

The impact of intensity of invasion of *Ixodes ricinus* and *Dermacentor reticulatus* on the course of the parasitic phase

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Abstract

Investigations into the effect of the intensity of invasion on tick-questing behaviour were conducted on 3 groups of rabbits: 1) 10 females and 5 males, 2) 20 females and 10 males, 3) 40 females and 20 males in the case of *I. ricinus*, whereas in the case of *D. reticulatus*, another three groups were used: 1) 10 females and 15 males, 2) 20 females and 15 males, 3) 40 females and 15 males. Given the different invasion intensities, no significant difference was found in the time of attachment to the host ($p=0.3773$) or in the feeding period ($p=0.1051$) for *Ixodes ricinus* females. In turn, body weight of engorged females exhibited highly significant differences ($p=0.0021$) between the groups, particularly in the least (0.3551 ± 0.0739 mg) ($p=0.0027$) and most numerous groups (0.2752 ± 0.0964 g) ($p=0.0017$). In the case of *D. reticulatus*, the length of the skin attachment period did not show statistically significant differences ($p=0.4036$) between the groups consisting of different numbers of ticks. However, invasion intensity had a statistically significant effect of the length of the feeding period ($p=0.0037$). In the least numerous female group, the length of the feeding period was significantly lower than in the groups composed of 20 ($p=0.0091$) and 40 ($p=0.0042$) specimens. No significant differences in the length of the feeding period ($p=1.0000$) were found between the most numerous groups of 20 and 40 females. The body weight in engorged females of this species exhibited highly significant differences ($p=0.0006$) in the most numerous group (0.2511 ± 0.1135), and in the other groups (0.3559 ± 0.0654 and 0.3554 ± 0.0380 mg, respectively, in the invasion of 20 and 10 females). Both the investigated species showed highly significant differences in the attachment period in the groups with 20 ($p=0.0017$) and 40 ($p<0.0001$) females, and in the feeding period in all the experimental groups: ($p=0.0001$), ($p<0.0001$) and ($p=0.0088$), respectively. Furthermore, decreased feeding success and 12.5% female death were reported from the most numerous group of *I. ricinus* females. The differences between the feeding course in *I. ricinus* and *D. reticulatus* may be explained by the presence of various interactions in the parasite-host system at the different intensities of invasion by the 2 tick species.

Key words

Ixodes ricinus, *Dermacentor reticulatus*, ticks, intensity of invasion, parasitic phase

INTRODUCTION

In the climate conditions prevailing in Central and Eastern Europe, the entire developmental cycle of *Ixodes ricinus* (Linnaeus) usually lasts 2-3 years [1, 2, 3, 4] and approximately 1 year in the case of *Dermacentor reticulatus* (Fabricius) [5]. It is composed of 3 active stages, the development of which depends on environmental conditions [6, 7] as well as the species [8, own unpublished data], and the physiological status of the host [9, 10, 11, 12, own unpublished data]. Although shorter than the length of the life cycle, the parasitic phase is an important stage in tick development. Each *I. ricinus* and *D. reticulatus* stage ingests only once host blood that is indispensable for the development and function of their organs. The amount and composition of ingested food have an impact on the course of all physiological processes, e.g. survival in the environment, activity, and reproduction. Therefore, identification of factors that exert an effect on the questing of *I. ricinus* and *D. reticulatus* may play a pivotal role in the elucidation of the mechanisms of regulation of

population abundance and, consequently, in limiting the risk of attack and transmission of diseases by these arthropods.

Since natural hosts may be infested by a variable number of ticks [13, 14, 15, 16, 17, own unpublished data], investigations into the intensity of invasion during the parasitic phase are fully justified and important from both the scientific and a practical point of view.

The aim of these experiments was to investigate whether the intensity of invasion by adult *I. ricinus* and *D. reticulatus* has an effect on their questing behaviour, and to determine what number of ticks can reduce their questing efficiency. Investigation of the possibility to reduce the population abundance by the effect of tick density on their questing success is vital for understanding the ecology of diseases transmitted by these arthropods.

MATERIAL AND METHODS

Tick hosts. The study involved 9 albino New Zealand rabbits (*Oryctolagus cuniculus*) weighing on average 3-3.5 kg. Six animals were used for analyses of the course of a homogeneous *I. ricinus* invasion, and 3 for similar investigations on *D. reticulatus*. The animals were kept under standard conditions and were given *ad libitum* access to food and water.

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Tick collection and rearing. Unengorged specimens of 2 tick species, *I. ricinus* and *D. reticulatus*, were collected in the vicinity of Lublin in May 2010 and September 2011, respectively, i.e. during periods of their activity peaks identified by Bartosik et al. [18] and Buczek et al. (unpublished data). Tick specimens were collected using the commonly applied flagging method, which involves sweeping the vegetation with an approximately 1m² cloth. In the laboratory, the ticks were kept in glass containers stored in the refrigerator at a temperature ca. 5 °C and humidity of ca. 90-100% until the beginning of the experiments. Prior to the analyses, the specimens were transferred into chambers of 90-100% humidity for *I. ricinus* and 92.5% for *D. reticulatus* at an ambient temperature of ca. 20 °C. The appropriate humidity ranges were maintained using distilled H₂O (90-100%) and a saturated KNO₃ solution (92.5 %) in accordance with the method developed by Winston and Bates [19]. The experiments were carried out on 140 females and 70 males of *I. ricinus* and 70 females and 45 males of *D. reticulatus*.

In order to investigate the course of tick attachment and feeding, cloth bags with unengorged specimens were attached to an area of ca. 10-cm-diameter of shaved skin on the spine of each host. The experiments were conducted at ca. 18.5±1 °C room temperature and ca. 38±1% humidity. The methodology of animal infestation and tick rearing was elaborated by A. Buczek (oral communication, unpublished data).

Experiment groups. Two experiments on the course of attachment and feeding of the 2 tick species, *I. ricinus* and *D. reticulatus*, were carried out using one host species. In each of the experiments, the course of the parasitic phase was observed in 3 tick groups differing in the number of females and males placed on different hosts. The respective groups of *I. ricinus* consisted of 10 females and 5 males, 20 females and 10 males, and 40 females and 20 males. In the case of *D. reticulatus* infestations, the groups were composed of 10 females and 15 males, 20 females and 15 males, and 40 females and 15 males, respectively.

The 3 *D. reticulatus* experimental groups consisted of the same number of males, which were attached to the skin of the host obligatorily prior to mating. This eliminated their possible impact on the course of the experiments, and facilitated comparison of the attachment and feeding course of females in the samples differing in tick numbers.

The number of males in each experimental *I. ricinus* group was two-fold lower than that of females. This created the possibility of fertilization of the females in case they had not been mating with males prior to being placed on the host. *I. ricinus* males do not typically attach to host skin. Only in a few cases and for a short period were specimens observed attached to the skin of the host.

The course of experiments on attachment and feeding of adult stages of *Ixodes ricinus* and *Dermacentor reticulatus*. Before the experiments were started, the *I. ricinus* and *D. reticulatus* females were weighed using the RADWAG XA 110 analytical balance with the accuracy of 0.0001 g. In the case of *I. ricinus*, tick behaviour on the host in species homogeneous groups differing in the numbers of specimens was observed every half-an-hour during the first 3 hr of the experiments; next, the observations were carried out at 24 hr intervals at the same time of the day until cessation of feeding by all the females. In turn, the behaviour of *D. reticulatus*

ticks feeding in species homogeneous groups consisting of varied numbers of specimens was observed every half-an-hour during the first 3 hr of the experiments, every hour during the subsequent 4 hr, and next at 24 hr intervals at the same time of day until all the tick specimens detached from the rabbit skin. The frequency of assessment of the tick attachment course depended on the behaviour of both species on the host. Based on the observations of female and male behaviour, the dynamics of tick attachment to rabbit skin was determined. Immediately after they detached from the rabbit skin, the engorged females were collected, weighed, and placed in rearing chambers.

On the basis of the results obtained, the following parameters of the parasitic phase were determined: attachment dynamics (AD) – proportion of specimens attached to the host skin within a specific period (1 hr accuracy), attachment period (AP) – period between the beginning of infestation and first contact of unengorged ticks with the host, and the time of attachment of the specimens tested to the rabbit skin (expressed in hours; 1 hr accuracy), feeding period (FP) – period between the beginning of infestation and the moment of female detachment after cessation of feeding (expressed in days; 1 day accuracy), female engorgement weight (FEW) – weight of female ticks immediately after cessation of feeding (expressed in grams; 0.0001 g accuracy).

The above-mentioned parameters were used for calculation of coefficients characterizing the course of the parasitic phase in the life cycle of the tick groups examined, such as: feeding success (FS) – proportion of engorged females among the total number of the experimental females FS (%) = F / N × 100% (±1%), female death (FD) – proportion of females that died on the host during the invasion period (±1%): FD = FDI / N × 100% (±1%), tick yield (TY) – proportion of engorged females weighing at least 0.1 g among the total number of females that ceased feeding in the experimental group (±1%) (after Brown and Askenase [20], modified): TY (%) = FT / F × 100% and abnormal feeding coefficient (AF) – proportion of engorged females weighing less than 0.1 g at the time of feeding cessation among the total number of engorged females in the experimental group (±1%) (after Schorderet and Brossard [21], modified): AF = FT / F × 100%.

Statistical analysis. The results were analyzed using statistical package STATISTICA for Windows 9.0. Analysis of the differences in the distribution of the results depending on the tick group tested was performed using Spearman's rank correlations, the Kruskal-Wallis test, and Fisher's exact test. Analogous results of AP, FP and FEW in both tick species were compared with the use of the Mann-Whitney U test; Fisher's exact test was employed for analyses of feeding success and tick yield.

The test probability at p<0.05 was assumed significant and highly significant at p<0.01.

RESULTS

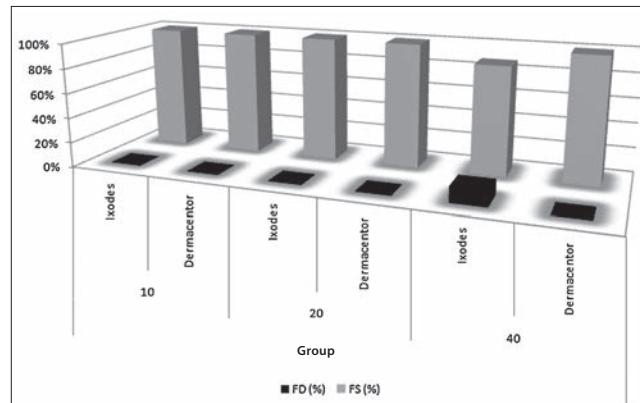
The course of the experiments on the behaviour of *I. ricinus* females on the host is presented in Table 1. On the basis of the results, no significant difference related to the number of female specimens in a given group was found in the length of the attachment (p=0.3773) and feeding (p=0.1051) periods. In turn, highly significant (p=0.0021) differences in the weight of

Table 1. Parameters of parasitic phase in *Ixodes ricinus* infesting the rabbit host in different abundance groups

Variable	Group	Descriptive statistics				Kruskal-Wallis test (p)
		M	SD	Min.	Max.	
AP	10♀ vs. 5♂	0.75	0.44	0.50	2.00	0.3773
	20♀ vs. 10♂	1.00	0.77	0.50	3.00	
	40♀ vs. 20♂	0.76	0.42	0.50	3.00	
FP	10♀ vs. 5♂	8.85	0.75	8.00	10.00	0.1051
	20♀ vs. 10♂	8.48	0.68	8.00	10.00	
	40♀ vs. 20♂	9.14	1.84	8.00	16.00	
FEW	10♀ vs. 5♂	0.3551	0.0739	0.1857	0.4990	0.0021
	20♀ vs. 10♂	0.3104	0.0675	0.1760	0.4260	
	40♀ vs. 20♂	0.2752	0.0964	0.0480	0.4810	

M – mean; SD – standard deviation; AP – attachment period (hours); FP – feeding period (days); FEW – female engorged weight (grams).

engorged *I. ricinus* females were found between the respective groups; they were mainly visible ($p=0.0017$) between the least numerous group consisting of 10 females with the highest engorgement weight (mean 0.3551 ± 0.0739 mg), and the most numerous group (40 females) with the lowest body weight (0.2752 ± 0.0964 mg). Exclusively in the case of infestation by 40 *I. ricinus* females, the feeding success was reduced to 87.5% and dead specimens constituting 12.5% of all the tested specimens were found (Fig. 1). In this female group, the tick yield was reduced to 91.43%, and the abnormal feeding coefficient was 8.57%.

**Figure 1.** Feeding success (FS) and female death (FD) in *Ixodes ricinus* and *Dermacentor reticulatus* feeding on species homogeneous groups differing in abundance

Likewise in *I. ricinus*, the length of the questing and attachment periods in *D. reticulatus* females did not exhibit statistically significant differences ($p=0.4036$) in groups differing in the tick numbers (Tab. 2). In turn, tick density exerted a statistically significant effect ($p=0.0037$) on the feeding period. The feeding period in the least numerous female group was significantly shorter than in the groups consisting of 20 ($p=0.0091$) and 40 females ($p=0.0042$). No significant differences in the feeding period length ($p=1.0000$) were found between the most abundant groups of 20 and 40 females. In the case of body weight of engorged *D. reticulatus* females, highly significant ($p=0.0006$) differences were found between the groups, primarily between the most numerous group and the others. The females from the group of 40 reached the lowest statistically significant ($p=0.0027$) body weight, compared to those from the group of 20 and 10

Table 2. Parameters of parasitic phase in *Dermacentor reticulatus* infesting the rabbit host in different abundance groups

Variable	Group	Descriptive statistics				Kruskal-Wallis test (p)
		M	SD	Min.	Max.	
AP	10♀ vs. 15♂	10.20	19.92	0.50	48.00	0.4036
	20♀ vs. 15♂	14.15	17.55	0.50	48.00	
	40♀ vs. 15♂	14.01	16.21	0.50	48.00	
FP	10♀ vs. 15♂	7.30	0.82	6.00	9.00	0.0037
	20♀ vs. 15♂	11.55	2.54	8.00	16.00	
	40♀ vs. 15♂	12.68	5.45	6.00	23.00	
FEW	10♀ vs. 15♂	0.3554	0.0380	0.2850	0.4144	0.0006
	20♀ vs. 15♂	0.3559	0.0654	0.2372	0.4849	
	40♀ vs. 15♂	0.2511	0.1135	0.0108	0.4102	

M – mean; SD – standard deviation; AP – attachment period (hours); FP – feeding period (days); FEW – female engorged weight (grams).

females ($p=0.0177$). The females from the groups of 10 and 20 did not exhibit statistically significant differences between each other ($p=1.0000$). In *D. reticulatus* females, the feeding indices, e.g. feeding success and abnormal feeding, were constant, irrespective of the group abundance (Fig. 1). During feeding, the large female group (40 specimens) exhibited reduced tick yield (87.5%) and increased coefficient of abnormal feeding (12.5%).

Comparison of the results of the experiments carried out on the same host species for both tick species did not reveal a significant difference ($p=0.1943$) in the attachment period between the least numerous groups of *I. ricinus*

Table 3. Comparison of values of parasitic phase parameters of *Ixodes ricinus* and *Dermacentor reticulatus* feeding on rabbits in groups of varied abundance

Variable	Group	Species	Descriptive statistics				Mann-Whitney U test (p)
			M	SD	Min.	Max.	
AP	10	I	0.75	0.44	0.50	2.00	0.1943
		D	10.20	19.92	0.50	48.00	
	20	I	1.00	0.77	0.50	3.00	0.0017
		D	14.15	17.55	0.50	48.00	
	40	I	0.76	0.42	0.50	3.00	<0.0001
		D	14.01	16.21	0.50	48.00	
FP	10	I	8.85	0.75	8.00	10.00	0.0004
		D	7.30	0.82	6.00	9.00	
	20	I	8.48	0.68	8.00	10.00	<0.0001
		D	11.55	2.54	8.00	16.00	
	40	I	9.14	1.84	8.00	16.00	0.0088
		D	12.68	5.45	6.00	23.00	
FEW	10	I	0.3551	0.0739	0.1857	0.4990	0.9824
		D	0.3554	0.0380	0.2850	0.4144	
	20	I	0.3104	0.0675	0.1760	0.4260	0.0281
		D	0.3559	0.0654	0.2372	0.4849	
	40	I	0.2752	0.0964	0.0480	0.4810	0.4465
		D	0.2511	0.1135	0.0108	0.4102	

I – *Ixodes ricinus*; D – *Dermacentor reticulatus*; M – mean; SD – standard deviation; AP – attachment period (hours); FP – feeding period (days); FEW – female engorged weight (grams).

and *D. reticulatus* (10 females) (Tab. 3). In turn, the more abundant groups (20 females) revealed a highly significant ($p=0.0017$) difference in the attachment period between the species. The *D. reticulatus* females remained attached for a longer period (14.15 ± 17.55 hrs) than the *I. ricinus* females (1.00 ± 0.77 hr). Similarly, a highly significant difference ($p<0.0001$) in the attachment period correlated with the species was found in the group of 40 females. The attachment period was substantially longer in *D. reticulatus*.

The length of the feeding period exhibited statistically significant differences ($p=0.0001$) between the species in the case of the least numerous groups of 10 females. The feeding period in *I. ricinus* was longer, reaching 8.85 ± 0.75 days, on average, whereas in *D. reticulatus* it was 7.30 ± 0.82 days, on average (Tab. 3). In the female groups consisting of 20 specimens, the feeding period was shorter in *I. ricinus* (8.48 ± 0.68 days, on average) than in *D. reticulatus* (11.55 ± 2.54 days, on average). The difference was statistically highly significant ($p<0.0001$). Similarly, a highly significant difference ($p=0.0088$) in the feeding period was found between both species in the groups consisting of 40 females. The feeding period in *I. ricinus* females was shorter than in *D. reticulatus* females, i.e. 9.14 ± 1.84 and 12.68 ± 5.45 days, respectively. Body weight of engorged females did not differ significantly between both species in the group of the lowest ($p=0.9824$) and highest ($p=0.4465$) number of specimens. Significant differences ($p=0.0281$) between the species were only found in the group of 20 females. Higher body weight was reached by the *D. reticulatus* females.

During the investigations, a reduced 87.5% feeding success was found, and a 12.5% female death rate only at the highest intensity of *I. ricinus* female invasion (Fig. 1). The difference in the feeding success and female death rates between *I. ricinus* and *D. reticulatus* was statistically significant (Fisher's test $p=0.0297$). The other parameters of the parasitic phase tested, i.e. tick yield and abnormal feeding, differed between the 2 tick species tested in the groups consisting of 40 specimens infesting the host, but the difference was not statistically significant (Fisher's test $p=0.5243$).

DISCUSSION

The interspecies differences in the course of attachment and feeding in *I. ricinus* and *D. reticulatus* may be associated with the different biological features of both species. *I. ricinus* females attach to the host more readily, as the process is dependent on the presence of males on the host to a lesser extent than in the case of *D. reticulatus* females, whose feeding period depends on mating with males on the host. As shown in own studies, *D. reticulatus* females cease feeding only after they are fertilized by engorged males.

The differences in the duration of the parasitic phase may reflect adaptation of individual species to diverse environmental conditions prevailing over their large occurrence range. In Europe, both species are distributed sympatrically, although *I. ricinus* prefers areas covered by trees or forests [1], while *D. reticulatus* usually occurs in open meadow ecosystems [22, 23, 24]. The shorter parasitic phase observed in a small number of *D. reticulatus* female ticks contributes to a reduced risk of substantial fluid loss through body surfaces in an environment with high exposure to sunlight and large fluctuations of temperature

and humidity. The prolonged feeding period observed at a higher density of *D. reticulatus* females on the host may be related to changes in the conditions at the attachment site induced by the large amount of saliva injected during food ingestion and the properties of its components, as well as by mechanical damage inflicted on the skin of the host by tick mouthparts. Although adult *D. reticulatus* stages, likewise other representatives of the genus *Dermacentor*, have a short hypostome and penetrate the epidermis only, they cause large lesions in the dermis [25]. This phenomenon can be explained not only by the composition of the saliva of these ticks, but also the manner of hypostome insertion into the skin and the structure of the cement cone in particular. The hypostome of *I. ricinus* is sufficiently long to reach the dermis and cement is formed on its sides. Therefore, skin lesions appear beneath the tip of the hypostome and reach deeper layers of the skin.

I. ricinus and *D. reticulatus* females in the groups differing in abundance exhibited similar body weight after feeding on a rabbit host, although hungry specimens of these species differed in terms of their size and weight. Ingestion of large amounts of food by the tick species examined is associated with their similar life styles. Both species are non-nidicolous ticks; therefore, they may be exposed to adverse environmental conditions through a long part of the life cycle. Oviposition of a large number of eggs related to the amount of ingested food [1, 12, 26, 27] enhances the chance of species survival.

In both tick species investigated, the larger the female group, the lower the body weight they reached after feeding. However, a considerable reduction was observed in the feeding success of *I. ricinus* and *D. reticulatus* only at the tick density of 40 females per area of 78.5 cm^2 . During infestation by large numbers of ticks, great amounts of antigens are injected into the skin, thus causing skin reactions. In consequence, the quality of the blood composition has a lower nutritional value, which affects the feeding success in females.

In contrast to the presented investigations, no impact has been reported of the density of *Ixodes scapularis* Say larvae feeding on *Peromyscus maniculatus bairdii* and *Peromyscus leucopus* mice on the tick engorged weight. However, differences were found in the course of moulting of the larvae after feeding on both hosts. The intensity of invasion of *I. scapularis* larvae on *Peromyscus leucopus* did not affect the moulting process, whereas in the case of feeding on *Peromyscus maniculatus*, the moulting success increased in a statistically significant manner, together with the increase in tick density. According to the authors, these differences result from the different character of both parasite-host systems rather than from tick density [28]. In the same species, Davidar et al. [29] found that the engorgement index (body length/scutum length) in *I. scapularis* larvae collected from *P. leucopus* mice caught in natural habitats increased with increasing tick density, which range from 1-35 on one host. In turn, the rate of infestation with *Dermacentor variabilis* (Say) (1-100 specimens on one host) did not have an effect on the course of feeding on *Microtus pennsylvanicus*, *Peromyscus leucopus* and *Rattus norvegicus* [30]. Randolph [31] demonstrated that the engorgement rate and moulting success of *Ixodes trianguliceps* Birula larvae decreased with the increased number of ticks attached to a previously non-infested bank vole (*Clethrionomys glareolus*).

The survival rate of *Rhipicephalus microplus* (Canestrini) (=*Boophilus microplus*) larvae feeding on previously non-infested cattle did not depend on tick density [32], but

decreased with the increasing level of infestation of hosts with acquired immunity [32, 33].

Numerous investigations show that the tick feeding success depends on the host species [30, 34, 35] and its immunological status [9, 10, 11, 21]. The results of an unpublished study by the authors of the presented study corroborate the effect of the host species on the feeding course and reproduction performance in *D. reticulatus*. Compared to feeding on guinea pigs, infestation of a rabbit host resulted in higher body weight and tick yield (ratio of engorged female weight and feeding duration), as well as a 100% feeding success and higher female fecundity. In previous investigations, an effect of host immunity was also found on the course of the parasitic and non-parasitic phases in the 2 most common tick species in Poland. The weight of engorged *I. ricinus* and *D. reticulatus* females decreased during 3 consecutive infestations of the same host. Additionally, the number of oviposited eggs decreased, while disturbances in larval hatching were more pronounced [12].

In the presented study, only at a high density of *I. ricinus* females on the rabbits during the first tick invasion, were disturbances found in the feeding course, manifested by a decrease in the number of specimens that ceased feeding and by the death of females during the parasitic phase. High tick density leads to high levels of antigens injected during feeding, which modify the immune response and lower the feeding success of females of this tick species. In contrast to *I. ricinus*, all *D. reticulatus* females in the biggest group ceased feeding. The course of feeding may be promoted by the components of saliva which, if present in sufficient quantities, inhibit the inflammatory response and prevent haemostasis. At the density of 20 specimens per area of 78.5 cm², no decrease was found in feeding success. In another species – *Rh. microplus* – it was reported that specimen death was dependent on tick density on cattle only after feeding on hosts previously infested by these ticks [32, 33].

The presented study demonstrates the great complexity of the dependencies between the parasite and its host. The differences in the course of the parasitic phase in *I. ricinus* and *D. reticulatus* females may be caused by other interactions taking place between these species and their hosts at various invasion intensities.

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