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Changes in population abundance of adult *Dermacentor reticulatus* (Acari: Amblyommidae) in long-term investigations in eastern Poland

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Abstract
Investigations into the abundance of adult *D. reticulatus* in relation to the effect of climatic conditions (temperature, humidity) on host-seeking behaviour were conducted during the autumn (September-October) and spring (May) activity peaks in 2008–2009 in eastern Poland (51°25′N). The study was conducted in the same habitat where similar examinations were performed in 1999–2000. A comparative analysis revealed that the abundance of *D. reticulatus* had almost doubled within 10 years. Analysis of data on the humidity and temperature conditions prevailing during tick collection, and on tick abundance in the respective study periods in 1999–2000 and 2008–2009, as well as absence of climatic changes over many years, indicate that the increase in the numbers of ticks may have been a result of a multitude of other factors, e.g. weather or environmental conditions favourable for ticks and their hosts. The substantial differences in *D. reticulatus* abundance observed during the autumn activity peak (an increase from 126 and 128.6 specimens per collection in 1999 and 2000, respectively, to 247.3 in 2008) demonstrate the considerable effect of the biotic and abiotic conditions prevailing during the development of young and adult stages on the abundance of this tick species. The activity of adult *D. reticulatus* ticks in the autumn was 2.7-fold higher in comparison to that observed during the spring collection; the difference was statistically significant ($p<0.0001$). Females dominated in both seasonal activity peaks. The ratio between females and males during the spring and autumn peaks was 3.31:1 and 1.05:1, respectively. The increase in the abundance of the *D. reticulatus* population implies a higher risk of transmission of tick-borne diseases in the study area, and the necessity to develop and implement effective prevention methods and tick control programmes.

Key words
Dermacentor reticulatus, changes of tick population, seasonal activity, sex ratio, climatic conditions

INTRODUCTION

The three-host meadow tick *Dermacentor reticulatus* is widely distributed in the Palearctic region. It parasitizes primarily wild and domestic mammals [1, 2, 3, 4, 5], although it has been found infrequently on human skin [6, 7]. *D. reticulatus* is a reservoir and vector of numerous pathogens that cause bacterial [8, 9, 10], rickettsial [11, 12, 13, 14] and protozoal diseases [15, 16].

The activity of *D. reticulatus* begins in early spring and persists until late autumn; in winter months, the species diapause [17, 18, 19]. Typically, the entire developmental cycle in this tick lasts one year [20].

As demonstrated by various investigations [13, 21, 22, 23, 24, 25, 26, 27], the distribution range of *D. reticulatus* in Europe has expanded and includes suburban and recreational areas, which in turn arouses interest in the biology and ecology of the species. A particularly important task from the epidemiological point of view involves permanent monitoring of the environment through assessment of the dynamics of *D. reticulatus* abundance in various habitats. This paper presents the results of studies on changes in the *D. reticulatus* abundance in eastern Poland occurring over 10 years in relation to environmental conditions. No similar comparative studies have been conducted in Poland to-date. Tick-borne encephalitis virus [28] and *Babesia microti* protozoa [29] have been detected in *D. reticulatus* adult stages in the study area.

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MATERIAL AND METHODS

Study area. The study was conducted in 2008–2009 in the vicinity of Ostrów Lubelski (eastern Poland, 51°25'N) in a habitat where the seasonal activity of *D. reticulatus* was investigated in 1999–2000 [19]. The 0.8 ha meadow is overgrown by vegetation typical of ecosystems composed of dense shrub-land and short trees and bushes that have developed around and within the habitat. The study area is situated near large forest complexes that are rich in small and large mammal fauna – hosts for the various developmental stages of the tick species. Between spring and autumn, the meadow is used for grazing cattle.

Tick collection. As in our previous study [19], ticks were collected during the autumn (September-October 2008) and spring (May 2009) activity peaks. Tick specimens were collected using the flagging method, which involved sweeping the vegetation with a 1 × 1m white cloth attached to a 1.5 m long bamboo pole. The collection methodology employed was identical to that in the previous study, which facilitated comparison of the results. Ticks were collected by the same person for two hours in each round between 14:00–16:00, i.e. during the diurnal activity peak of adult ticks [30]. After the cloth had been swept over the plants several times, its surface was viewed and tick specimens attached to it were transferred to tubes with forceps. Tick abundance was assessed based on the number of specimens collected during one collection round. Simultaneously, temperature with an accuracy of 1°C and humidity with an accuracy of 1% were measured electronically at the height of 25–30cm above the ground. The field studies were performed on sunny days only. No tick collections were undertaken when the grass was wet after rainfall. Ticks were collected 6 times in this study, i.e. 3 times during the autumn activity peak and 3 times during the spring activity peak.

In the laboratory, the ticks were submerged in 70% ETOH, and then viewed under a stereoscopic microscope in order to recognise the species and the gender of the specimens. The key developed by Siuda [31] was used to identify the species.

Statistical analysis. The results were elaborated using the STATISTICA statistical package for Windows. All correlation analyses were performed by calculation of Spearman's correlation coefficients. Probability was regarded as significant at $p \leq 0.05$ and as highly significant at $p \leq 0.01$.

RESULTS AND DISCUSSION

A total of 1,018 adult *D. reticulatus* specimens were collected during the study – 593 females (58.2%) and 425 males (41.8%). No larvae and nymphs of *D. reticulatus* were found. Tick abundance had almost doubled in the study habitat, compared to that reported 10 years before (Tab. 1). In the present study, more specimens of the species were found during both the autumn and spring activity peaks than in the respective periods of 1999 and 2000 [19] (Tab. 1, Fig. 1).

During the autumn activity period, 742 specimens were collected; the number of ticks collected in the spring activity period was considerably lower (276 specimens) (Tab. 1). The average number of ticks obtained in one collection period in the autumn months was 247.3, and 92 in the spring (Fig. 1), and the difference was statistically significant ($p < 0.0001$). The temperature during the field study ranged from 18.2–24°C, and humidity persisted in the range of 53.6–76% (Tab. 1). The mean temperature and humidity during the autumn and spring activity peaks were 19.7°C and 65.8% and 21.3°C and 72%, respectively.

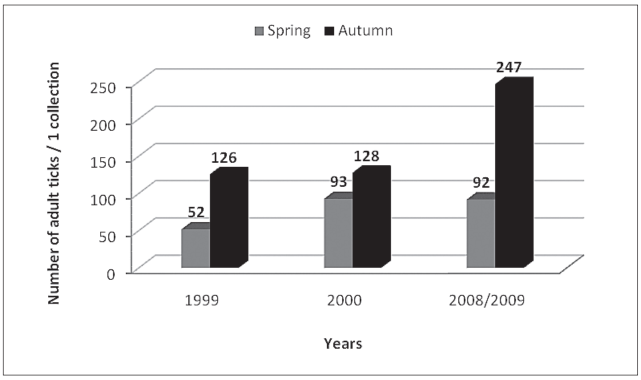

Figure 1. Average number of adult ticks collected during one collection in spring and autumn activity peaks in particular years of the study.

The trend towards expansion of the distribution range of ticks and increased incidence of tick-borne diseases has been reported worldwide [25, 26, 32, 33, 34]. Yet, the causes of this phenomenon are still little known and are probably dependent on a variety of co-factors – including climatic changes (climate warming) – which are considered to have a substantial role in the expansion of e.g. *Ixodes ricinus* [34, 35, 36, 37, 38, 39, 40] in Europe, *Ixodes scapularis* in North America [41], and *Rhipicephalus sanguineus* ticks in the Mediterranean Sea Basin [42]. Long-term research data from eastern Poland do not provide unequivocal evidence of climatic changes in the region in the second half of the 20th century [43]. There was a clearer trend towards an increase in the mean annual temperature, mainly resulting from the temperature rise in spring and winter. The differences between the mean temperatures of December, January, February, and March reported at the end of the 20th century and those recorded in the previous century, were 0.4, 0.1, 0.3, and 0°C, respectively. In turn, the precipitation amounts declined in the study area. The mean differences in precipitation sums in December, January, February, and March were by 1, 4, 0 and

Table 1. Tick abundance in particular seasons and years in the study period in context of temperature and relative humidity.

1999						2000						2008/2009					
Spring																	
date	A	F	M	T	RH	date	A	F	M	T	RH	date	A	F	M	T	RH
06.05	75	54 (72%)	21 (28%)	10	72	17.04	119	71 (59.6%)	48 (40.4%)	17	78	14.05	118	90 (76.2%)	28 (23.8%)	24	72
20.05	46	30 (65.2%)	16 (34.8%)	25	40	30.04	60	42 (70%)	18 (30%)	32	48	17.05	112	84 (75%)	28 (25%)	19	76
27.05	35	24 (68.5%)	11 (31.5%)	31	45	11.05	100	79 (79%)	21 (21%)	25	39	21.05	46	38 (82.6%)	8 (17.4%)	21	68
Total	156	108 (69.2%)	48 (30.8%)	22*	52.3·	Total	279	192 (68.8%)	87 (31.2%)	25*	55·	Total	276	212 (76.8%)	64 (33.2%)	21.3*	72·
Autumn																	
date	A	F	M	T	RH	date	A	F	M	T	RH	date	A	F	M	T	RH
11.09	129	60 (46.5%)	69 (53.5%)	21	95	07.09	93	45 (48.3%)	48 (51.7%)	18	65	09.09	267	133 (49.8%)	134 (50.2%)	22.4	53.6
10.10	131	70 (53.4%)	61 (46.6%)	12	100	05.10	168	104 (61.9%)	64 (38.1%)	23	65	05.10	202	110 (54.4%)	92 (45.6%)	18.6	72
13.10	118	54 (45.7%)	64 (54.3%)	9	85	13.10	125	65 (52%)	60 (48%)	22	79	08.10	273	138 (50.5%)	135 (49.5%)	18.2	72
Total	378	184 (48.6%)	194 (51.4%)	14*	93.3·	Total	386	214 (55.4%)	172 (44.6%)	21	70	Total	742	381 (51.3%)	361 (48.7%)	19.7*	65.8·
Total	**534**	**292 (54.6%)**	**242 (45.4%)**	-	-	**Total**	**665**	**406 (61%)**	**259 (39%)**	-	-	**Total**	**1018**	**593 (58.2%)**	**425 (41.8%)**	-	-

A – adults; F – females; M – males; T – temperature; RH – relative humidity
* average temperature; · – average RH

2mm lower in the late 20th century, compared to the previous century. The changes were not accompanied by significant changes in other climate elements, with the exception of the number of days with snow cover [44]. Given the high ecological plasticity of *D. reticulatus* [19], climate warming (an average increase in the annual temperature by 0.2°C) does not seem to be the major direct factor producing the increase in the population of the tick species.

Analysis of temperature and humidity values recorded in the same habitat during the autumn and spring activity peaks, as well as the number of specimens collected in the two study periods, do not demonstrate simple correlations between them. For instance, during the autumn activity peak in 1999 at mean temperature 14°C and mean humidity 93.3%, similar mean specimen numbers per one collection round were recorded in 2000 at 21°C and 70% humidity. In turn, in 2000, at mean temperature 21°C and 70% humidity, lower numbers of ticks (146.6 specimens/1 collection round) were collected than in 2008 (249.9 specimens/1 collection round), when similar thermal and humidity conditions prevailed (19.7°C and 65.8%). Similar mean numbers of ticks were reported during the spring activity peaks in 2000 and 2009 at similar temperature and humidity. The lower mean number of ticks per one collection round in the period of 1999 may have been the result of weather conditions prevailing during their winter diapauses, or in early spring.

The climatic conditions of eastern Poland [44], as well as our long-term field observations, indicate the participation of various biotic and abiotic factors that contribute to changes in tick abundance in the ecosystem studied. Over the 10-year period, changes were observed in the character of the habitat induced by a succession of new plants (abundance of shrubs along the edge of meadows), which offer favourable living conditions for the ticks and their hosts. The significance of the habitat type in the maintenance of tick populations has been indicated in investigations conducted in, e.g. southern Moravia (Slovakia) and north-eastern Poland, which showed differences in the development of *D. reticulatus* in different biotopes and microclimate conditions [45]. Natural conditions of a given habitat affect the species composition and number of tick hosts [46, 47]. The correlation between tick population density and host abundance has been reported in numerous investigations conducted worldwide [48, 49].

A similar abundance of *D. reticulatus* adults was reported during the spring activity periods in 2000 and 2009 at similar mean temperature and humidity values (Tab. 1). In turn, the lower mean number of specimens per one collection round in the same period of 1999 may have resulted from weather conditions and/or other factors operating in wintertime and early spring that affected the number of active ticks [19].

The greatest numbers of adult *D. reticulatus* in the years 1999, 2000, and 2008 were found during the autumn activity peak, reaching on average 126, 128.6 and 247.3 per one collection round, respectively (Fig. 1). This implies that factors operating during the development of the particular tick stages, rather than conditions prevailing during their winter diapause, exert the most considerable effect on maintenance of high abundance in tick populations. In natural conditions, oviposition in *D. reticulatus* females and larval hatching takes place in June and July. Young stages reach their activity peak within a period of a few weeks, which ends in August-September in the case of larvae and August-October in the case of nymphs [20, 50, 51].

Other factors, which have not been taken into account in the presented study, i.e. weather conditions during the seasonal activity period, presence and behaviour of its hosts, and changes in the habitat caused by human activity (e.g. frequency of mowing the grass, use of fertilizers and pesticides), may have induced the changes in tick abundance through their effect on female fertility, effectiveness of development of larvae, nymphs, and adults, and on the survival of young and adult *D. reticulatus* stages. As indicated by the results our (unpublished) investigation, the fertility of *D. reticulatus* females is affected by the species-specific mode and success of questing, as well as the degree of host immunization and density of ticks on the host. In turn, the laboratory investigations conducted by Zahler and Gothe [52] showed that the life span of unengorged adults and oviposition in engorged *D. reticulatus* females depended on temperature and humidity. These parameters exert an effect on larval hatching, moulting, and the survival of larvae and nymphs of this tick species [53].

Female specimens dominated during the collection rounds in both periods of seasonal activity peaks, although the female-to-male ratios differed. The female-to-male ratios during the spring and autumn activity peaks were 3.31:1 and 1.05:1, respectively (Tab. 2). The higher activity of females than males during the spring rather than autumn peak can be explained by the biological characteristics of the species. Host blood ingestion is indispensable for females for copulation with males, which takes place on the host exclusively during the second phase of feeding, and for the normal process of maturation and development of eggs (own unpublished data). Similar tendencies were found in our previous study in 1999 and 2000, but the female-to-male ratio during the spring activity was lower, i.e. 2.25:1 and 2.20:1 respectively [19]. These differences in the gender ratio are probably caused by different atmospheric conditions prevailing during the winter and early spring in 1999–2000, which may have affected survival and host feeding and host- seeking behaviour in representatives of both genders. In observations carried out in Puszcza Białowieska (eastern Poland), Karbowiak et al. [54] reported that *D. reticulatus* males persisted on bison longer than females after the winter diapause; however, the authors did not investigate the effect of weather conditions on the behaviour of these ticks on the host.

Table 2. Gender ratio of *Dermacentor reticulatus* ticks collected in spring and autumn activity peaks in 1999, 2000 and 2008–2009.

Years	Gender ratio (Females/Males)	
	Spring	Autumn
1999	2.25: 1	0.94: 1
2000	2.20: 1	1.24: 1
2008–2009	**3.31: 1**	1.05: 1

The presented long-term field studies demonstrate the complexity of interrelations between organisms and weather, climatic, and habitat conditions, which is an inspiration for further research aimed at elucidation of the causes of changes in the distribution and density of *D. reticulatus*. Given the increase in tick abundance and the possibility of transmission of human and animal tick-borne disease pathogens, permanent monitoring of the area of south-eastern Poland is indispensable.

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