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Locomotor activity of adult *Dermacentor reticulatus* ticks (Ixodida: Ixodidae) in natural conditions

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Abstract

Introduction and objective. Expansion into new areas and the great epidemiological significance of the *D. reticulatus* tick in Europe prompts investigations of its ethology. Therefore, the locomotor activity of *D. reticulatus* adult stages in an optimal habitat during the spring and autumn activity periods was analysed.

Materials and method. Marked *D. reticulatus* adults were placed at the central point of each experimental plot. At regular time intervals, specimens attached to the cloth used in the flagging method were collected, and the distance covered by the ticks was measured. In each collection round, the temperature and humidity level in the habitat was also measured.

Results. Within 7 weeks, adult *D. reticulatus* ticks can cover an average distance of 60.71±44 cm. The locomotor activity of adult stages is greater during the spring than the autumn activity period. Questing, females cover a greater distance (66.35±100 cm) than male ticks (54.85±45 cm). Adult stages are characterised by greater aggressiveness 24 hours after being released, i.e. 30% of females and 19% of males attempt to attach to host skin. The locomotor activity in adult ticks depends on the humidity of the habitat (Z=-1.198; p=0.050). The temperature does not affect tick walking.

Conclusions. Given the low rates of horizontal locomotion of adult *D. reticulatus* ticks, the prevalence of the species in nature is determined by the presence of their hosts and humidity conditions ensuring their further development and survival. The dependence of *D. reticulatus* locomotor activity and aggressiveness on the humidity level implies an increased risk of host attacks in locations and periods that offer favourable humidity conditions for this species.

Key words

Dermacentor reticulatus, locomotor activity, host-seeking activity, tick aggressiveness

INTRODUCTION

In recent years, *D. reticulatus* ticks have been noted in new localities in Europe [1], including areas that are strongly affected by anthropogenic factors [2, 3]. The extension of the distribution range and increased abundance of *D. reticulatus*, probably caused by changes in the climate [4], weather [5, 6], and habitat conditions [7–9], increase the risk of host infestations by these ticks, and the incidence of tick-borne diseases. Therefore, investigation of the ethology of adult *D. reticulatus* stages in natural conditions is fundamental for determination of the risk posed to host species in tick habitats, and the prevalence of tick-borne diseases.

OBJECTIVE

The aim of this study was to investigate the locomotor activity of adult *D. reticulatus* ticks in their habitat during the spring and autumn peak activity periods from the aspect of health threats posed by this tick species to their hosts.

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MATERIALS AND METHOD

Study area and locality. The investigations were carried out in 2013–2014 in the Polesie National Park in central-eastern Poland during the spring and autumn activity peak in this part of the *D. reticulatus* geographical range [10]. The study locality was in a meadow overgrown by grass species representing the *Poa pratensis-Festuca rubra* community, with the dominance of *Poa pratensis*, *P. trivialis, Festuca rubra*, *F. pratensis*, and *Alopecurus pratensis,* as well as heather patches and low shrubs, mainly *Betula* spp. Earlier studies by the authors showed that this type of habitat offers the most favourable living conditions to the investigated tick species [11].

In the meadow, 8 circular, 2,826-m^2 experimental plots were established, i.e. 4 for each of the spring and autumn activity peak periods in 2013 (C, D, and G, H) and in 2014 (K, L, and O, P). The plots were located at a distance of ca. 30 m from each other and did not overlap.

Course of the experiments. Hungry *D. reticulatus* adults were collected by the flagging method in the area of the Park. In both years, ticks were collected twice, i.e. during the spring and autumn *D. reticulatus* activity peaks. The specimens were transferred to the laboratory in transport chambers, and males and females were separated in accordance with the identification key developed by Siuda [12]. Eight groups of ticks, each comprising 50 females and 50 males, were made, and the ticks marked with a marker on the dorsal side.

Figure 1. A- *Dermacentor reticulatus* habitat studied. B- central part of the experimental plot on which ticks were released

Ticks in each group were marked with a different colour. In total, 800 hungry *D. reticulatus* adults – 400 females and 400 males – were marked.

The marked ticks were released in the centre of each plot (Fig. 1A, B). At regular time intervals, a ca. 1-m^2 flannel cloth was swept over the vegetation with a circular movement from the point of release of the ticks. The cloth was checked 2–3 times per one circle and, after measuring the distance covered by the ticks, attached specimens were collected and transported to the laboratory where they were preserved in a 70% ethanol solution.

Each time during the observations in the experimental plots, air temperature and humidity was measured at 3 heights, i.e. at ground level (0 cm), at the average height of plants growing in the habitat (30 cm above ground), and at the height of the shrubs present in the study area (70 cm). The measurements were averaged during the analysis of the experimental results.

In each plot, the investigations were conducted every day for the first 7 days following the release of the ticks, and next at 10–14-day intervals for 5 weeks in spring and 7 weeks in autumn at the highest diurnal activity peak of the species, i.e. at 13.00 and 14.00 [13]. The ticks were collected only on sunny, rainless days. No collection was carried out on days after heavy rains when the grass was wet.

Statistical analysis. Based on the data obtained, the arithmetic mean was calculated of the distance covered by adult ticks (females and males) in each experimental plot, and the collective distance in all localities in 2013 and 2014. The calculations were carried out using the Excel and Word programmes from the MS Office 2013 package.

The differences between the two groups were tested by the Mann-Whitney U test. A greater number of groups were compared with the Kruskal-Wallis chi-square test. A level of significance p=0.05 was assumed.

Correlation between the tick locomotor activity and humidity, as well as temperature, was assessed using Spearman's rho-test.

RESULTS

As shown in the authors' investigations, *D. reticulatus* ticks present in the analysed habitat were able to move an average distance of 60.71±44.0 cm during 7 weeks, in an average temperature of 18.3 °C and 56.7% humidity (Fig. 2). The

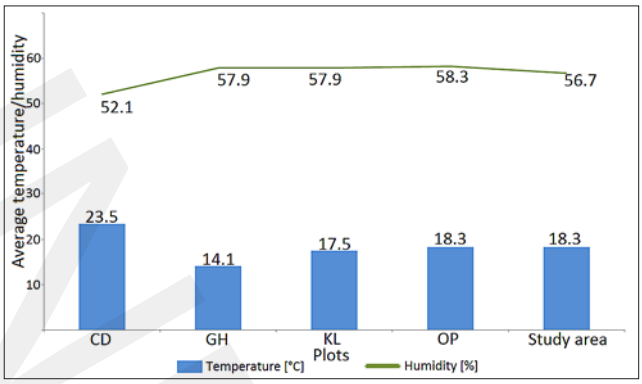

Figure 2. Average temperature and humidity in the experimental plots during the spring (CD-2013, KL-2014) and autumn (GH-2013, OP- 2014) activity peak of adult *Dermacentor reticulatus* ticks

females covered greater distances (mean 66.35±100.0 cm) than males (mean 54.85±45.0 cm). Additionally, single adult specimens were found at a distance of 23 m from the release site after 21 days.

The locomotor activity of *D. reticulatus* adults differed statistically significantly between the spring and autumn of 2013 (Z=-3.388; p=0.001) and between the spring periods in 2013 and in 2014 (Z=-5.355; p<0.001). In 2013, ticks covered greater distances in both the spring and the autumn activity periods than in 2014 (Tab. 1).

The locomotor activity of *D. reticulatus* adults differed in both periods of seasonal activity and in both study years (Fig. 2). In 2013, the locomotor activity of females was approximately two-fold higher in spring than in autumn. In 2014, higher female activity was noted in autumn. Similar trends were noted for males.

In spring 2014, the average distance covered by ticks was three-fold shorter (mean 35.60 cm) than that in the analogous period of 2013 (mean 109.61 cm). A statistical test confirmed the statistically significant differences in tick walking between spring 2013 and spring 2014 in experimental plots C and K (Z=-4.2851; p<0.001), C and L (Z=-3.495; p<0.001), and D and K (Z=-4.549; p<0.001).

In contrast, there were no statistically significant differences in the mean distances covered by adult *D. reticulatus* ticks which, on average, covered a radius of 54.82 cm and 42.80 cm (Z=-0.828; p=0.408) during the autumn periods in both years. In the autumn of both years, differences in the locomotor activity between females and males were noted (Tab. 2). 1.68%–4.60% of adult specimens (including 2.82%–4.88%

Table 1. Average distance (cm) covered by adult *D. reticulatus* ticks during 5 weeks (spring) and 7 weeks (autumn) in experimental plots

	Season	Plots	N	M	SD	Min	Max
Females	spring 2013	CD	77.00	139.87	287.35	0.00	600.00
	spring 2014	KL	54.00	29.11	42.09	0.00	210.00
	autumn 2013	GH	56.00	61.25	62.84	0.00	240.00
	autumn 2014	OP	50.00	35.20	41.47	0.00	150.00
	average		237.00	66.35	100.04	0.00	600.00
Males	spring 2013	CD	77.00	79.35	152.68	0.00	960.00
	spring 2014	KL	54.00	41.25	34.22	0.00	150.00
	autumn 2013	GH	56.00	48.39	86.38	0.00	450.00
	autumn2014	OP	50.00	50.40	42.52	0.00	170.00
	average		237.00	54.85	45.01	0.00	960.00
Total	spring 2013	CD	77.00	109.61	120.33	0.00	600.00
	spring 2014	KL	54.00	35.60	40.00	0.00	280.00
	autumn 2013	GH	56.00	54.82	58.11	0.00	450.00
	autumn 2014	OP	50.00	42.80	50.00	0.00	240.00
	average		237.00	60.71	44.00	0.00	600.00

N – number of samples; M – mean; SD – standard deviation

of females and 0–4.35% of males), and 4.26%–7.89% of specimens (including 1.96%–8.57% of females and 1.96%–6.98% of males) remained at the release site during the spring and autumn periods, respectively (Tab. 2).

The highest locomotor activity in the spring and autumn seasonal activity periods was noted after 24 hours of the experiments (Tab. 3). The high locomotor activity of adult *D. reticulatus* ticks persisted for 120 hours in spring and 96 hours in autumn (Tab. 3).

The greatest horizontal walking ability was exhibited by adult stages in a humidity range of 55%–65%. Statistical analysis showed a significant effect of humidity (Z=-1.198; p=0.050) on the locomotor activity of adult *D. reticulatus* stages. In turn, no correlation was found between the temperature and locomotor activity of the ticks in the analysed experimental plots (Spearman's rho-test, from Z=-0.258; p=0.084 to Z=-0.019; p=0.921).

Table 3. Host-seeking activity of adult *D. reticulatus* ticks in experimental plots during seasonal activity peaks

Plots (season)	Average temperature [°C]	Average humidity [%]	Time [h]	Active ticks		
				Females (N=50) [%]	Males (N=50) [%]	Total [%]
CD (spring 2013)	23.5	52.1	24	30.00	19.00	24.50
			72	16.00	15.00	15.50
			96	9.00	6.00	7.50
			120	11.00	7.00	9.00
			144	5.00	1.00	3.00
			336	1.00	2.00	1.50
			Total	72.00	50.00	61.00
GH (autumn 2013)	14.1	57.9	24	21.00	17.00	19.00
			72	10.00	10.00	10.00
			96	8.00	1.00	4.50
			144	3.00	4.00	3.50
			552	3.00	3.00	3.00
			Total	45.00	35.00	40.00
KL (spring 2014)	17.5	57.9	24	16.00	15.00	15.5
			48	4.00	7.00	5.50
			72	8.00	9.00	8.50
			96	6.00	6.00	6.00
			120	5.00	4.00	4.50
			552	2.00	3.00	2.50
			Total	41.00	44.00	42.5
OP (autumn 2014)	18.3	58.3	24	12.00	18.00	15.00
			96	9.00	11.00	10.00
			120	5.00	8.00	6.50
			144	3.00	1.00	2.00
			168	1.00	5.00	3.00
			Total	30.00	43.00	36.00

N – number of samples

Table 2. Locomotor activity of adult *D. reticulatus* ticks in experimental plots

Plots	CD			GH			KL			OP		
Distance [cm]	Females (N=50) [%]	Males (N=50) [%]	Total [%]	Females (N=50) [%]	Males (N=50) [%]	Total [%]	Females [%] (N=50)	Males [%] (N=50)	Total[%] (N=100)	Females (N=50) [%]	Males (N=50) [%]	Total [%]
0	2.82	0.00	1.68	1.96	6.98	4.26	4.88	4.35	4.60	8.57	7.84	8.14
10	8.45	8.33	8.40	7.84	20.93	13.83	12.20	6.52	9.20	0.00	1.96	1.16
20	9.86	10.42	10.08	5.88	13.95	9.57	14.63	6.52	10.34	8.57	13.73	11.63
30	11.27	8.33	10.08	5.88	13.95	9.57	19.51	15.22	17.24	8.57	9.80	9.30
40	9.86	6.25	8.40	15.69	11.63	13.83	21.95	17.39	19.54	22.86	21.57	22.09
50	5.63	6.25	5.88	7.84	0.00	4.26	4.88	10.87	8.05	11.43	7.84	9.30
60	11.27	8.33	10.08	9.80	9.30	9.57	7.32	17.39	12.64	17.14	11.76	13.95
70	2.82	4.17	3.36	3.92	0.00	2.13	7.32	10.87	9.20	8.57	9.80	9.30
80	1.41	12.50	5.88	15.69	2.33	9.57	4.88	6.52	5.75	5.71	3.92	4.65
90	1.41	4.17	2.52	3.92	0.00	2.13	0.00	4.35	2.30	0.00	1.96	1.16
100	4.23	0.00	2.52	3.92	6.98	5.32	2.44	0.00	1.15	2.86	3.92	3.49

CD – spring 2013; GH – autumn 2013; KL – spring 2014; OP – autumn 2014

DISCUSSION

Tick locomotor activity is an important element of the life strategy developed by these arthropods in order to quest for hosts and survive in natural conditions. The presented study shows that in field conditions hungry *D. reticulatus* adults cover a short distance (on average ca. 60 cm) before attempting to attack a host during the spring and autumn activity peaks. The distance covered by ticks, however, may be substantially longer, as the route is not a straight line and, like other tick species [14–17], they move both horizontally and vertically.

The presence of hungry specimens after 3 weeks at a distance of 23 m from the site of release, thereof may indicate involvement of animal species or humans who are not specific hosts of these ticks in the transfer of this species to new habitats. A significant role may also be played by host animals, which may drop unattached tick specimens from their skin while self-cleaning.

The locomotor activity of adult *D. reticulatus* ticks is lower than that of other non-nidicolous ticks with an ambushing life strategy, e.g. representatives of the genus *Ixodes*. In field conditions, *Ixodes scapularis* nymphs moved an average distance of 2–3 m within 2–3 weeks. Adult stages covered a greater distance. Some specimens were collected at a distance from 5–6 m from the site of tick release after 3–4 weeks [18]. The limited walking capability of adult *D. reticulatus* ticks in natural conditions probably, determines the mosaic distribution of these ticks, and their presence in habitats ensuring appropriate humidity and food supply through host availability. The poor mobility of adult *D. reticulatus* stages is compensated for by their considerable aggressiveness facilitating questing. The majority of analysed ticks attempted to attach to the cloth already after 24 hours in a new locality.

The differences in the behaviour of the *D. reticulatus* ticks noted in this study may be determined by the different physiological age or status of the ticks, e.g. the degree of body hydration.

The distance covered by ticks may also depend on habitat and climate conditions prevailing in the area of their occurrence. In both study years, differences in the weather were noted in the Polesie National Park (Tab. 4). In 2014, the conditions in the *D. reticulatus* habitat changed due to land management work (cutting down shrubs and trees, mainly birch specimens) aimed at exposing heath land. These treatments contributed to all-day insolation of an area that had previously been partially shaded; this may have changed the habitat conditions and determined tick behaviour. In unfavourable conditions, ticks stayed in the lower layers of vegetation, thereby having protection against water loss [19].

The correlation between the locomotor activity of adult *D. reticulatus* ticks and humidity implies a higher risk of host attack in the humid conditions preferred by this species. The greatest locomotor activity is was exhibited by adult *D. reticulatus* stages in various habitats of central-eastern Poland at a humidity of 55–65%.

Tick host-seeking behaviour is also influenced by other factors that were not analysed in the presented study, including some host-produced substances [14, 20–24].

In spring and autumn, the host-seeking activity of adult *D. reticulatus* ticks differs. In spring, the ticks cover greater distances than in autumn, which is associated with the physiology of these mites. The greater locomotor activity of adult *D. reticulatus* ticks in the quest for hosts noted in this study during the spring months, compared with that in the autumn months, increased the chance of finding a host, and producing the next generation in favourable environmental conditions. Host blood ingestion is obligatory for the normal development of female and male germ cells, insemination, and oviposition. The weaker locomotor activity of adult stages noted in the autumn period might also have resulted from the fact that the group of specimens examined may have physiologically comprised young adults moulted from nymphs shortly before being collected in the habitat in this field study.

Table 4. Weather conditions in the area of Polesie National Park (2012–2014) (www.tutiempo.net)

	Average temp. [°C]	Average max. temp. [°C]	Average min. temp. [°C]	Total precipitation [mm]	No. of rainy days	No. of days with snow cover	No. of days with fog
2012	8.2	12.8	3.5	499.56	183	74	45
2013	8.4	12.6	4.1	674.62	163	72	56
2014	9.0	13.7	4.6	738.42	181	37	61

As indicated by the field observations, tick females are characterised by greater locomotor activity than males, which follow the source of pheromones secreted by host-infesting females [25]. In Metastriata ticks, represented by *D. reticulatus*, mating takes place on the host.

The presented study demonstrating the relationship between the locomotor activity of *D. reticulatus* and the level of humidity in the habitat are in agreement with the results reported by Crooks and Randolph [24], who observed movement of *I. ricinus* ticks towards water-saturated air in the case of even minimal water loss. Loss of water through the evaporation process may lead to lowered tick activity and reduce the chance of finding a host.

CONCLUSIONS

The study shows that *D. reticulatus* adults move within a small area, but are an aggressive species. Therefore, it seems that a population of these ticks can only survive in habitats ensuring the presence of hosts and suitable moisture conditions. The greater locomotor activity in the favourable humidity range of 55%-65% indicates increased threats to host health posed by tick attacks.

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