncatum* ticks also need to conserve water particularly given the dry areas which they inhabit. While data obtained in this study has been interpreted along the understanding that DGC reduce respiratory water loss in ticks, it is important to acknowledge that a different school of thought which proposes that DGC contributes little towards respiratory water loss exist (see Lighton 1996 and Fielden *et al.* 1999). The conflicting data with regard to the existence or non-

existence of discontinuous gas exchange in engorged ticks highlights the need to further investigate the respirometry of engorged ticks.

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CHAPTER FIVE

Concluding Remarks

According to Balashov (1972) ticks are second only to mosquitoes as vectors of disease-causing agents to humans and are the most important arthropod transmitting pathogens to other animal species. Tick-host interaction as a subject, has been reviewed recently by many authors (e.g. Waladde *et al.* 1996; Wikel 1996; Wikel and Bergman 1997; Randolph 1998; Wikel 1999; Willadsen and Jongejan 1999). These reviews highlight the recognition of the importance of ticks as disease agents themselves and as vectors of pathogens of human and veterinary importance. However, despite this recognition, knowledge on many aspects of tick biology and their interaction with hosts remain poorly understood. These include the life patterns of ticks on various hosts, the phenomenon of tick embedment and the respirometry of ticks. The goal of this thesis has been to examine these issues using natural and non-natural tick-host models.

Data presented in Chapter 2 demonstrate the capacity of *H. truncatum* and *H. marginatum rufipes* to develop through a mixed two-host and three-host patterns on guinea-pigs and exclusively as two-host ticks on rabbits. However, from the results of this study, *H. marginatum rufipes* appears to have proceeded more towards a two-host life pattern than *H. truncatum*. The elimination of an off-host phase in the development from the non-engorged larva to an engorged nymph in a two-host life pattern has far reaching implications, particularly with regard to water conservation. The need to conserve water is more critical in the host-

seeking (off-host) ticks than in those that are engorged (Knulle and Randolph 1991). In engorging or engorged ticks the need to conserve water is eliminated or reduced since vertebrate host blood is hyposmotic to tick fluids (Kaufman and Sauer 1991). Thus, problems of facing prolonged survival on the ground before finding a new host and the need to maintain body water for long periods are eliminated in nymphs emerging through a two-host life pattern. Considering the fact that *H. truncatum* and *H. marginatum rufipes* are ticks of dry areas (Matthysse and Colbo 1987), it is reasonable to suggest that a two-host life pattern could be a survival strategy for these ticks.

Craine *et al.* (1995) suggested that ticks occurring in drier climatic conditions quest lower in vegetation, and as a result would be in contact with small mammals. This could be true for *H. truncatum* whose immature stages feed successfully on small rodents as demonstrated in this study and elsewhere (Els *et al.* 1988, Els 1992, Rechav and Fielden 1997). The poor attachment of *H. marginatum rufipes* on *R. pumilio* and *L. rosalia* demonstrated in this study, provides evidence that these rodents are less attractive as hosts to the larvae of this tick species. This view, coupled with the observation that the nymphs of *H. marginatum rufipes* attached and fed successfully on *R. pumilio* and *L. rosalia* suggest the presence of factors at the larval stage which isolate these rodents from being hosts of *H. marginatum rufipes*.

Expressed in terms of the mean blood-meal quantity, the feeding performance of the nymphs of *H. truncatum* and *H. marginatum rufipes* which developed through a two-host pattern on guinea-pigs and rabbits was lower than that of similar ticks which developed through a three-host pattern on the filed mice *R. pumilio* and *L. rosalia*. However, the reduction in the feeding performance of nymphs which developed through a two-host pattern had little influence on the moulting success of these nymphs. Reducing the blood-meal ingested by ticks without negatively affecting the tick survivorship may have been a necessary adaptation towards a two-host life pattern.

In Chapter 3 of this study, it is clear that embedment was the most common method of attachment following infestation of *R. pumilio* and *L. rosalia* with nymphs of *H. truncatum* and *H. marginatum rufipes*. Relatively few nymphs of *H. truncatum* embedded on white laboratory rats when compared to those that fed without embedding. This observation, together with the result that embedment was the most prevalent type of attachment on *R. pumilio* and *L. rosalia*, suggest that tick embedment is a host-dependent phenomenon. Although previous studies have examined embedment of *H. truncatum* nymphs on *R. pumilio* (Els *et al.* 1988 and Els 1992 and Molala 1998) and *T. brantsii* (Els *et al.* 1988, Els 1992), this study is the first to report on observations of embedded nymphs of *H. truncatum* and *H. marginatum rufipes* on *L. rosalia*. Except for spending longer periods on the hosts, embedded nymphs performed similar to non-embedded ones with regard to feeding. Els *et al.* (1988), Els (1992) and Molala (1998) found

similar results when comparing the feeding performance of embedded and non-embedded nymphs of *H. truncatum*. These results differ from those of Lebeda (1962) and Tovornik (1984) who established that embedded females of *I. ricinus* could not feed to full engorgement as a result of the restriction imposed on the tick by the host skin. It appears from these conflicting results that different tick species respond differently to embedment.

Ticks are limited in movement and thus move only for short distances when off-host (Varma 1993); when on the other hand rodent hosts such as *R. pumilio* and *L. rosalia* can cover wider distances in their habitats (Kindon 1974, de Graaf 1981). Spending prolonged periods on the host, as it was the case for embedded nymphs of *H. truncatum* and *H. marginatum rufipes* in this study, could be a compensation for the inability of these tick species to move long distances and thus enhance their distribution.

It was also shown in this study that embedment occurred even during post-primary infestations of *H. truncatum* nymphs on *R. pumilio* and *L. rosalia*. These hosts failed to express resistance to *H. truncatum* nymphs, allowing these ticks to feed to full engorgement even during post-primary infestations. On the other hand, the white laboratory rats expressed resistance to nymphs of *H. truncatum* which was characterized by a significant increase in mean γ-globulin concentration in the blood of these hosts during the second and tertiary infestations. The increase in γ-globulins concentration during the second

infestation coincided with a significant decrease in mean engorgement weights of fed nymphs of *H. truncatum*. These results support the view held by many authors (e.g. George *et al.* 1985, Fielden *et al.* 1992 and Wikel and Bergman 1997) that natural tick-host interactions, unlike non-natural ones, are often characterized by reduced or non-existent host immune responses. The fact that embedment occurred during the primary infestation on tick naïve hosts, eliminates acquired resistance to tick infestation by the host as a possible cause of tick embedment. This view is further supported by the lack of *H. truncatum* nymphs to embed in the skin of white laboratory rats during post-primary infestations.

The nymphs of *H. truncatum* and *H. marginatum rufipes* in this study also showed the capacity to concentrate the blood-meal. According to Akov (1982), the process of concentrating the blood-meal during tick feeding forms the first step in the digestion of blood by ixodid ticks. Water loss is significant in the process of concentrating the blood-meal in ixodid ticks. Although intermittent ejaculation of salivary secretions into the host by ixodid ticks is known to be the primary rout through which feeding ixodid ticks rid themselves of excess water, the possible contribution of respiratory water loss to blood-meal concentration should not be ignored.

Fielden *et al.* (1999) examined gas exchange in feeding ticks which were removed from their hosts before completing their blood-meal. In this study, I

presented for the first time data on CO_2 release in ticks while they were attached to their hosts. Also, this study is the first to report on observations of discontinuous CO_2 release in fully engorged nymphs and females of *H. truncatum*. This observation could be a special feature of *H. truncatum* ticks since Lighton *et al.* (1993) and Rechav and Fielden (1995) could not find the occurrence of DGCs in engorged ticks of *A. marmoreum* and *R. eversti evertsi*. Furthermore, Lighton *et al.* (1993) and Fielden *et al.* (1994) could not distinguish between the closed and flutter phases during the inter-bursts of unfed *A. marmoreum* and *A. hebraeum* ticks. In this study, a distinction between these two phases of the inter-burst was made in two females as well as in engorged nymphs (n=4) and females (n=10) of *H. truncatum* which were examined.

It is clear from the above account that the present study has revealed valuable insights into tick life patterns, tick embedment, acquired resistance to tick infestations and gas exchange in engorged and non-engorged ticks. There has been a trend over the years towards studying tick-host interactions using laboratory animals (non-natural hosts). As a result of this, little information exists on the interaction of ticks and their natural hosts. While acknowledging the valuable insights provided by studies involving laboratory animals (non-natural hosts) in tick-host interactions, the overemphasis of this approach may defeat the ultimate aim of tick research which includes the development of tick control measures which will be effective when applied in the natural environment. The interaction between ticks and their natural hosts often differs from that of similar

ticks on non-natural hosts as demonstrated in this study and elsewhere (George *et al.* 1985, Fielden *et al.* 1992).

Apart from being hosts to ticks, rodents play a significant role in enzootic cycles of tick-borne pathogens. Under natural conditions, disease-causing agents are commonly transferred when uninfected vectors feed on an infected vertebrate host, then the pathogens that are taken up with the blood-meal propagate inside the vector, and in a later stage are injected into uninfected hosts, usually with infected saliva from the salivary glands of the feeding ticks (Scott *et al.* 1993, Zeller *et al.* 1994). In the light of this, features which prolong contact between ticks and their hosts, as in the case of two-host development and embedment, may favour the transmission of pathogens between ticks and their hosts. Having expressed the above views, it is important once more to note that in this study, the larvae of *H. marginatum rufipes* attached poorly on *R. pumilio* and *L. rosalia*. In view of this result and those from field studies by Horak and MacIvor (1987), it is unlikely that *R. pumilio* and *L. rosalia* could serve as reservoir hosts of pathogens transmitted by *H. marginatum rufipes*.

Knowledge on the life patterns, embedment and respirometry of ticks has far reaching implications particularly with regard to the design of tick control measures. For example, factors influencing the selection and degree of resistance to acaricides in ticks include the frequency of exposure to and the concentration of acaricides (Cotton *et al.* 1984). The prolonged contact between

ticks and their hosts in a two-host life pattern, coupled with the fact that such ticks can have several generations per year is likely to permit excessive exposure to acaricides, particularly if treatment of animals with acaricides is repeated in intervals shorter than the duration of development from flat larvae to engorged nymphs. In the light of this, it is not surprising that the development of resistance to acaricides in ticks was first observed in single-host ticks of the genus *Boophilus*, which may have up to five generations per year (Wharton 1976). Furthermore, tick control measures which incorporate deferred grazing, are less likely to be effective in the control of three-host ticks since the immature stages of these ticks can be sustained by feeding on small vertebrate hosts even in the absence of livestock from the grazing field. Also, when designing control methods which include treatment of rodents for control of ticks which can embed (e.g. *H. truncatum* and *H. marginatum rufipes* nymphs), it must be recognized that such ticks enjoy some degree of cover by the host skin. Lastly, air-borne acaricides may have less effect in the control of *H. truncatum* ticks since this tick species can undergo extended discontinuous gas exchange cycles.

Although this study has revealed valuable insights on aspects of tick-host interaction in *Hyalomma* ticks, the need for further research in this field cannot be overemphasized. Future research should aim at studying the life patterns of the southern African *Hyalomma* ticks on other natural hosts including birds and scrub hare which have been identified as common hosts for these tick species. Also, the development of *Hyalomma* immature stages through a mixed two-host and

three-host pattern on guinea-pigs should be further investigated. It will be interesting to determine the cause for some larvae to develop through a two-host pattern when others from the same infestation and same host, develop through a three-host pattern. Information from such investigations will undoubtedly clarify the situation on the life patterns of *Hyalomma* ticks much more. Tick embedment should be further investigated since it appears to be a useful attachment strategy in *Hyalomma* ticks. The question why some nymphs of *H. truncatum* and *H. marginatum rufipes* embed when others from the same infestation and same host feed without embedding should be investigated. Lastly, studies on gas exchange in ticks should be intensified since valuable information regarding the physiological adaptations of ticks to their habitats could be established.

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