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Dermacentor reticulatus (Acari, Ixodidae) female feeding in laboratory

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The effects of host, size of tick males and tick density per host on feeding of *Dermacentor reticulatus* females were investigated in the laboratory. Comparison of feeding performances on laboratory animals suggests that mice and rabbits are more suitable hosts than guinea pigs. The size of male ticks did not influence the feeding rate of females. Density of tick pairs per host influenced finally the length of female feeding, however, other female characteristics were the same.

Key words: tick, *Dermacentor reticulatus*, laboratory hosts, feeding aggregation.

Introduction

It is generally known that host species influences the feeding rate of ticks (Amin, 1969; Musatov, 1970), however, data on this aspect for laboratory breeding of *Dermacentor reticulatus* (F., 1794) (syn. *D. pictus* Herman, 1804) (Acarina, Ixodidae) ticks are limited. Honzáková (1971) observed that the duration of *D. reticulatus* feeding was affected stronger by the season (spring and autumn: 7–9 and 11–14 days, respectively) than by the laboratory hosts (rabbit and white mouse: 10–14 and 11–14 days, respectively)

According to Nosek (1979), all adults of *D. reticulatus* and *D. marginatus* (Sulzer, 1776) that moult in spring are smaller in size. This variability is natural and can be observed in each species, or it may be caused by parthenogenesis or engorgement state, i.e. not fully fed nymphs moult into smaller adults. Moreover, overwintering engorged females are also smaller. Considerable differences in tick body size/weight (of both sexes) in laboratory colonies of this species were also recorded (Slovák et al., 2002). Wang et al. (1998) stated that males of *Rhipicephalus appendiculatus* Neumann, 1901 help mated females to feed by modulating the host immune response during male-female co-feeding. The question is if different sized males help to females in the same way.

Wang et al. (2001) noted that when more ticks of the same species (*R. appendiculatus*) were co-feeding on a host, they usually were gathered into one or more groups of different size. At group feeding, females gain weight more quickly than those feeding individually or in pairs. Preliminary observations indicated that this phenomenon is also valid for *D. reticulatus*.

In ixodids mating occurs during feeding, preceding the final phase of engorgement (Aeschlimann & Grandjean, 1973), thus mating is necessary to enable the full repletion of the female. As the minimum period of *D. reticulatus* male feeding before mating is 5 days (Slovák et al., 2002), we chose this day in female feeding for comparison.

The task of this study was to contribute to the knowledge how host species, size of tick males and tick density per host can influence the length of *D. reticulatus* females feeding and their weight at laboratory conditions to prepare homogenous material, e.g. for physiological studies.

Material and methods

A *D. reticulatus* colony has been maintained at the Institute of Zoology (Bratislava) (Slovák et al., 2002). In all experiments, unfed adult ticks were kept more than 2 months post-moulting. Tick naive guinea-pigs weighing 500–550 g, ICR mice weighing 15–20 g, and New Zealand white rabbits weighing 3.8–4.2 kg were obtained from breeding colonies of the Institute of Virology, Slovak Academy of Sciences.

For feeding experiments, neoprene retaining chambers (later on containing ticks) were attached to the backs of guinea-pigs or rabbits (Slovák et al., 2002). Cut-cone-shaped plastic chambers were used for feeding of ticks on mice (Slovák et al., 2000). The hosts

Table 1. Details of experimental design.

Experiment: Influence of host species on female feeding

Host	*n*	No. of ticks/host F/M	Weight of unfed females (mg) Replicate I.	II.	III.	Weighting
Guinea pig	4	5/5	4.2*	5.0	5.0	5 days fed
Mouse	3	5/5	4.2*	5.0	5.0	5 days fed
Rabbit	4	5/5	4.2*	5.0	5.0	5 days fed
Guinea pig	10	5/5	5.1			engorged
Mouse	5	5/5	5.1			engorged
Rabbit	4	5/5	5.1			engorged

Experiment: Influence of tick male size on female feeding

Host	*n*	No. of ticks/host F/M	Weight (in mg) of unfed females	unfed small males *n*	mean	range	unfed big males *n*	mean	range	Weighting
Mouse	6	5/5	4.6	15	2.12	1.4–2.5	15	7.90	7.5–8.8	5 days fed
Mouse	6	5/5	4.9	15	2.26	1.2–3.0	15	9.22	8.5–10.1	5 days fed
Mouse	6	5/5	4.8	15	2.37	1.8–2.8	15	6.75	6.3–8.4	5 days fed
Mouse	6	5/5	4.8	15	2.80	2.6–3.0	15	7.10	6.7–7.4	5 days fed
Rabbit	4	5/5	5.4	10	2.87	1.7–3.4	10	6.87	6.4–8.0	engorged
Rabbit	4	5/5	5.4	10	3.08	2.7–3.4	10	6.72	6.5–7.4	engorged

Experiment: Influence of tick density on female feeding

Host	*n*	No. of ticks/host F/M	Weight of unfed females (mg) Replicate I.	II.	III.	Weighting
Mouse	12	1/1	4.4	4.4	4.4	5 days fed
Mouse	4	3/3	4.4	4.4	4.4	5 days fed
Mouse	2–3	5/5	4.4	4.4	4.4	5 days fed
Guinea pig	3	12x1/1; 4x3/3; 2x5/5	4.8			engorged
Mouse	15	1 F	4.3	5.0*	5.2*	5 days fed
Mouse	6	3 F	4.3	5.0*	5.2*	5 days fed
Mouse	3	5 F	4.3	5.0*	5.2*	5 days fed

Key: F – female; M – male; *n* – number of used animals per experiment; * data of this replicate are not shown, however, similar trends were observed like in published results.

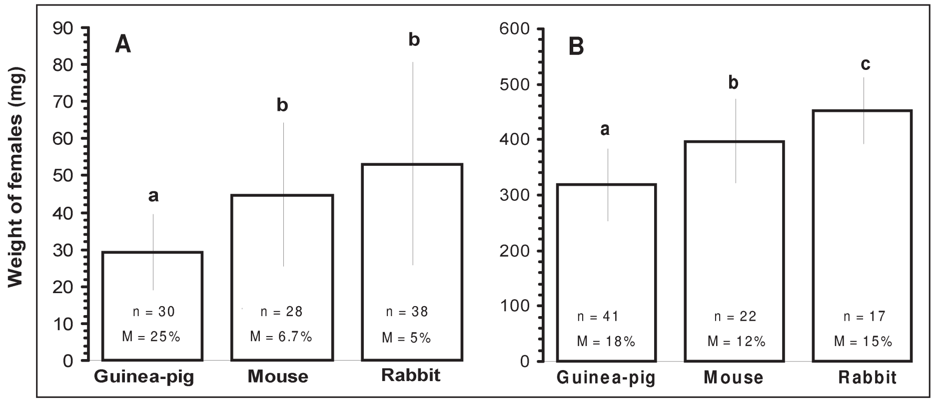

Fig. 1. Weight differences in 5 days fed (A) and engorged (B) *Dermacentor reticulatus* females after feeding on different hosts. After 5 days of feeding, the results of two experiments with the same weight of unfed females (5.0 mg) were enough identical and so they were pooled. Columns represent mean weights ± SD; M – mortality; n – number of individuals; means followed by the same letter beyond column (particularly in experiments A and B) are not significantly different at $P = 0.05$ (Duncan's test). $F = 10.46$; $P < 0.001$ and $F = 28.84$; $P < 0.001$ were calculated for results in experiments A and B, respectively.

with feeding ticks were kept individually at laboratory conditions as described by Slovák et al. (2002). The modified method of Wang et al. (2001) using guinea-pigs was applied for the investigation of engorgement of females feeding individually and in groups. Details of experimental conditions are summarized in Table 1.

Engorged females were weighed and then stored in modified plastic syringes (Slovák et al., 2002) at 24℃, 80% relative humidity and darkness in an incubator (Binder). Except their engorged weight and feeding time, preoviposition, fecundity (calculated from weight of deposited eggs) and egg incubation periods were observed. The same characteristics were also estimated in all experiments.

Results were statistically evaluated by one-way ANOVA, followed by Duncan multiple range test or Student's t-test using Statgraphic or Microsoft Excel softwares, respectively. Differences at $P < 0.05$ were considered as significant.

Results

Tick feeding success depended on the laboratory host. By day 5 of feeding, *D. reticulatus* females engorged (as defined by increase of body weight) most slowly when fed on guinea-pigs (29.3 ± 10.9 mg; mean ± SD), significantly (~1.5 times) faster on mice ($P < 0.001$, t-test) and most quickly on rabbits (~ 1.8 times faster when compared with females on guinea-pigs) (Fig. 1A). The highest mortality of tick females was observed when fed on guinea-pigs 25%. When ticks fed on rabbits or mice, their mortality (M) was considerably lower (Fig. 1). Feeding on different hosts strongly influenced the weight of tick females ($F = 10.46$; $P < 0.001$, ANOVA). The influence of host was even more significant after tick engorgement ($F = 28.84$; $P < 0.001$, ANOVA). In comparison to feeding on mice (396.9 ± 76.1 mg; mean ± SD), the weight of females fed on guinea-pigs was ~1.2 times lower and of those fed on rabbits ~ 1.2 times higher ($P < 0.001$, t-test; Fig. 1B).

Although the time required for tick feeding from attachment to detachment was similar on all observed hosts, females fed on mice oviposited on average 1.60 or 1.71 days earlier and laid 1.5 or 1.3 times more eggs than females fed on guinea-pigs or rabbits, respectively (Tab. 2). Significantly shorter incubation time was recorded in egg masses of females fed on mice compared to rabbits.

The results of experiments where different sized males fed together with females indicated that generally the size of males did not influence significantly the weight and other characteristics of co-feeding females.

On day 5 of engorgement females feeding in groups of five or three and with the same number of males showed an increase in weight by 40.6 or 26.2%, respectively, in comparison with females feeding in pairs (Fig. 2A). While differences in the weight of females feeding in pairs (35.5 mg) in comparison with weights of females feeding in three (44.8 mg) and five (49.9 mg) pairs were statistically significant ($P < 0.05$ and $P < 0.01$, respectively, t-test), the differences in weights of females feeding in 3 and 5 pairs were not significant. In all experiments mortality of females decreased from 19.4 to 12.5% with increasing num-

Table 2. Engorged *Dermacentor reticulatus* female characteristics after feeding on different host species.

Host	Development (in days) from attachment to									Egg batch (no. of eggs)		
	Detachment			Egg laying			Hatching of larvae					
	n	mean ± SEM	min–max.	*n*	mean ± SEM	min–max.	*n*	mean ± SEM	min–max.	*n*	mean ± SEM	min–max.
Guinea-pig	41	9.97 ± 0.36 a	7–16	39	14.49 ± 0.32 a	11–20	35	27.57 ± 0.24 ab	25–31	39	3655 ± 120 a	2236–5644
Mouse	22	9.91 ± 0.28 a	8–13	19	12.89 ± 0.29 b	11–15	19	26.74 ± 0.29 a	25–29	19	5458 ± 271 b	3256–7201
Rabbit	17	10.06 ± 0.29 a	8–12	15	14.60 ± 0.35 a	13–17	15	27.67 ± 0.39 b	26–31	15	4182 ± 255 a	2536–5626
ANOVA	$F = 0.27$; $P = 0.763$			$F = 5.48$; $P = 0.006$			$F = 2.68$; $P = 0.076$			$F = 24.48$; $P = 8.68E\text{-}09$		

Key: *n* – number of observed individuals; means followed by the same letter are not significantly different at $P = 0.05$.

bers of tick pairs per mouse. Almost all group-fed females (100% and 92% among 5 and 3 feeding pairs, respectively) attached after 24 h (day 1), which was more rapid than female attachment in individually fed pairs (60%). Repletion and detachment occurred at day 8 in 33.3 and 50.0% of females feeding in groups of 5 and 3 pairs, respectively, but only in 11.1% of females fed in individual pairs. Group feeding increased the feeding rate as measured by repletion time compared to single tick pairs (11.78 ± 0.62 days; mean ± SEM), however, significantly in three feeding pairs (9.92 ± 0.42 days; $P < 0.05$, *t*-test) and not significantly in 5 feeding pairs (10.33 ± 0.41 days). Females in group-fed and individually fed pairs finally engorged with similar body weight (Fig. 2B).

The same trends as observed for tick pairs have been observed for females feeding solitarily (1) and gregariously (3 and 5), but without males (Fig. 3). After 5 days of feeding, the weights of females feeding in groups of 3 or 5 were 1.4 (not significantly) or 1.7 times bigger ($P < 0.01$; *t*-test), respectively, in comparison with solitarily fed females.

The observed factors influencing laboratory feeding of *D. reticulatus* were ranked according to descending significance and Fisher's coefficients (Tab. 3). Based on this arrangement the grade of influence of a factor on female development in laboratory can be anticipated.

Discussion

HONZÁKOVÁ (1971) observed minimal influence of host species (rabbit and white mouse) on the duration of *D. reticulatus* feeding. Our results on times required for engorgement of females on rabbits (10.06 days), mice (9.91 days) and guinea-pigs (9.97 days) confirmed the conclusions of HONZÁKOVÁ (1971). However, significant differences were observed in other aspects of female engorgement. Females feeding on mice deposited significantly more eggs, oviposition took place within the shortest time and the incubation time was shorter than in females feeding on rabbits or guinea-pigs. On the other hand, *D. reticulatus* females feeding on rabbits tended to take in significantly bigger volumes of blood than those feeding on mice and guinea pigs. These results indicated that rabbit and mouse, although laboratory animals, are more closely resembling natural hosts in relation to *D. reticulatus* feeding success than the guinea-pig, which might be attributed to its strong immune response to ticks (RECHAV et al., 1994; SZABO et al., 2003).

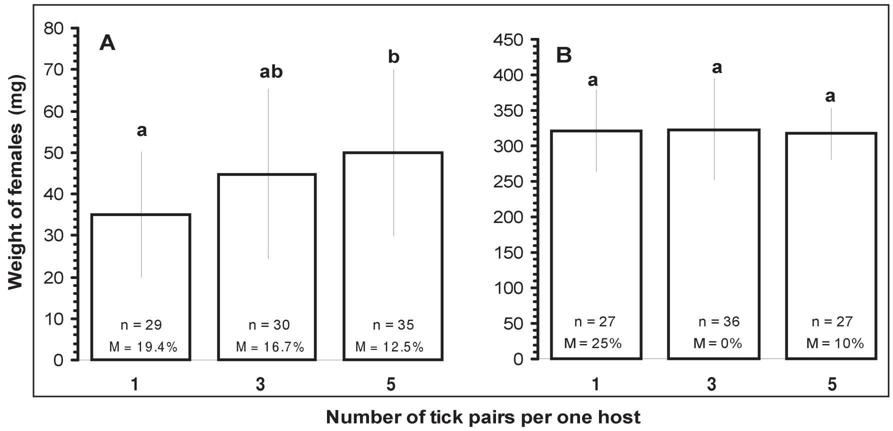

Fig. 2. Weight differences in 5 days fed (A) and engorged (B) *Dermacentor reticulatus* females which fed on mice (A) and guinea pigs (B) in 1, 3 or 5 pairs. $F = 4.33$; $P < 0.01$ and $F = 0.08$; $P < 0.9$ were calculated for results in experiments A and B, respectively (ANOVA); for others see Fig. 1.

Table 3. Ranking of factors affecting *D. reticulatus* female engorgement (as defined by mean body weight) in accordance with significance of influence and F – value (one-way ANOVA) on day 5 of feeding.

Rank Rank	Factor Factor	After 5 days of feeding		After engorgement	
		Significance	F–value	Significance	F–value
1	Host species	$P < 0.001$	10.46	$P < 0.001$	28.84
2	Density of tick pairs	$P < 0.016$	4.33	$P < 0.925$	0.08
3	Density of females only	$P < 0.044$	3.94	–	–
4	Size of males	$P < 0.119$*	2.46*	$P < 0.211$**	1.81**

Key: *Counted by ANOVA for all data of experiments on mice; **counted by ANOVA for all data of experiments on rabbits.

The size of co-feeding males did not influence the weight and other characteristics of *D. reticulatus* females. In parallel experiments with *R. appendiculatus*, the differences (3.0, 3.7 and 4.8 multiple) between mean body weight of "small" and "big" males also did not influence the weight and other female characteristics (unpubl.).

At day 5, after feeding in groups (3 or 5 tick pairs), *D. reticulatus* females attained significantly bigger weights compared to solitarily feeding pairs. Wang et al. (2001) showed that group feeding in *R. appendiculatus* adults increased their blood-feeding rate compared with feeding of paired adults. The authors suggested that ticks could support each other during the feeding process. This reciprocal help while feeding is usually influenced by the size of group. The more ticks are feeding in a group, the more expressive is the reciprocal support in spite of the fact that in larger groups usually one or two females are increasing their body weight more slowly. The benefits of feeding aggregation indicate direct reciprocity between ticks, most likely resulting from the shared activities of their bioactive saliva. Wang et al. (1998) stated that male *R. appendiculatus* helps its mated female to feed by modulating the host immune response during male-female co-feeding. Partially fed males produce specific salivary gland immunoglobulin-binding proteins (IGBP) (Wang & Nuttall, 1995). At least one of these male-specific proteins (IGBP-MC) functions to help mated females to feed (Wang et al., 1998). As shown by our results, in *D. reticulatus* ticks gregariousness can be advantageous compared to solitariness when only females are present on host. This suggests that, except the male-specific immunoglobulin G – binding proteins, support in gregariously feeding *D. reticulatus* females is also secured by other salivary compound(s). The mechanisms that ticks use to depress host immunity are

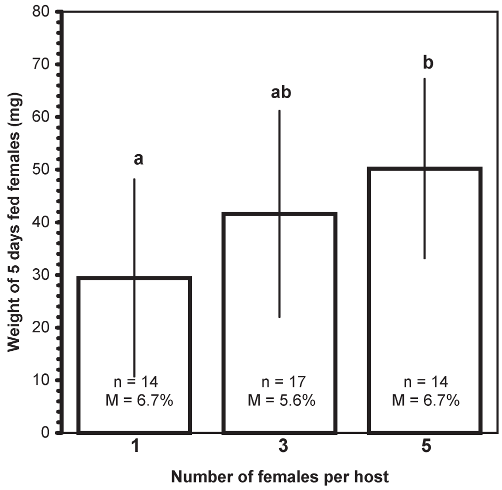

Fig. 3. Weight differences in 5 days fed *Dermacentor reticulatus* females which fed on host solitarily (1) or in group of 3 or 5 individuals. $F = 3.94$; $P < 0.04$ (ANOVA); for others see Fig. 1.

not clear yet but some research has been done in this area (Barriga, 1999).

Wang et al. (2001) observed that all group-fed *R. appendiculatus* ticks attached after 24 h (day 1), which was significantly more rapid than attachment of individually fed ticks. Repletion and detachment occurred significantly earlier in group-fed females compared with individually-fed females (at day 7), but group-fed and individually fed females finally engorged with similar body weight. Although at day 5 *D. reticulatus* group- and solitary- feeding females significantly differed in weight, these differences were not significant after females engorgement, similarly as in *R. appendiculatus.* The patterns of attachment and detachment of females also appears to correspond in *R. appendiculatus* and *D. reticulatus* at day 8.

In conclusion, the observed factors influenced the laboratory feeding of *D. reticulatus* females in various ways. The weight of engorged females differed when fed on different hosts, however, the rate of feeding was similar. On the other hand, tick density per host influenced the duration of feeding while the weight of engorged females was similar. The size of co-feeding tick males did not influence female feeding.

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