



UDC: 595.2:576.895.2

ECTOPARASITISM OF CASTOR BEAN TICKS *IXODES RICINUS* (LINNAEUS, 1758) ON SAND LIZARDS *LACERTA AGILIS* (LINNAEUS, 1758) IN WESTERN POLAND

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Wieczorek, M., & Najbar, B. (2022). Ectoparasitism of castor bean ticks *Ixodes ricinus* (Linnaeus, 1758) on sand lizards *Lacerta agilis* (Linnaeus, 1758) in western Poland. *Studia Biologica*, 16(1): 27–34 • DOI: <https://doi.org/10.30970/sbi.1601.678>

Background. Over 700 species of ticks are characterised by moderate to high host specificity, and a handful by very high host specificity, parasitizing upon a single host species. The rest are not host specific. One of them is the castor bean tick, which parasitizes all available terrestrial vertebrates including humans. Poland lacks tick species that are reptile-specific, since, as reported by K. Siuda (1993), these ectoparasites generally prefer endotherms, namely birds and mammals. However, the literature reports that the prevalence of castor bean tick infestation of local sand lizard populations is as high as 13.2 % – 40.4 %. Observations of different authors also indicate that outside the period of regular tick appearance on lizards, individual lizards can have very high tick loads, varying between several to over 100.

Materials and Methods. The research was conducted in three separate study areas in western Poland: Żary, Zielona Góra and Nowa Sól. 606 specimens of sand lizards were captured, analysed and photographed. 222 (36.6 %) of them were infested with ticks. With each capture ticks were checked and removed. The lizards were caught by hand. Inter-group two-way and three-way ANOVA and Pearson correlation coefficients were calculated with the use of IBM SPSS Statistics 23 software. Threshold of statistical significance was set to $\alpha = 0.05$.

Results. In total 1339 ticks were obtained from lizards, all of which were castor bean ticks (*Ixodes ricinus*). 914 (68.2 %) were larvae, 424 (31.7 %) were nymphs and 1 (0.1 %) was an adult female. The highest number of ticks (778) was collected from lizards at Żary site. The most infested male from Żary was parasitized upon by 55 ticks, from Zielona Góra – by 46 and from Nowa Sól – by 15. In regard to females these



numbers were 36, 34 and 15, respectively. The average infestation (defined as number of ticks) was higher for lizards from Żary than for those from Zielona Góra ($p < 0.001$) and Nowa Sól ($p < 0.001$), which also differed from each other in a statistically significant way ($p = 0.001$). Main effect of sex/age group turned out to be statistically significant, $F(2.796) = 67.75$; $p < 0.001$; $\eta^2 = 0.15$. Hence post-hoc analysis was carried out and showed that males carried higher number of ticks than females and juveniles ($p < 0.001$). Females, on the other hand, carried more ticks than juveniles ($p < 0.001$).

Conclusion. The research confirmed a significant role of sand lizards in propagation of *Ixodes ricinus*. It was shown that lizards were affected mainly by early developmental stages of ticks. The prevalence of tick infestation was 36.6 % – 58.1 % for Żary, 28.1 % for Zielona Góra and 13.8 % for Nowa Sól. The average tick load on sand lizards was 2.5 individuals. The highest number of them was collected from 58.5 % of males, 36.4 % of females and 5.1 % of juveniles. High tick infestation of males can be explained by their higher mobility, especially during mating season.

Keywords: *Lacerta agilis*, *Ixodes ricinus*, ectoparasitism

INTRODUCTION

900 species of ticks have been described worldwide (Barker & Murrell, 2004; Dunlop *et al.*, 2016). Over 700 of them are characterized by moderate to high host specificity, and a handful by very high host specificity, parasitizing upon a single host species. The rest are not host specific. One of them is the castor bean tick, which parasitizes all available terrestrial vertebrates including humans (Siuda, 1993). Poland lacks tick species that are reptile-specific, since, as reported by the aforementioned author, these ectoparasites generally prefer endotherms, namely birds and mammals. However, the literature reports that the prevalence of castor bean tick infestation of local sand lizard populations is as high as 13.2% – 40.4 % (Gryczyńska-Sięmiątkowska, Siedlecka, Stańczak, & Barkowska, 2007; Ekner *et al.*, 2011; Kurczewski, 2014; Dudek *et al.*, 2016). In the Netherlands the prevalence is 46.8 % and in Germany even 71.4 % (Rahmel & Meyer, 1988).

Ticks are of significant epidemiologic importance. They are natural reservoirs and vectors of about 130 species of arboviruses, 30 *Rickettsia* spp., 20 species of Spirochaetales, other bacteria and fungi, 200 species of Piroplasmida, other protozoa and also Filariidae (nematodes). Pathogenic organisms usually settle in mites' gastrointestinal tract, salivary glands, the Malpighian tubule system and reproductive system. The pathogens usually do not change vector's biology. However, in the case of significant infestation, they can impede functioning of reproductive organs, salivary glands and the development of internal organs (Boczek & Błaszak, 2016).

Ticks are important for medicine and veterinary science because specific cells of their salivary glands secrete neurotoxins which can cause tick paralysis. *Ixodes ricinus*, the most common European ectoparasite, is the single greatest causal agent of that disease. Toxin production increases with attachment time and peaks on the 5th or 6th day. The tick paralysis can occur in humans, pet and wild animals (Boczek & Błaszak, 2016). High tick infestation can impede hunting and escaping predators. Moreover, skin damages heal slowly because ticks secrete anticoagulants (Bauwens, Strijbosch, & Stumpel, 1983; Siuda, 1993; Stanek, 2009).

Ectoparasitism of sand lizards from western Poland has not been studied thoroughly. Therefore, the goal of our study was to assess the extent of infestation of these lizards with different developmental stages of ticks.

MATERIALS AND METHODS

606 specimens of sand lizards were captured, analyzed and photographed. 222 (36.6 %) of them were infested with ticks. In total we collected 1339 individual ticks (**Table 1**). Some lizards (199 specimens) were captured more than once in the course of the research. With each capture ticks were checked and removed. In statistical analyses we have 805 samples due to subsequent captures being treated as independent samples (**Table 2**). The lizards were caught by hand. Their age and sex (adult female, adult male, or juvenile) were determined. Fieldwork was mainly conducted during warm and sunny days, since in such conditions activity of the studied species peaks. Ectoparasites were detached and preserved in 70 % ethanol. Species and developmental stage were determined in a laboratory using Ixodida diagnostic key (Siuda 1993) and Nikon SMZ 3000 stereomicroscope with Nikon NIS Elements-D software.

The research was conducted in three separate study areas in western Poland: Żary, Zielona Góra and Nowa Sól between 28 March 2016 and 3 October 2016 and between 12 April 2017 and 19 October 2017. The specific coordinates of each location are: (1) Żary (51°37.690'N, 15°05.387'E), (2) Zielona Góra (51°52.188'N, 15°27.122'E) and (3) Nowa Sól (51°43.850'N, 15°43.816'E).

The area selected in Żary is a gravel pit. Approximately 42 % of the area is covered in trees such as young scots pines (*Pinus sylvestris*) and common aspen (*Populus tremula*). In Zielona Góra, a post-agricultural location with a development of small housing estates was selected. 69 % of the selected land is barren or covered in meadows (*Arrhenatheretalia elatioris*). Nowa Sól is a transformed, post-mining area with a lot of sunlight. 83 % of the land is covered by grassland that grows on high hills. Detailed description of the research sites is given in M. Wiczorek & B. Najbar (2020).

Inter-group two-way and three-way ANOVA and Pearson correlation coefficients were calculated with the use of IBM SPSS Statistics 23 software. Threshold of statistical significance was set to $\alpha = 0.05$.

RESULTS

In total 1339 ticks were obtained from lizards, all of which were castor bean ticks (*Ixodes ricinus*). 914 were larvae (68.2 %), 424 (31.7 %) were nymphs and 1 (0.1 %) was an adult female. The highest number of ticks (778) was collected from lizards at Żary site. Lizards from Zielona Góra carried 376 ticks and those from Nowa Sól – 185. The most infested male from Żary was parasitized upon by 55 ticks, from Zielona Góra – by 46 and from Nowa Sól – by 15. In regard to females, these numbers were 36, 34 and 15, respectively (**Table 1**).

All samples ($N = 805$) were analyzed to explore the relationship between infestation and lizards' sex/age group and location. The results of these calculations are shown in **Table 2**.

Statistically significant main effect of site was observed, $F(2.796) = 59.05$; $p < 0.001$; $\eta^2 = 0.13$. Therefore, post-hoc analysis with Sidak's test was carried out and revealed three statistically significant differences. The average infestation (defined as number of ticks) was higher for lizards from Żary than for those from Zielona Góra ($p < 0.001$) and Nowa Sól ($p < 0.001$), which also differed from each other in a statistically significant way ($p = 0.001$) (**Fig. 1**).

Table 1. Infestation by larvae, nymphs and adults of castor bean ticks (*I. ricinus*) on sand lizards (*L. agilis*) (N = 606) in relation to their sex/age group and location

Sex/age group	Number of lizards	Number of lizards with ticks	Number of all ticks min/max	Number of tick larvae	Number of tick nymphs	Number of adult ticks
Zielona Góra						
Males	45	31	243 / 1 / 46	124	119	
Females	48	21	124 / 1 / 34	85	39	
Juveniles	57	4	9 / 1 / 5	4	5	
Żary						
Males	48	38	466 / 1 / 55	338	128	
Females	54	41	278 / 1 / 36	225	53	
Juveniles	71	20	34/1/8	26	8	
Nowa Sól						
Males	91	29	75 / 1 / 15	39	35	1 (female)
Females	107	27	85 / 1 / 15	55	30	
Juveniles	85	11	25 / 1 / 10	18	7	

Table 2. The average number of castor bean ticks (*I. ricinus*) taking into account all sand lizards (*L. agilis*), including those which did not have any ticks, in relation to sex/age group and location. SD – standard deviation, N – number of lizards

Site	Sex/age group	Average number of ticks	SD	N
Zielona Góra	Males	4.61	8.65	70
	Females	2.34	5.99	59
	Juveniles	0.10	0.58	89
	All	2.16	6.10	218
Żary	Males	11.10	12.93	63
	Females	5.67	7.54	58
	Juveniles	0.30	0.95	120
	All	4.41	8.82	241
Nowa Sól	Males	0.85	2.22	105
	Females	0,95	2,62	110
	Juveniles	0.29	1.18	131
	All	0.67	2.07	346
In total	Males	4.67	9.23	238
	Females	2.52	5.53	227
	Juveniles	0.24	0.97	340

Main effect of sex/age group turned out to be statistically significant, $F(2.796) = 67.75$; $p < 0.001$; $\eta^2 = 0.15$. Hence, post-hoc analysis was carried out and showed that males carried higher number of ticks than females and juveniles ($p < 0.001$). Females, on the other hand, carried more ticks than juveniles ($p < 0.001$) (**Fig. 2**).

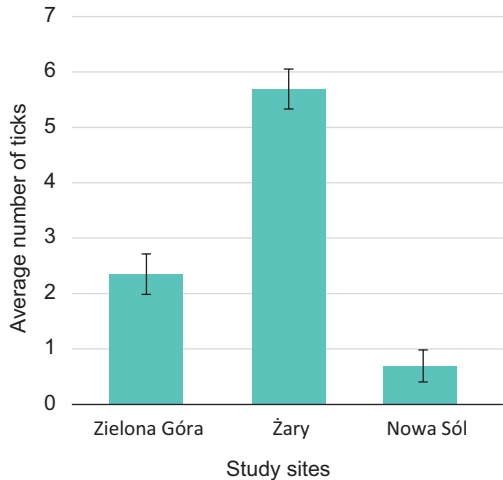


Fig. 1. The average number of ticks parasitizing upon sand lizards in the study sites

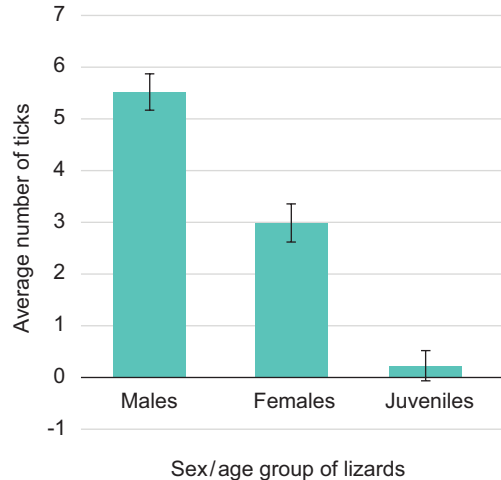


Fig. 2. The average number of ticks parasitizing upon sand lizards in relation to sex/age group

DISCUSSION

Our results show that there are big differences of *Ixodes ricinus* infestation between the three studied populations of sand lizards. We can conclude that lizards help to subsist *I. ricinus* population by feeding all developmental stages – mainly larvae and nymphs, while adults are reported very rarely. Similarly, R. Kurczewski (2014), who studied sand lizards in Wielkopolski National Park (WNP), reported only three cases of adult tick parasitism. Our research also found that lizards are predominantly affected by *I. ricinus* larvae (68.2 %), even though radiotelemetric studies of 16 lizards in Zielona Góra (Wieczorek, Rektor, Najbar, & Morelli, 2020) and observations of other authors indicate that sometimes nymphs are more common (e.g. Bauwens *et al.*, 1983; Rahmel & Meyer, 1988; Baruš & Oliva, 1992). According to K. Siuda (1993), high larvae load in a small area can be related to the presence of the so-called nests – aggregations in a place of oviposition. Lizards inhabiting such places are vulnerable to intensified ectoparasitism, especially during motionless sun basking (Kurczewski, 2014).

Observations of different authors also indicate that outside the period of regular tick appearance on lizards, individual lizards can have very high tick loads, varying between several to over 100 (Jansen, 2002). The highest tick load reported in a single lizard by R. Kurczewski (2014) from WNP was 35 and the host was a male. The maximum reported by U. Rahmel and S. Meyer (1988) was 76 ectoparasites. The highest tick load in a single male lizard from Żary, Zielona Góra and Nowa Sól was 55, 46 and 15, respectively. In regard to females it was 36, 34 and 15, respectively (**Table 1**). However, we speculate that these numbers can be even higher, as evidenced by a male captured 5 km away from our study site in Zielona Góra which carried 230 ticks (**Fig. 3**).



Fig. 3. Male of the sand lizard with ticks (photo B. Najbar)

The research also allowed us to test a hypothesis stipulating that different habitat types influence tick load. Analyses demonstrated statistically significant differences in tick load between the study sites. The highest tick load was found in Żary $M = 4.4 \pm 8.82$, in Zielona Góra $M = 2.16 \pm 6.10$, and the lowest in Nowa Sól $M = 0.67 \pm 2.07$ (Table 2). Therefore we can conclude that the hypothesis is correct. The highest infestation being reported in Żary could be due to several reasons. The habitat (humid meadows, bushes, depressions of land, intermittent water bodies) was characterized by the lowest mean and maximum temperatures of air and ground both in 2016 and 2017 when compared to other study sites. Moreover, the relative humidity of air just above ground was the highest in that study site. These conditions were more optimal for activity and survivability of ticks, which do not tolerate temperatures above 39–41 °C. Moreover, relative air humidity was about 80–100 % while below these values ticks die due to desiccation (Boczek & Błaszak, 2016).

Statistical analysis demonstrated that infestation depends not only on the study site but also on sex and age group. The highest number of ticks was obtained from 58.5 % of males ($M = 4.67 \pm 9.23$), 36.4 % of females ($M = 2.52 \pm 5.53$) and only 5.1 % of juveniles ($M = 0.24 \pm 0.97$). Studies of other European populations of sand lizards also confirm that tick load can be different in adults and juveniles (Chilton, Bull, & Andrews, 1992; Schall, Prendeville, & Hanley, 2000). U. Rahmel and S. Meyer (1988) in Germany observed infestation of 94.4 % males, 83.2 % females and 44.5 % juveniles. For WNP it was 40 %, 29.2 % and 13.6 % respectively (Kurczewski, 2014). A higher tick load of males is often explained by their higher mobility, especially during mating season. As to females, as speculated by R. Kurczewski (2014), a larger body surface might play a role because of potentially larger skin area for ticks to attach.

CONCLUSION

The research confirmed a significant role of sand lizards in propagation of *Ixodes ricinus*. It was shown that lizards were affected mainly by early developmental stages of ticks. The prevalence of tick infestation was 36.6 % – 58.1 % for Żary, 28.1 % for Zielona Góra and 13.8 % for Nowa Sól. These intersite differences are explained by habitat type and local conditions. The average tick load on sand lizards was 2.5 individuals. The highest number of them was collected from 58.5 % of males, 36.4 % of females and 5.1 % of juveniles. The high tick infestation of males can be explained by their higher mobility, especially during mating season.

We are thankful to Mr. Michał Szkudlarek for help in translation of the manuscript.

This research was approved by the Regional Directorate for Environmental Protection (permit No. WPN-I.6401.206.2015.JK) and the Local Ethics Commission for Animal Experiments (Resolution No. 70/2016).

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Human Rights: This article does not contain any studies with human subjects performed by any of the authors.

Animal studies: All institutional, national and institutional guidelines for the care and use of laboratory animals were followed.

AUTHOR CONTRIBUTIONS

Conceptualization, [M.W.; B.N.]; methodology, [M.W.; B.N.]; validation, [M.W.; B.N.]; formal analysis [M.W.; B.N.]; investigation, [M.W.; B.N.]; investigation, [M.W.; B.N.]; resources, [M.W.; B.N.]; data curation, [M.W.; B.N.]; writing – original draft preparation, [M.W.; B.N.]; writing – review and editing, [M.W.; B.N.]; visualization, [M.W.; B.N.]; supervision, [M.W.; B.N.]; project administration, [M.W.; B.N.]; funding acquisition, [M.W.; B.N.]

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