Differential feeding success of two economically important tick species *Rhipicephalus warburtoni* and *Ixodes rubicundus* on two sympatric small mammal species *Micaelamys namaquensis* and *Elephantulus myurus*

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Abstract

Rodents are recognised as important hosts of ixodid ticks and as reservoirs of tick-borne pathogens across the world. Sympatric insectivores are inconspicuous and often overlooked as hosts of ticks and reservoirs of disease. Elephant shrews or sengis of the order Macroscelidea are small insectivores that often live in sympatry with rodents in South Africa. Sengis are invariably parasitised by large numbers of immature ticks while sympatric rodents are infested with very few. The reason for the difference in tick parasitism rates between these hosts is unknown. While a number of mechanisms are possible, we hypothesised that ticks would exhibit "true host specificity" and as such would only attach and feed successfully on their preferred host. To investigate this, we conducted feeding experiments using two economically important tick species, the brown paralysis tick, *Rhipicephalus warburtoni* and the Karoo paralysis tick, *Ixodes rubicundus* using two sympatric small mammal species as potential hosts, the eastern rock sengi, *Elephantulus myurus* and the Namaqua rock mouse, *Micaelamys namaquensis*. Ticks attached and fed readily on *E.*

myurus but did not attach or feed successfully on *M. namaquensis* suggesting that these ticks exhibit true host specificity. We suggest that a kairomonal cue originating from the odour *E. myurus* stimulates attachment and feeding of these ticks and they possess immunosuppressive mechanisms specific to *E. myurus*, allowing them to feed on this host species but not on *M. namaquensis*. This study highlights the importance of small mammalian insectivores as hosts of ixodid tick species and their potential as reservoirs of tick-borne pathogens.

Keywords

Toxic paralysis; Host specificity; Host choice; *Ixodes*; *Rhipicephalus*; Insectivore; Rodent

1. Introduction

Small mammals are important hosts for the immature stages of ixodid ticks throughout the world (Soneshine, 1991). The importance of rodents in supporting these life stages is well documented (Norval, 1979; Talleklint and Jaenson, 1997; Clark *et al.*, 1998) and the role of rodents as reservoir hosts of tick-borne pathogens is similarly well known (Donahue *et al.*, 1987; Randolph *et al.*, 1999; Bown *et al.*, 2003; Karbowiak, 2004). However, the importance of sympatric insectivore species in supporting tick populations, and as reservoirs of tick-borne pathogens, is poorly understood. Available data suggest that insectivores may be more important hosts for immature ticks than sympatric rodents and be important reservoirs of tick-borne pathogens. For example, in a forest in Dutchess County, NY, Brisson *et al.* (2008) concluded that 2 shrew species, *Blarina brevicauda* and *Sorex cinereus*, fed 35% of larval blacklegged ticks, *Ixodes scapularis*, and 55% of all larvae infected with the Lyme spirochete, *Borrelia burgdorferi*. In contrast, white-footed mice, *Peromyscus leucopus*, fed 10% of larvae and 25% of infected larvae. Similar results were observed in the UK, where

common shrews, *Sorex araneus*, carried six times more *Ixodes trianguliceps* larvae and twice as many *I. ricinus* larvae than sympatric field voles, *Microtus agrestis* and had a higher prevalence of infection with *Anaplasma phagocytophilum* (Bown *et al.*, 2011).

In southern Africa, rodents are typically infested with small numbers of ticks. In contrast, elephant shrews or sengis of the order Macroscelidea (small insectivores endemic to Africa) are invariably parasitised by large numbers of immature ticks (Fourie *et al.*, 1992; Fourie *et al.*, 2005; Horak *et al.*, 2011; Harrison *et al.*, in press, hosting approximately 27 species in 6 genera (Fourie *et al.*, 1995).

Harrison *et al.* (in press) found that the eastern rock sengi, *E. myurus*, had an overall mean tick burden of 399 compared to 1 for the Namaqua rock mouse, *Micaelamys namaquensis*, in Limpopo province, South Africa. Similar differences in tick parasitism rates have also been recorded for these two species at other locations in South Africa and in Zambia (MacLeod, 1970; Colbo and MacLeod, 1976; Fourie *et al.*, 1992).

The reason for such large differences in tick parasitism rates between these hosts is currently unknown but a number of non-mutually exclusive mechanisms may be possible. *M. namaquensis* is social living in family groups (Smithers, 1971; Choate, 1972) while *E. myurus* is solitary (Ribble and Perrin, 2005), therefore, *M. namaquensis* may benefit from allogrooming. Differences in immunity may contribute; some small mammal species develop immunity to ticks while others do not (Du Toit *et al.*, 1994; Dizij and Kurtenbach, 1995). *M. namaquensis* is nocturnal (Fleming and Nicolson, 2004) while *E. myurus* is active both day and night (Ribble and Perrin, 2005). Ticks also exhibit daily activity patterns (Belozerov, 1982, Madden and Madden, 2005), therefore, differences in the activity patterns of ticks and their hosts may also affect their contact rates with ticks; Moreover, ticks may exhibit "true or physiological host specificity" were ticks go through a process of host recognition, often mediated through specific chemical compounds associated with host odour, and possess

mechanisms to suppress the hemostasis or immune response of the specific host (Soneshine, 2006).

In the current study investigated differences in tick parasitism rates between *E. myurus* and *M. namaquensis*. Given the pronounced differences in parasitism rates observed previously for these hosts, we hypothesised that ticks would exhibit "true host specificity" and would only attach and feed successfully on *E. myurus* but would not attach or feed successfully on *M. namaquensis*. To test this hypothesis we conducted feeding experiments using *E. myurus* and *M. namaquensis* as potential host species for 2 economically import tick species, the Karoo paralysis tick, *Ixodes rubicundus* and the brown paralysis tick, *Rhipicephalus warburtoni*. Adults of these tick species feed on sheep and goats causing toxic paralysis in central and southern South Africa, with many thousands of animals lost annually (Spickett and Heyne, 1998), while larvae and nymphs of these tick species feed on *E. myurus* (Fourie *et al.*, 1992).

2. Materials and Methods

2.1. Initial small mammal and tick collection

Small mammals were trapped across 7 consecutive nights between 7th and 13th December 2010 at the farms "Nooitgedacht" (-29º37'40''S, 25º38'20''E) and "Langberg" (-29º36'15'', 25º28'10''E) located in the south western Free State, South Africa. These locations were chosen as they were located central to the distribution of *R. warburtoni*, *I. rubicundus* and to the distribution of toxic paralysis they are known to cause (Spickett and Heyne, 1988; Walker *et al.*, 2000). The farms also had sheep and goats as livestock and contained suitable rocky habitat for *E. myurus* and *M. namaquensis*. One hundred and twenty Sherman live traps, baited with a peanut butter, oats and sardine mix were set on rocky outcrops at 10-m intervals. Depending on the terrain, either 2 transects of 60 or 4 transects of 30 were set in

parallel lines spaced 10-m apart. All traps were set after 16.00 and collected before 08.00 the following day. The body of each individual was searched for the presence of ticks with particular attention given to the ears, base of the tail, and legs where ticks were found to aggregate. The remainder of the body was searched by back combing the fur. Ticks were removed using fine forceps and placed in 70% ethanol for processing. Animals were housed in standard rodent containers, with a sawdust substrate, supplied with water and, depending on the species, fed cat food, grated apple and grated carrot (*E. myurus*) or mouse pellets (*M. namaquensis*). Animals were then transported to a climate controlled room in the department of zoology, University of Pretoria. Collections in the Free State were authorised by the Department of Economic Development, Tourism and Environmental affairs under permit number 01/7675 and their import to Gauteng was authorised by the Gauteng Direcotate of Nature Conservation permit number CPB6-003501. Ticks were identified by I.G.H. using descriptions provided by Apanaskevich *et al.* (2007), Walker *et al.* (2000) and by comparison to reference material. Once identified, ticks were counted and their developmental stage recorded as larva or nymph. A second trapping bout took place across 5 consecutive nights between 3rd and the 7th of April 2011 at the farm Langberg. This was required in order to collect live, engorged, larval ticks from *E. myurus* which could then be allowed to moult into unfed nymphs for use in feeding experiments and to obtain additional *E. myurus* to increase their sample size for experiments. Animals were trapped as described above but housed in small mammal containers with a wire mesh floor suspended over a tray covered in moist tissue paper to facilitate the collection and survival of engorged, detaching ticks. The edge of the tray was covered in a thin layer of petroleum jelly to prohibit ticks from leaving the housing area. Tissue paper was inspected each morning and each evening for the presence of detached ticks and the tissue replaced. Detached ticks were removed using fine forceps, placed in glass vials plugged with moist cotton wool and kept shaded from

direct sunlight. Of the 14 *E. myurus* captured in the second trapping bout, 4 were transported back to the climate room at the University of Pretoria while the remaining 10 were released to their respective trapping sites. These animals were collected and transported under permit numbers 01/9031 (Free State) and CPB6-003470 (Gauteng) respectively. Ticks were transported to the University of Pretoria and housed in an acaridarium consisting of a glass tank filled to one tenth its volume with saturated salt solution in order to maintain an approximate relative humidity of 70% to facilitate the survival and development of ticks. The acaridarium was located in the same climate room as small mammals which was maintained at a temperature of 23ºC and 12 hour light (06.00-18.00), 12 hour dark (18.00-06.00) cycle. Engorged larval ticks were allowed to moult to nymphs and were given a minimum of 2 weeks for their mouthparts to harden before use in feeding experiments.

2.2. Tick feeding experiments

Unfed nymphs that had moulted were examined under a dissecting microscope, identified to species and divided into groups of 30 of either *R. warburtoni* or *I. rubicundus*. In experiment 1, 30 unfed *R. warburtoni* nymphs were emptied into a petri dish containing water to restrict the ticks movement. A fine bristle brush was then used to place the ticks on the fur of the lower back of a single *E. myurus* which had been manually restrained. The animal was then placed in a holding cage set up as described above for the collection of engorged larvae. This procedure was repeated for 4 males and 4 females each of *E. myurus* and *M. namaquensis*, therefore, a total of 240 nymphs of *R. warburtoni* were used to infest each potential host species. Although every measure was taken to ensure the welfare of the animals involved, 2 female *E. myurus* died suddenly on days 6 and 8 of experiment 1. Both animals were kept for an additional day to allow any engorged ticks to detach at which point the bodies were searched for ticks as described above. 2 used an identical protocol to experiment 1 but instead

used *I. rubicundus* nymphs. However, this experiment was replicated for 3 males and 2 females of *E. myurus* and 3 females and 2 males of *M. namaquensis,* therefore, 150 *I. rubicundus* were used to infest each potential host species. Experiments were run consecutively and 2 males and 1 female from the first experiment were also used in the second experiment, however a 2 week break was left between experiments to ensure any ticks not accounted for in the first experiment did not affect the second experiment. To give an indication of any potential effects of immunity on tick-host relationships, the experiment was also replicated for *R. warburtoni* using a single female each of *E. myurus* and *M. namaquensis* that had been born in the laboratory, and thus had not been exposed to ticks previously. The lid, inside and outside of the holding tank and the tissue paper was searched daily for the presence of ticks. All ticks were collected and examined under the microscope to verify the species and to assess the level of engorgement of the tick. Ticks were deemed to be "partially/fully engorged" if there was a visible presence of blood in the gut, characterised by a dark mass and a swelling of the tick body. Ticks in which there was no presence of blood were designated "unengorged". Experiments continued until engorged ticks were not recorded for 4 consecutive days, at which point animals were searched manually, as described above, for any ticks that had not detached. Ticks that consumed blood were weighed and placed in glass vials plugged with cotton wool and placed in the acaridarium to allow moulting. For each engorged tick the day of detachment and time to moult was recorded. All work was carried out under approval from the Animal Use and Care Committee, University of Pretoria, under permit number EC056-10

3. Results

3.1. Field data

A total of 10 *E. myurus* (3 males and 7 females) and 24 *M. namaquensis* (11 males and 13 females) were captured and sampled for ticks during the first trapping session. There was a marked difference in the tick fauna and tick abundance between *E. myurus* and *M. namaquensis* (Table 1). *E. myurus* was heavily and predominantly parasitised by *R. warburtoni* but this tick species was almost absent from *M. namaquensis*. To give an indication of the magnitude of this effect, *E. myurus* had, on average, 2440 times more larvae of *R. warburtoni* than did *M. namaquensis* and *E. myurus* had a mean nymphal abundance of 18.10 for *R. warburtoni* while *M. namaquensis* had 0. *M. namaquensis* was parasitised at very low levels by *Haemaphysalis elliptica*, *Hyalomma truncatum* and *Rhipicephalus gertrudae*, however, these tick species were completely absent from *E. myurus*. In contrast, *E. myurus* was parasitised at very low levels by *Amblyomma marmoreum* while this tick was absent from *M. namaquensis*. During the second trapping session 14 animals were captured yielding approximately 780 moulted *R. warburtoni* nymphs and 730 moulted *I. rubicundus* nymphs as a result of 7 days engorged larvae collection. No other tick species were recovered from moulted larvae.

Table 1. Mean larval and nymphal tick abundance (±SE) collected from *Elephantulus myurus* and *Micaelamys namaquensis* in the south western Free State, South Africa (LL=Larvae, NN=Nymphs)

Tick species	Stage	*E. myurus* n=10	*M. namaquensis* n= 24
R. warburtoni	LL	195.20 ± 61.20	0.08 ± 0.06
	NN	18.10 ± 5.72	0
H. elliptica	LL	0	0.08 ± 0.06
	NN	0	0.42 ± 0.17
H. truncatum	LL	0	1.08 ± 0.40
	NN	0	0
R. gertrudae	LL	0	10.96 ± 0.23
	NN	0	0.50 ± 0.19
A. marmoreum	LL	0.20 ± 0.06	0
	NN	0.20 ± 0.06	0

3.2. Tick feeding experiments

3.2.1. Recovery of unengorged ticks

3.2.1.1. Experiment 1

There was a marked difference in the behaviour of ticks in response to each host. On the first day post infestation, 14 (5.8%) unengorged *R. warburtoni* nymphs left the bodies of *E. myurus* and were recovered from the housing or under-floor tissue as opposed to 128 (53.3%) recovered from *M. namaquensis*. This represented mean tick collections of 1.8 ± 0.6 (SE) and 16.0 ± 1.3 for *E. myurus* and *M. namaquensis*, respectively (Fig. 1). On days 2–5, 7 unengorged nymphs were collected from the housing or under-floor tissue of *E. myurus* as opposed to 47 from those of *M. namaquensis*, while a further 3 unengorged ticks were collected from *E. myurus* and 7 from *M. namaquensis* during the remaining 13 days of experiment 1. Overall, significantly more unengorged *R. warburtoni* nymphs were recovered from *M. namaquensis* than from *E. myurus* (χ^2 = 80.83, df = 1, P < 0.001, Table 2). The same pattern was observed for *R. warburtoni* nymphs used to infest the laboratory reared, naive *E. myurus* and *M. namaquensis*. No unengorged ticks were recovered from the naive experimentally infested *E. myurus* throughout the whole study period while a total of 24 (80%) unengorged *R. warburtoni* nymphs were collected from a single naive *M. namaquensis*, 19 (60%) of which were recovered on the first day after infestation.

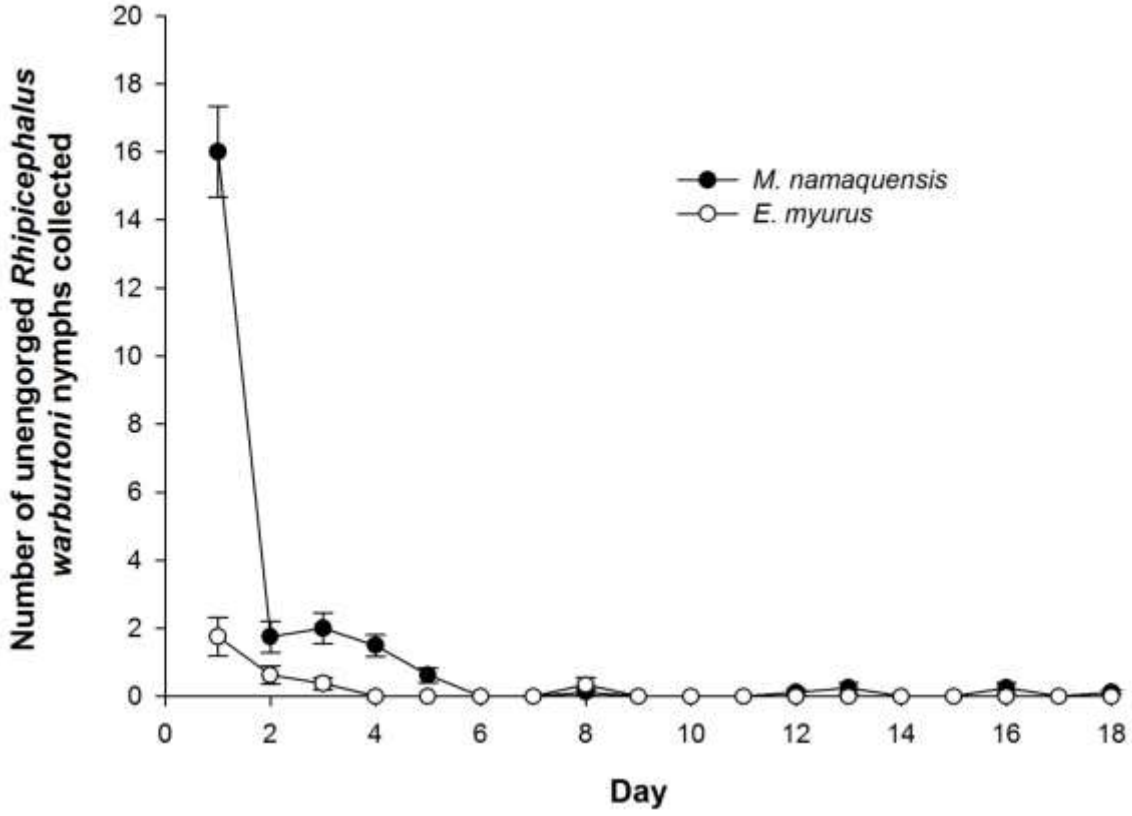

Figure 1. Numbers of unengorged *Rhipicephalus warburtoni* nymphs collected from the holding tanks and under tank substrate of *Micaelamys namaquensis* and *Elephantulus myurus* on each consecutive day post infestation with 30 unengorged ticks. (*M. namaquensis* n=8, *E. myurus*: day 1, n=8; day 6, n=7; day 8 n=6).

Table 2. Total numbers of engorged and unengorged *Rhipicephalus warburtoni* and *Ixodes rubicundus* nymphs recovered from *Elephantulus myurus* and *Micaelamys namaquensis* after infestation with 30 unengorged nymphs.

	E. myurus		*M. namaquensis*	
	R. warburtoni n=240	*I. rubicundus* n=150	*R. warburtoni* n=240	*I. rubicundus* n=150
Unengorged	25 (10.4%)	9 (6.0%)	182 (75.8%)	87 (58.0%)
Engorged	99 (41.3%)*	33 (22.0%)	1 (0.4%)	0 (0.0%)

*excluding data from 2 deceased females=56 (23.3%); excluding data from ticks collected by manual searching at the end of experiment 1=88 (26.7%); excluding both groups, i.e. natural detachment from live hosts=45 (18.8%).

3.2.1.2. Experiment 2

A similar pattern was observed for *I. rubicundus*. On the first day post infestation, 8 (5.3%) unengorged *I. rubicundus* nymphs were collected from the cages of *E. myurus* as opposed to 63 (42.0%) from those of *M. namaquensis*, representing mean tick collections of 1.6 ± 0.4 and 11.8 ± 0.6 for *E. myurus* and *M. namaquensis*, respectively (Fig. 2). On days 2–5 post infestation, no unengorged ticks were collected from the cages of *E. myurus* while a total of 23 unengorged nymphs were collected from those of *M. namaquensis*. During the remaining 9 days of experiment 2, a single tick was recovered from a cage of each host species. Overall, significantly more unengorged *I. rubicundus* nymphs were recovered from *M. namaquensis* than from *E. myurus* (χ^2 = 93.19, df = 1, P < 0.001, Table 2).

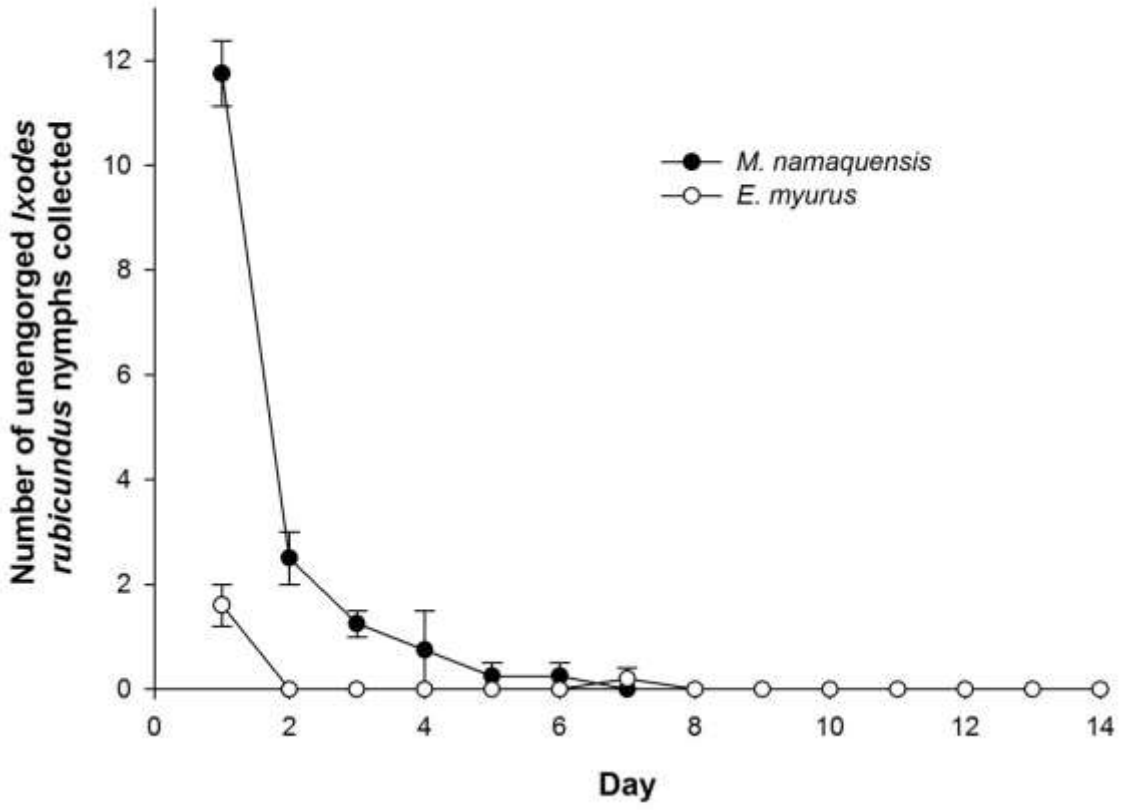

Figure 2. Numbers of unengorged *Ixodes rubicundus* nymphs collected from the holding tanks and under tank substrate of *Micaelamys namaquensis* and *Elephantulus myurus* on consecutive days post infestation with 30 unengorged ticks, n=5.

3.2.2. Recovery of engorged or partially engorged ticks

3.2.2.1. Experiment 1

Again marked differences were observed in the engorgement of ticks in response to each host species. In total, 99 (41.3%) of the unengorged *R. warburtoni* used to infest *E. myurus* were recovered as engorged nymphs while only a single, partially engorged nymph was recovered from *M. namquensis*. Of these 99, 23 detached from a deceased female on day 7, 20 from a deceased female on day 9 while 11 were collected manually from the animals on day 19 when experiment 1. Therefore, the total number of engorged ticks that naturally detached from live animals was 45 (18.8%) (Table 1). These ticks began to detach from *E. myurus* on day 7 and continued until day 14 (figure 3.) Overall the mean number of engorged *R. warburtoni* ticks that detached naturally from *E. myurus* across days 7-14 was 0.9 ± 0.6. The mean time to detachment was 10.1 ± 0.3 days with most ticks detaching on day 9. When taking all engorged ticks into account, regardless of when or how they detached, their overall mean mass was 5.8 ± 0.4 mg. There was a significant difference in mass between ticks that detached on different days (Kruskall-Wallis test adjusted for ties, $H=49.6$, $df=7$, $P<0.001$) with ticks that detached on the first day of detachment, day 7, being significantly lighter (1.3 ± 0.5 mg) than ticks that detached on any other day (5.9 ± 0.7 to 9.2 ± 0.8 (means ± SE), Mann-Whitney U tests, $W=354.5$ to 378.0, $P<0.001$ to $P<0.01$, $df=7$). A total of 4 engorged *R. warburtoni* nymphs were collected from the single laboratory reared, naive *E. myurus*, 1 on day 10, 2 on day 11, and 1 on day 12 with a mean mass of 8.8 ± 0.6 mg. No engorged ticks were collected from the naive *M. namaquensis*.

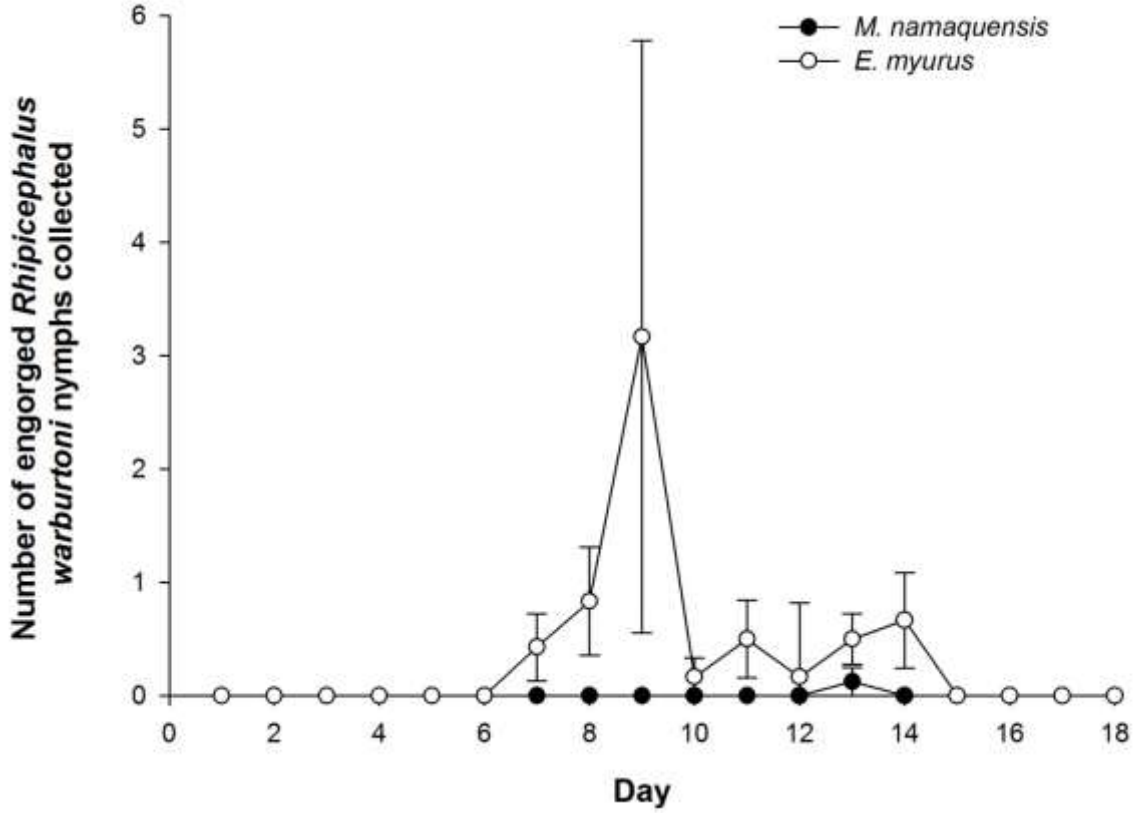

Figure 3. Mean numbers of engorged *Rhipicephalus warburtoni* nymphs (± SE) collected from the holding tanks and under tank substrate of *Micaelamys namaquensis* and *Elephantulus myurus* on consecutive days post infestation with 30 unengorged *Rhipicephalus warburtoni* nymphs (only data from ticks that detached naturally is presented, data from 2 deceased female *E. myurus* and from manual collections on day 19 are omitted. *M. namaquensis* n=8, *E. myurus*: day 1, n=8; day 6, n=7; day 8, n=6).

3.2.2.2. Experiment 2

In total 33 (22%) of the unengorged *I rubicundus* nymphs used to infest *E. myurus* were collected as engorged nymphs. No engorged nymphs were recovered from *M. namaquensis*. Ticks started to detach from *E. myurus* on day 6 and continued until day 10 (figure 4). No ticks were recovered from the manual search at the termination of the experiment. The mean number of ticks that detached across days 6-10 was 1.3 ± 0.6. The mean time to detachment for *I. rubicundus* nymphs was 7.5 ± 0.2 days with most ticks detaching on day 8. The overall

mass of ticks collected was 3.8 ± 0.2 mg. There was a significant difference in the mass of ticks that detached on different days (Kruskall-Wallis test adjusted for ties, H=13.87, df=3, P<0.01) with ticks on day 9 being heavier (5.1 ± 0.1) than ticks that detached on all other days (3.1 ± 0.2 to 3.8 ± 0.3 (means ± SE), Mann-Whitney U tests, W=23.5 to 85.5, P<0.01 to P<0.05, df=3). Day 10 was omitted from the analysis as only one engorged tick was recovered.

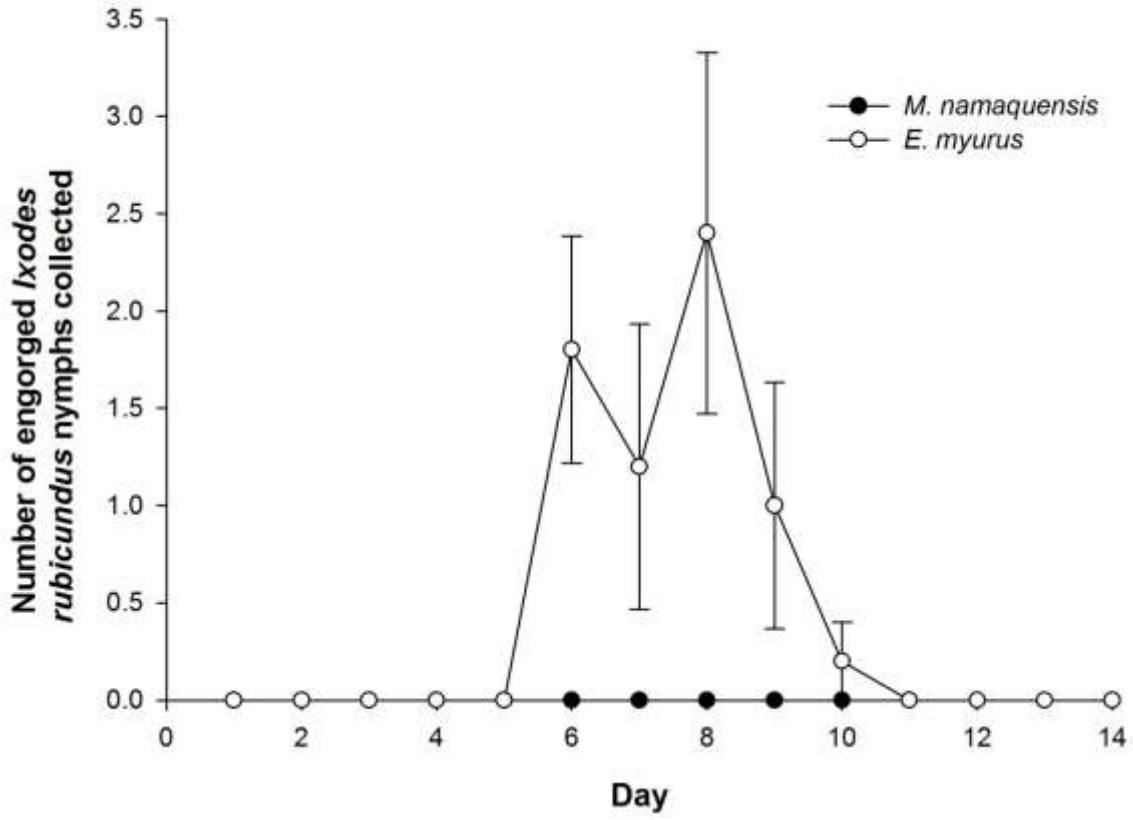

Figure 4. Mean numbers of engorged *Ixodes rubicundus* nymphs (± SE) collected from the holding tanks and under tank substrate of *Micaelamys namaquensis* and *Elephantulus myurus* on consecutive days post infestation with 30 unengorged *Ixodes rubicundus* nymphs, n=5)

3.3. Moult success

Of the 99 engorged *R. warburtoni* nymphs collected from *E. myurus*, 21 moulted successfully to the adult stage. Mean time to moult was 39.6 ± 0.4 (SE) with most ticks moulting on day 39. As the period from engorgement of nymphs of *I. rubicundus* until moulting varies

between 2 and 9 months (Fourie and Horak, 1994) the same data were not recorded for this tick species, however, after a period of 5 months, 26 of the 33 engorged *I. rubicundus* nymphs collected from *E. myurus* had moulted successfully.

3.4. Personal observations

A number of personal observations were made during the course of the experiments. Firstly, when infesting animals with ticks, it was observed that when the ticks were applied to the base of the fur of *M. namquensis*, *R. warburtoni* ticks would immediately begin to move away from the site of infestation. The same phenomenon was observed for *I. rubicundus*, however, this species activity was less than that of *R. warburtoni*. When applied to *E. myurus*, ticks of both species remained stationary at the point of infestation and rarely moved. Secondly, the single *R. warburtoni* nymph that was classified as "engorged or partially engorged" collected from *M. namquensis* had consumed a very small amount of blood so that a dark mass in the body was barely visible and the body not swollen. Moreover, the exoskeleton of this tick had become unattached dorsally and anteriorly in a manner not consistent with mechanical damage.

4. Discussion

The current study highlights the importance of small mammalian insectivores in the life cycle of ixodid ticks, a group often overlooked with respect to their importance as hosts to immature ticks and as reservoirs of tick-borne pathogens. Nothing is known regarding the reservoir competence of the Macroscelidea for tick-borne pathogens in South Africa but many of the tick species which they support in their juvenile stages have been implicated in the transmission of tick-borne pathogens (Walker *et al.*, 2000) For example, *R. evertsi evertsi*

can transmit a variety of tick-borne pathogens to livestock, such as *Anaplasma marginale*, *Ehrlichi ovina*, and *Babeis equi*. This tick species has also been implicated in the transmission of human infectious diseases including the virus responsible for Crimean-Congo haemorrhagic fever in humans and of *Rickettsia conori*, the cause of tick-bite fever. Recently, Harrison *et al.* (in press) detected *Anaplasma bovis*, a tick-borne pathogen of wild and domestic stock, in a previously undescribed species of *Rhipicephalus* collected from *E. myurus* in Limpopo province, South Africa. The importance of Macroscelidea and other small mammal insectivores in the life cycle of ixodid ticks and of reservoirs of tick-borne pathogens should be more fully investigated globally.

Both *R. warburoni* and *I. rubicudus* were able to attach and feed upon *E. myurus* but did not attach or feed on *M. namaquensis*. This is consistent with patterns observed in the field data, where *E. myurus* hosted large numbers of *R. warburtoni* larvae and nymphs while *M. namaquensis* hosted only 2 incidental larvae. The absence of *I. rubicundus* in field data collected in December was as result of seasonality, this tick species is not found on *E. myurus* during the summer, while *R. warburtoni* is relatively abundant throughout the year (Fourie *et al.*, 1992). By placing ticks directly on solitary hosts during feeding experiments, effects other than "true host specificity" that could affect the abundance of ticks on hosts, such as differences in habitat selection, tick and host activity patterns and allogrooming, were removed. Moreover, the response of both tick species to captive reared hosts, naive to ticks, was almost identical to hosts that had been previously exposed, suggesting that acquired immunity did not play a part in the ability of ticks to feed on *E. myurus*. We suggest that both *R. warburtoni* and *I. rubicundus* exhibit true host specificity and that some cue delivered by the host triggers attachment and feeding of these ticks on *E. myurus*. A variety of host stimuli are responsible for the appetence response of ticks. Carbon dioxide, radiant heat, vibration, visual images, shadowing and host odour can all induce an appetence response in ticks

(Waladde and Rice, 1982). Fourie *et al.* (1993) observed that adult female *R. warburtoni* (then called *R. punctatus*) and *I. rubicundus* exhibited appetence responses, in varying degrees, to radiant heat, shadow, carbon dioxide and host odour (in the form of goat hair). However, nothing is known regarding the appetence responses of the juvenile stages of these ticks. Of all potential host stimuli, host odour is the most specific, with others acting as general attractants mostly acting at long range (Waladde and Rice, 1982). Whilst some or all of these stimuli may cause an initial appetence it is likely that some kairomonal aspect of the host odour is responsible for the particular cue for *R. warburtoni* and *I. rubicundus* to attach and to feed on *E. myurus*. While species-specific identification of potential mates via pheromones has been widely researched (Soneshine, 2004) the use of kairomones for the specific identification of hosts is poorly understood. Rechav *et al.*, (1978) observed that *Ixodes neitzi* would aggregate on vegetation that had been scent marked by their preferred host, the klipspringer *Oreotragus oreotragus*, using its pre-orbital gland and demonstrated that adult ticks could detect this scent in the laboratory. Moreover, Osterkamp *et al.*, (1999) observed that *Boophilus microplus*, a 1 host tick specific to cattle, was highly sensitive to the cattle-associated phenolic compounds, 1-octen-3-ol and 2-nitrophenol, in the host odour. Therefore, it is likely that specific chemical compounds associated with the odour of *E. myurus* are detected by *R. warburtoni* and *I. rubicundus* that initiate an attachment and feeding response.

Ticks also have highly developed and diverse mechanisms to suppress both the innate and acquired immune responses of hosts on which they commonly feed (Soneshine, 1993; Wikel, 1999). For example, the black-legged tick, *Ixodes dammini* can feed very successfully on its preferred host, the white footed mouse, *Peromyscus leucopus*, due to its ability to evade and suppress the host immune response (Ribeiro, 1989 and references therein). In contrast the same ticks fed on guinea pigs are rapidly rejected on repeated exposure. It was shown that

successful feeding of *I. dammini* on *P. leucopus* was mediated through a number of pharmacologically active compounds that inhibit platelet and neutrophil aggregation and T-cell activation. Therefore, it is likely that *R. warburtoni* and *I. rubicundus* have evolved immunosupressive mechanisms in order to avoid rejection by *E. myurus* and to continue feeding once attached. This notion is supported by the collection of the single *R. warburtoni* which had attempted to feed on *M. namaquensis*. This tick obtained a small amount of blood and then detached and died. Although the only example, it may suggest that these ticks do not have the appropriate mechanisms to evade the host responses of *M. namaquensis* to feed successfully, even when they are able to attach.

While the current study has provided some insight into the mechanisms behind differences in tick parasitism rates between *E. myurus* and *M. namaquensis*, the question remains as to why juvenile stages of *R. warburtoni* and *I. rubicundus* are specific to *E. myurus* but do not, or cannot, feed on *M. namaquensis*. It has been noted that ticks feeding on more primitive animals tend to more host specific. For example, some species of *Amblyomma* feed only on snakes, others only on lizards while others feed only on primitive Australian monotremes and marsupials (Soneshine, 1993). The Macroscelidea are believed to be an ancient monophyletic group within the Superorder Afrotheria (Rathbun, 2009). It may be that ticks specific to sengis have coevolved with their hosts over long periods of time and as a result have become highly specialised as appears to be the case with ticks found on other primitive animals. *E. myurus* is an insectivore and *M. namaquensis* a herbivore and as such occupy different trophic levels and the chemical composition their tissues will be different (see Kelly, 2000 for a review), for example, *E. myurus* may have greater levels of nitrogen than *M. namaquensis*. Should this blood be more nutritious, it would be advantageous for ticks to feed on those hosts at this trophic level as opposed to those at a lower trophic level which could lead to the evolution of mechanisms resulting in host-specificity.

The current study demonstrates that *R. warburtoni* and *I. rubicundus* exhibit true host specificity which is likely mediated through specific chemical compounds within the odour of the host. This has important implications for the potential control of these tick species if these chemical compounds could be isolated and utilised in attractant traps. The study also highlights the importance of small mammalian insectivores as host of ixodid ticks and as potential reservoirs of tick-borne pathogens of medical and veterinary importance.

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