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Sequential feeding of *Ixodes rubicundus* on its natural host, *Elephantulus myurus*: effects on tick mass and on engorgement and moulting success

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ABSTRACT

DU TOIT, J.S., FOURIE, L.J. & HORAK, I.G. 1994. Sequential feeding of *Ixodes rubicundus* on its natural host, *Elephantulus myurus*: effects on tick mass and on engorgement and moulting success. *Onderstepoort Journal of Veterinary Research*, 61:143–147

Engorgement, mass at repletion and moulting success of immature *Ixodes rubicundus* after sequential infestations of their natural hosts, rock elephant shrews (*Elephantulus myurus*), were investigated under laboratory conditions. The reaction of *E. myurus* is characterized by inefficient or non-existent anti-tick immunity which enables immature *I. rubicundus* to attach and engorge successfully and ensures a high moulting success rate.

INTRODUCTION

A wide range of host adaptations have evolved to minimize the effects of tick infestations. These adaptations are collectively known as immunity (Need & Butler 1991) and are either innate or acquired (Kim 1985).

The development of acquired immunity to tick feeding is well documented in a variety of laboratory and domesticated animals and has been reviewed by Wikel & Allen (1982), Brown (1985), Kim (1985) and Willadsen (1980). The adaptations of natural hosts to tick challenge are largely unknown and poorly understood (Trager 1939; Doube 1974; Randolph 1979; Ribeiro 1989). The manifestation of acquired resistance in these hosts appears to vary considerably, depending on the type of host and the species of tick.

Resistance to ticks is generally expressed as an increase in the number which fails to complete their blood meal, and a decrease in the mean mass of replete ticks (Rechav & Dauth 1987). Resistance in unnatural hosts may cause a decline in the fertilization of female ticks and in egg production (Fivaz 1982), as well as a decrease in the moulting success of subadults. Immunity to tick infestations in natural hosts, however, may be inefficient or non-existent (Ribeiro 1989). *Elephantulus myurus*, the rock elephant shrew, is considered to be the principal natural host of immature Karoo paralysis ticks (*Ixodes rubicundus*) (Fourie, Horak & Van den Heever 1992). These animals may harbour consistently heavy burdens from winter to spring (Fourie *et al.* 1992). The objectives of this study were to determine engorgement and moulting success as well as mass at repletion of *I. rubicundus* after sequential infestations of rock elephant shrews.

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MATERIALS AND METHODS

Ticks

Engorged female *I. rubicundus* were collected in the field from sheep on the farm "Preezfontein" in the Fauresmith district of the Orange Free State during July 1990. These ticks were placed in 10-mℓ glass vials, plugged with cotton wool and kept in total darkness in a temperature-controlled cabinet at 25 ± 1 °C (86 ± 4 % RH) to oviposit. After the eggs had hatched, the larvae were maintained in the dark at 20 ± 1 °C (86 ± 4 % RH). Larvae were fed on caged *E. myurus* kept in a temperature-controlled cabinet at 20 ± 1 °C (12L:12D light regime). After detachment the ticks were collected and maintained in darkness at 25 ± 1 °C (86 ± 4 % RH) until they moulted. Nymphs were maintained under similar conditions.

Hosts

Four subadult *E. myurus* were captured during January 1990 in the Bloemfontein District. The mean mass of these elephant shrews was 44,12 (S.D. ± 1,21 g), so they were probably born during December 1989. Rock elephant shrews breed only from November to March (Van der Horst 1946; Tripp 1972). These four animals were assumed to be immunologically naive in terms of exposure to *I. rubicundus* since larvae are active from March to September and nymphs from May to November (Fourie *et al.* 1992).

The elephant shrews were individually housed in plastic cages and acclimated for two months in an environmental room at 20 ± 1 °C (43 ± 4 % RH) with a photoperiod of 12L:12D before they were infested with ticks. Water and food (puppy food, stage 2 with Vitagen, Epol, South Africa) were provided *ad lib*.

Infestation procedure

Infestation levels of 100 larvae and 50 nymphs were initially used. These numbers approximated natural burdens (Fourie *et al.* 1992). Three sequential infestations with 100 larvae, 2–3 weeks apart, were made. The ticks were placed on the backs of the elephant shrews with the aid of a small paint brush and allowed to feed at a temperature of 20 ± 1 °C and a photoperiod of 12L:12D. The floor of each cage consisted of wire mesh which allowed detached ticks to fall through. These ticks were collected daily from water-filled trays placed beneath each cage. They were dried, counted and subsequently weighed on a Sartorius single-pan balance to the nearest 0,01 mg. Engorged larvae (n = 20 for each batch) were put into 10-mℓ glass vials plugged with cotton wool. These were placed inside a high-humidity container in a temperature-controlled cabinet at 25 ± 1 °C (86 ± 4 % RH) in the dark. Weekly observations were made until the first larvae moulted, followed by daily observations, and the success rate of moulting was recorded once all the larvae had either moulted or died.

Similar procedures were followed when the same four elephant shrews were infested with 50 nymphs on each of three consecutive occasions at intervals of 3–4 weeks. This was done 2 months after the last infestations with larvae.

Since infestation levels above a certain threshold level are necessary to stimulate an immunogenic response (Dineen 1963), the whole procedure was repeated immediately after the completion of the initial infestation programme, with 300 larvae and 200 nymphs per individual per infestation, respectively.

All ticks were 2–3 weeks old at the commencement of the experiments.

RESULTS

The engorgement, moulting success and engorgement masses of the larvae and nymphs of *I. rubicundus* on the artificially-infested elephant shrews are summarized in Tables 1a and b. Engorgement success for both larval infestation levels (100 and 300 larvae) remained above 80 % and moulting success above 95 %. There was no indication of a decrease in engorgement mass between consecutive infestations. An ANOVA test indicated that there were no statistically significant differences ($P > 0,05$) between the different infestations for any of the parameters measured. However, significant differences (Student t-test; $P < 0,05$) were recorded between engorgement success of larvae at the low (100) and at the high (300) infestation levels (Table 1a). This was also true for the nymphs (Table 1b).

The engorgement success of nymphs (Table 1b) was significantly lower (Student t-test; $P < 0,05$) than that of larvae. The values varied between 74 and 79 % for infestations with 50 nymphs and 67 and 74 % for infestations with 200 nymphs.

The engorgement times (i.e. the time from infestation to the first detaching tick) for larvae and nymphs were 3–4 d and 4–5 d, respectively. The detachment periods (i.e. the period from the first to the last detaching tick) was 2–3 d for larvae and 3–4 d for nymphs. Both the engorgement times and detachment periods remained stable during the entire experiment.

DISCUSSION

This study indicates that the immature stages of *I. rubicundus* do not induce an effective immune response in their natural hosts, *E. myurus*. Laboratory rabbits, however, develop effective immunity to the feeding of both larvae and nymphs of *I. rubicundus* (Arthur M. Spickett, personal communication 1992). Nymphs feeding on rabbits may induce paralysis at an infestation level of about 200 ticks per animal

TABLE 1 Engorgement success (%) mass (g), time (days) to first moulting, and moulting success of immature *Ixodes rubicundus* parasitizing on *Elephantulus myurus*

Animal	Sex	Ticks/ infestation	First infestation				Second infestation				Third infestation			
			Engorgement success (%)	Engorgement mass ($\times 10^{-5}$ g)	Time to first moulting (d)	Moulting success (%)	Engorgement success (%)	Engorgement mass ($\times 10^{-5}$ g)	Time to first moulting (d)	Moulting success (%)	Engorgement success (%)	Engorgement mass ($\times 10^{-5}$-g)	Time to first moulting (d)	Moulting success (%)
(a) *Larvae*														
(i)														
1	M	100	92	18,60	34	95	89	21,70	31	100	93	20,80	28	100
2	F	100	84	20,20	31	100	86	17,50	22	95	87	21,00	43	100
3	F	100	89	20,00	28	100	91	21,20	27	95	86	23,10	31	100
4	M	100	90	22,10	27	100	90	23,10	38	95	91	24,20	32	95
$\bar{x}$			88,75	19,35	30,00	98,75	89,00	20,88	29,50	96,25	89,25	22,28	33,50	98,75
S.D.			2,95	0,75	2,74	2,17	5,55	0,37	5,80	2,50	2,86	1,43	15,68	2,17
(ii)														
1	M	300	83	19,90	33	90	81	22,20	34	100	84	21,10	33	95
2	F	300	79	20,10	37	95	83	20,00	28	95	79	23,30	37	100
3	F	300	87	20,90	28	95	79	22,10	23	95	83	21,90	28	100
4	M	300	91	23,10	21	100	87	18,60	32	100	88	18,60	22	100
$\bar{x}$			85,00	21,00	29,80	95,00	82,50	20,73	29,30	97,50	83,50	21,23	30,00	98,75
S.D.			3,56	0,27	1,07	2,50	2,96	1,51	4,21	2,50	3,20	1,71	5,61	2,17

Animal	Sex	Ticks/ infestation	First infestation				Second infestation				Third infestation			
			Engorgement success (%)	Engorgement mass ($\times 10^{-4}$ g)	Time to first moulting (d)	Moulting success (%)	Engorgement success (%)	Engorgement mass ($\times 10^{-4}$ g)	Time to first moulting (d)	Moulting success (%)	Engorgement success (%)	Engorgement mass ($\times 10^{-4}$ g)	Time to first moulting (d)	Moulting success (%)
(b) *Nymphs*														
(i)														
1	M	50	79	43,60	143	95	81	42,40	157	95	73	43,10	150	95
2	F	50	68	42,80	150	100	83	42,90	171	95	76	42,60	157	100
3	F	50	72	43,30	143	100	71	42,10	147	100	83	42,20	143	90
4	M	50	78	42,90	164	100	71	43,00	164	100	82	43,70	150	95
$\bar{x}$			74,25	43,15	150,00	98,75	76,50	42,60	162,30	97,50	78,50	42,90	150,00	95,00
S.D.			4,49	0,32	8,57	2,17	5,55	0,37	5,80	2,50	4,15	0,56	4,95	3,54
(ii)														
1	M	200	64	42,80	171	95	69	43,10	136	100	71	42,50	150	95
2	F	200	72	42,60	150	90	71	42,80	157	95	74	42,70	164	90
3	F	200	63	42,70	143	90	62	44,10	164	95	69	13,10	171	95
4	M	200	68	42,10	164	95	68	43,70	185	90	81	44,00	178	95
$\bar{x}$			66,75	42,55	157,00	92,50	67,50	43,43	160,50	95,00	73,75	43,08	165,80	93,75
S.D.			3,56	0,27	11,07	2,50	3,35	0,51	17,50	3,54	4,55	0,58	10,35	2,17

(Spickett, Elliot, Heyne & Neser 1989). This level of infestation, however, failed to induce paralysis in *E. myurus*.

Although no histological examinations were done during the present study, inflammatory lesions surrounding the mouthparts of nymphs in the dermis of *E. myurus* were observed (J.S. du Toit, unpublished data 1993). Higher vertebrates normally respond to tissue damage caused by parasitic arthropods by adaptive immune responses mediated by serum or antibodies (Brown 1982; Kim 1985). The absence of any significant difference between the engorgement masses of larvae and nymphs, respectively, for consecutive infestations, indicates that the decrease in engorgement success between the light and heavy tick burdens does not have an immunological basis.

The differences in the engorgement success of larvae and nymphs at low and high infestation levels are probably related to competition for attachment sites. Larvae and nymphs of *I. rubicundus* attach to specific

body areas of the rock elephant shrew, notably to the sacral and anogenital areas, respectively (J.S. du Toit, J. Loubser, L.J. Fourie, unpublished data 1992). Preferred attachment sites of ticks may be influenced by a variety of factors such as grooming (Barnard, Morrison & Ervin 1989), density (Andrews & Petney 1981; Petney & Al-Yaman 1985), intra- and interspecific interaction between tick species (Andrews, Petney & Bull 1982), habitat (Balashov 1972; Wilkinson 1985) and season (Evans 1952). High infestation levels can therefore cause ticks to attach to non-preferred body areas, resulting in their removal by the scratching or grooming actions of the host (Barnard *et al.* 1989).

Randolph (1979) compared the attachment, engorgement and subsequent development of sequential infestations of *Ixodes trianguliceps* larvae and nymphs on natural hosts (*Apodemus sylvaticus*) and unnatural hosts (laboratory mice). Upon reinfestation of the laboratory mice, infestations above a threshold level of approximately ten ticks elicited an immunological response which reduced, in a density-dependent manner, the rate of successful tick engorgement. In comparison, successive infestations of *A. sylvaticus* with larvae resulted in unchanged or slightly improved survival rates of larvae to nymphs. Similarly, Doube (1974) found that bandicoots, which are the natural hosts for *Ixodes holocyclus*, did not become more resistant to either larvae or nymphs after successive infestations in the laboratory, and no obvious lesions were detected at the ticks' feeding sites. The present results are in accordance with these findings.

Although rock elephant shrews are easily maintained in the laboratory, they do not breed under these conditions (Du Toit 1993). Elephant shrews are also known to serve as hosts for a variety of ticks (Fourie *et al.* 1992). Potentially, therefore, they can be used as hosts for these ticks under laboratory conditions in preference to laboratory animals, in which the development of resistance to tick feeding is a real problem (Bowessidjaou, Brossard & Aeschlimann 1977; Rechav, Heller-Haupt & Varma 1989; Girardin & Brossard 1989; 1990; Brossard & Papatheodorou 1990).

In the natural, presumably long-established *E. myurus*-*I. rubicundus* relationship (Du Toit 1993), no evidence exists of effective acquired resistance, even at infestation levels which exceed peak natural tick burdens. Since several authors have reported that the immunological response of the host to ticks starts 7–14 d after primary infestation and that resistance may persist for at least 1–3 months (Trager 1939; Allen 1973), it is unlikely that any transient response by *I. rubicundus* was suspended by limiting the infestation intervals to 14–21 d. In addition, there is as yet no evidence of a critical level of infestation by *I. rubicundus* at which *E. myurus* suffers, or even dies, as a result of tick parasitism.

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