

ENVIRONMENTAL CONDITIONING OF INCIDENCE OF TICK-BORNE ENCEPHALITIS IN THE SOUTH-EASTERN POLAND IN 1996–2006

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Abstract: Epidemiological studies of tick-borne encephalitis (TBE) conducted in the Świętokrzyskie province (south-eastern Poland) in 1996–2006 demonstrated an increase in the number of registered TBE cases in the last 6 years of the study. The highest risk of TBE virus infections occurs in the summer-autumn period (seasonality factors 96.55% and 248.28%), i.e. in periods of the highest *Ixodes ricinus* activity in this region. An increased number of registered TBE cases were noted in the regions which provide ticks with favourable habitats and, simultaneously, where the TBE diagnosis and treatment for the inhabitants are more efficient. The increasing tendency in TBE incidence observed even in the non-endemic regions of the Świętokrzyskie province indicates the necessity of education in tick-borne disease prophylaxis and the need to elevate the level of public health care. Differences in the density and activity of various developmental *Ixodes ricinus* stages were observed in 4 localities in diverse biotopes located at different altitudes a.s.l. This justifies the necessity of environmental monitoring of the threats posed by ticks in various habitats, especially in those frequently visited by humans.

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INTRODUCTION

Tick-borne encephalitis (TBE) is a viral disease occurring on a big area ranging from Western Europe to China and Japan in Asia [24, 25, 40]. A virus from the genus *Flavivirus* (TBEV), family *Flaviviridae*, which occurs in one of several subtypes identified on the basis of molecular sequencing of the envelope gene, is known to be its etiological cause [19, 26] with *Ixodes ricinus* [8, 26] or *Ixodes persulcatus* ticks [33] as its vectors, depending on the locality.

The TBE viruses (TBEV) are usually focally distributed in forest ecosystems, where they circulate between tick populations and their vertebrate hosts [35, 54]. *I. ricinus*

has a wide range of hosts: small mammals are infested by its larvae [46, 56] while bigger wild-living and domestic mammals and humans are attacked by adults and nymphs. Although some rodent species respond to TBEV infection with low-level viremia this does not ensure efficient transmission of the virus to the feeding ticks, they play an important role in the enzootic TBE transmission. On these hosts, non-viremic transmission is possible when infected immature ticks co-feed in close proximity to infected specimens [27, 41]. However, big mammals, e.g. roe deer (*Capreolus capreolus* L.), which are the main hosts of adult *I. ricinus* in Europe, do not play a role in the virus transmission [42], but they contribute to a higher density

of tick population [26, 32] and maintenance of the virus in the environment. Therefore, assessment of the density and activity of *I. ricinus* ticks is indispensable for prediction of the threat of tick attack and tick-borne diseases, including TBE, in various regions.

The aim of our study was to investigate TBE incidence in chosen regions of the Świętokrzyskie province (south-eastern Poland) during 10 years, and the environmental conditioning of TBE in the region.

MATERIAL AND METHODS

The epidemiological studies of TBE were carried out in 1996–2006 in the Świętokrzyskie province (south-eastern Poland). The data about TBE cases were obtained from 8 out of the 11 regional sanitary-epidemiological stations in the region. We took into account the prevalence rate and the number of people hospitalized for TBE in particular regions and in the whole study area, and the seasonality of TBE incidence. On the basis of these data, we calculated the TBE incidence rate (number of cases/100 thousand inhabitants) and the seasonality factors (SF), which express the percentage differences between prevalence in successive months and the mean number of cases in the given area.

In order to estimate the diagnostic-treatment possibilities for the province inhabitants, data about medical care from the Regional Statistics Office in Kielce was analysed. It provided information about the number of the medical care institutions (hospitals and outpatient clinics) and the total number of doctors, the number of doctors of various specializations dealing with TBE diagnostics and treatment (physicians, internists, infectious diseases, neurologists, cardiologists, GPs and family doctors and epidemi-

ologists), the number of medical diagnosticians and nurses per 10 thousand inhabitants (Tab. 1).

The tick attack threat and the risk of viral infections were estimated on the basis of prevalence of various developmental stages of *Ixodes ricinus* (larvae, nymphs, females and males) in the habitats. Tick collections were performed between 16:00–18:00, each time for 1 hour, with the flagging method, which involves sweeping the vegetation and ground surfaces with a 100 × 70 cm white flannel cloth. The cloth was checked every minute and the attached specimens were placed in a test-tube containing 70% EtOH. The collected ticks were examined under a stereoscopic microscope in laboratory conditions in order to determine the developmental stage and the tick species.

During collection of ticks in their habitats, the temperature was measured with accuracy of ±1°C. The seasonal activity of the ticks was assessed on the basis of monthly field studies conducted from April–October 2000. The studies were carried out in 4 localities, which are frequently visited by the inhabitants of the region for recreational and occupational purposes (foresters, farmers and forest fruit and mushroom pickers).

- locality I: Kielce-Stadion (200 m a.s.l.) in a mixed forest with predominant pines, spruces and birches, and with cowberries in the undergrowth;

- locality II: Borków (200 m a.s.l.) in a mixed forest with predominant oaks, pines, spruces and birches, and with cowberries in the undergrowth;

- locality III: Ameliówka (400 m a.s.l.) in a mixed forest with predominant pines, spruces and beeches, and with numerous grasses and cowberries in the undergrowth;

- locality IV: Kielce-Skoczna (365 m a.s.l.) in a mixed forest with predominant pines, spruces and birches, and with numerous grasses and cowberries in the abundant undergrowth.

Table 1. Number of doctors and health care institutions (per 10,000 residents) in the area under surveillance of Regional Sanitary-Epidemiological Stations in Świętokrzyskie province.

Regional Sanitary-Epidemiological Stations	Physicians	Internal medicine physicians	Infectious disease physicians	Neurologists	Cardiologists	GPs doctors	Family doctors	Epidemiologists	Nurses	Diagnosticians	Outpatients clinics and hospitals
RSES Kielce	16.8	1.63	–	1.19	0.25	0.77	0.41	0.05	56.1	0.1	28
RSES Busko	40.6	6.9	0.05	0.36	0.26	2.6	1.6	0.13	71.3	0.13	15
RSES Końskie	16.2	3.76	0.57	–	0.11	0.23	0.46	–	56	0.1	12
RSES Jędrzejów	11.6	2.38	–	–	–	–	–	0.22	29.1	0.11	16
RSES Opatów	19.3	1.35	–	0.34	–	0.85	0.85	0.34	29.9	0.34	14
RSES Ostrowiec Świętokrzyski	10.5	4.69	–	0.74	0.25	0.41	–	–	50.6	0.08	15
RSES Skażysko-Kamienna	20.9	4.05	0.48	1.19	0.36	1.19	0.48	–	52.7	0.12	13
RSES Starachowice	17.8	2.74	0.30	0.61	0.10	0.71	1.42	0.10	50.6	0.3	17
RSES Włoszczowa	16.2	3.74	–	0.42	–	0.63	0.42	0.42	50.9	–	10
Świętokrzyskie province	17.6	3.2	0.20	0.7	0.3	0.23	0.27	0.7	51.6	–	–

‘GPs’ – general practitioners; ‘–’ –no data

We also examined the influence of geoclimatic conditions, with emphasis on the shape of terrain and temperature, on tick prevalence.

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 at $p \leq 0.05$ was regarded as significant and as highly significant at $p \leq 0.01$.

In order to compare the seasonal tendencies in the particular localities, the data were scaled to obtain values of similar magnitude. The scaling involved dividing each total number of ticks obtained by the mean number of ticks collected in a given locality (Fig. 3, 4, 5, 6).

RESULTS

TBE cases in the Świętokrzyskie province during 10 years. TBE cases were reported from the Świętokrzyskie province in the various years of the study period (Tab. 2). Between the years 2000–2006, there were more TBE cases reported in particular regions than previously. The incidence proportion ranged from 0 to 3.63 in that peri-

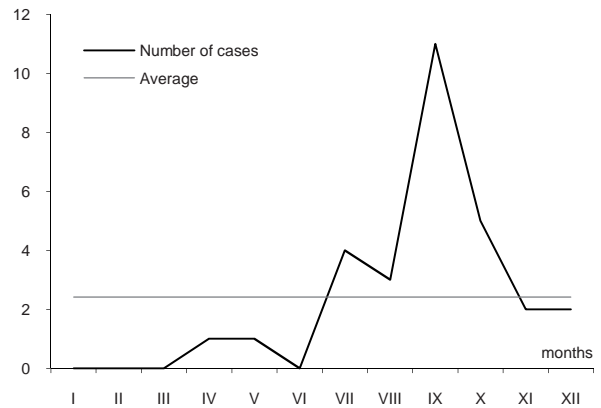


Figure 1. Seasonality of TBE in Świętokrzyskie province 1996–2007. Cumulative data for each month.

od. The highest incidence proportion was observed in the Świętokrzyskie province in 2006 – 2.23 and in 2005 – 2.11.

The highest incidence rate was reported by the Regional Sanitary-Epidemiological Stations in the area of Busko, Jędrzejów, Starachowice, and Opatów (Tab. 2) where, compared to other regions, better medical care is provided for the inhabitants by a larger number of medical staff employed in more numerous outpatient clinics and hospitals

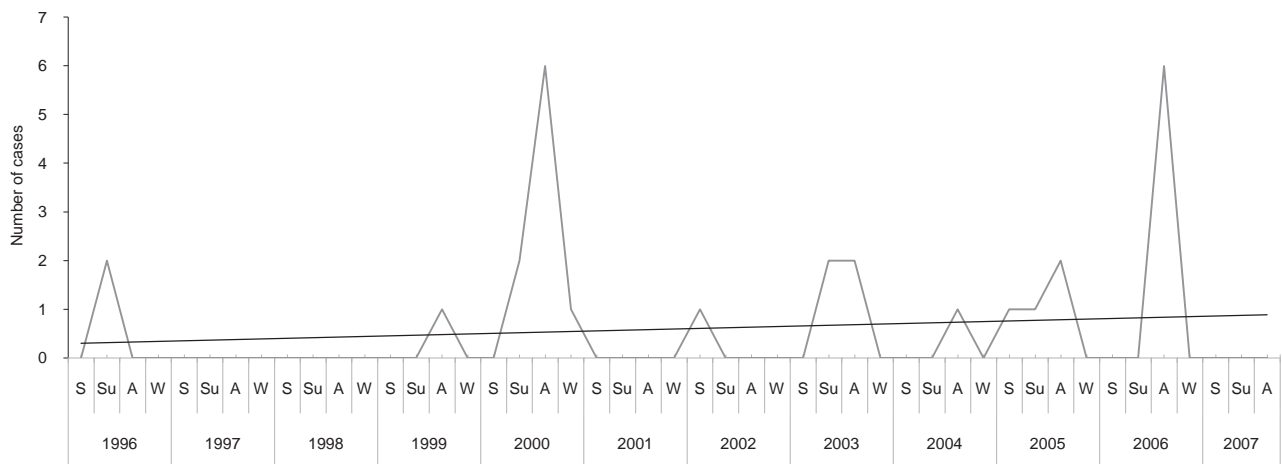


Figure 2. Trends of TBE incidence in time, in Świętokrzyskie province, in 1996–2007. S – spring, Su – summer, A – autumn, W – winter.

Table 2. TBE incidence in Świętokrzyskie province in 1996–2006.

PSSE	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
Busko	1.4	0	0	0	0	0	0	0.65	0	0.65	0
Jędrzejów	0	0	0	0	1.22	0	0	0	0	0	2.23
Kielce	0	0	0	0	0.59	0	0	0.25	0	0.25	0.25
Końskie	0	0	0	0	0	0	0	0	0	0	0
Opatów	0	0	0	0	0	0	0	0	0	0	1.76
Ostrowiec	0	0	0	0	1.75	0	0	0.85	0.85	0.85	1.71
Skarżysko	0	0	0	0	0	0	0.00	0.00	0	0	0
Starachowice	0	0	0	0.9	3.63	0	1.04	1.04	0	0	0
Włoszczowa	0	0	0	0	0	0	0	0	0.00	2.11	2.11

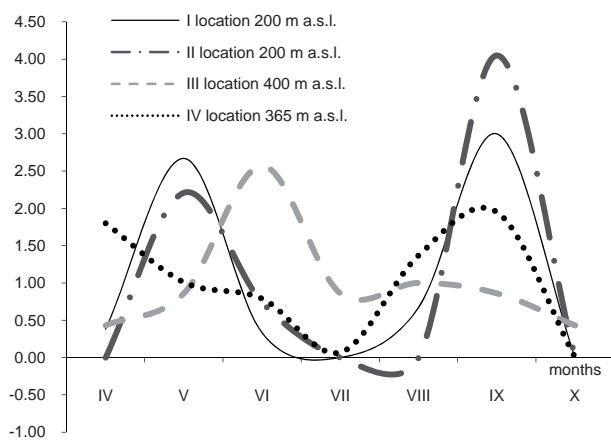


Figure 3. Seasonal dynamics of activity of *Ixodes ricinus* nymphs in 4 localities studied.

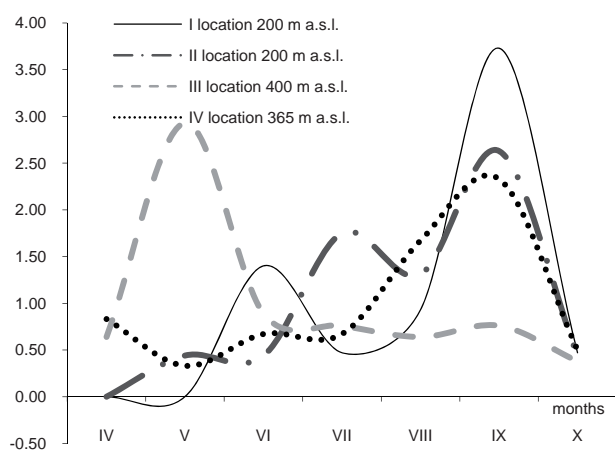


Figure 4. Seasonal dynamics of activity of *Ixodes ricinus* females in 4 localities studied.

(Tab. 1). Statistical test confirmed significant correlations between the registered TBE cases and the number of medical care institutions ($p < 0.05$, $r = 0.7468$).

The number of TBE cases varied in different seasons of the year (Fig. 1). It was markedly lower than the mean in spring and winter and significantly higher in autumn. The seasonality factor in spring, summer, autumn and winter was 27.59%, 96.55%, 248.28% and 27.59%, respectively. The highest number of cases was reported in September.

The linear model constructed to illustrate the trends of TBE incidence in time is represented by an equation: $y = 0.015 \times t - 0.26$, where t denotes time. The tendency towards an increase in TBE incidence persisted in the successive years (Fig. 2). It should be emphasised that all the TBE-diagnosed patients in the Świętokrzyskie province were hospitalized.

TBE incidence risk in the Świętokrzyskie province.

The density and structure of *I. ricinus* populations differed between the 4 study localities. The most numerous specimens were collected in localities III and IV, where the numbers were comparable: 35.86 ± 22.74 and 34.86 ± 24.97 , respectively, whereas the numbers in localities I and II were notably lower: 5.71 ± 7.41 and 6.14 ± 7.01 (Tab. 3). Active larvae were observed only in locality IV ($6.35 \pm 11.74/1h$ of collection). The biggest number of nymphs was reported from localities III and IV (9.04 ± 7 and $9.76 \pm 8.38/1h$ of collection, respectively). The highest occurrence frequency of females and males was noted in locality III (7.81 ± 6.81 and $9.19 \pm 11.58/1h$ of collection, respectively); it was lower in the other localities (Tab. 3). In spite of the differences observed, the statistical test indicated no correlation between tick density and structure of *I. ricinus* populations and the localities studied ($p > 0.05$).

Table 3. Mean number of *Ixodes ricinus* ticks collected in one hour in successive months and during the whole study period in various localities in Świętokrzyskie province.

Month	Kielce – Stadion	Borków	Ameliówka	Kielce – Skocznia
	Location I T/F/M/N/L	Location II T/F/M/N/L	Location III T/F/M/N/L	Location IV T/F/M/N/L
April	2/0/1/1/0	0/0/0/0/0	8.67/4.67/1/3/0	36/5/6/25
May	1/0/0/1/0	7/1/2/4/0	77/23/34/20/0	59/2/2/25/30
June	5/3/1/1/0	4/1/1/2/0	37/7/12/18/0	20/4/5/11/0
July	2/1/1/0/0	4/4/0/0/0	20/6/8/6/0	8/4/3/1/0
August	6/2/2/2/0	6/3/3/0/0	15.66/5/3.3/7.3/0	67/10/9/19/29
September	22/8/5/9/0	21/6/4/11	16/6/4/6/0	50/14/9/27/0
October	2/1/1/0/0	1/1/0/0/0	8/3/2/3/0	4/3/1/0/0
The mean from one collection throughout the study period	F 1.42 ± 1.51 M 0.92 ± 0.83 N 1.21 ± 1.52 L 0	F 1.35 ± 0.94 M 0.92 ± 0.93 N 1.64 ± 2.28 L 0	F 7.81 ± 6.81 M 9.19 ± 11.58 N 9.04 ± 7 L 0	F 3.81 ± 1.83 M 3.14 ± 1.54 N 9.76 ± 8.38 L 6.35 ± 11.74
Mean number of ticks throughout the study period	5.71 ± 7.41	6.14 ± 22.74	35.86 ± 22.74	34.86 ± 24.97
R ²	0.23	0.17	0.32	0.05

T – total, F – females, M – males, N – nymphs, L – larvae

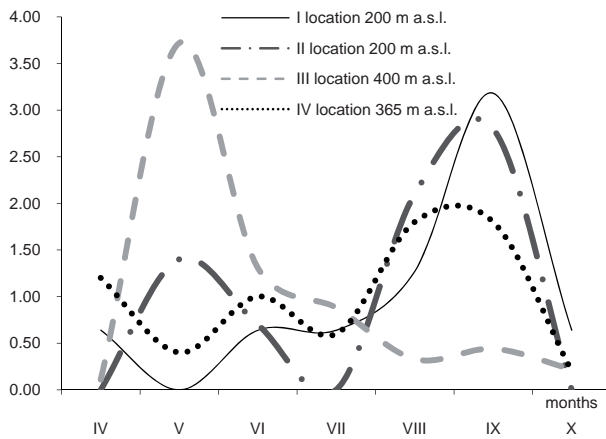


Figure 5. Seasonal dynamics of activity of *Ixodes ricinus* males in 4 localities studied.

The study habitats were characterised by varied dynamics of the activity of *Ixodes ricinus* larvae, nymphs and adult ticks (Fig. 3–6). In localities I, II and III, the particular stages displayed a single peak of activity; however, the increase in the activity was observed in different periods, i.e., in spring (locality III) or autumn (localities I and II). In locality IV, the activity dynamics was irregular.

The largest number of *I. ricinus* larvae were collected in May (Tab. 3). The peak of nymph activity was observed in May (locality III), in September (localities II and IV), or in May and September (locality IV) (Fig. 3). More pronounced differences were visible in the activity rhythm in the female ticks. Only in locality III was the peak of seasonal activity observed in spring (May). In the remaining localities, the highest seasonal activity was observed in September (Fig. 4). The highest single peak activity of males was noted in May in locality III. In the other localities, males displayed similar activity throughout the study period (Fig. 5).

The field study results demonstrate certain differences in the seasonal dynamics of tick activity. A marked increase in the total number of ticks in localities II and IV was observed in May, and a decline in June–July. In locality IV, there was a significant increase in the number of active ticks also in August, i.e. approximately a month earlier than in the other localities. A moderate increase was observed in locality III in July and August, and in localities I and II – in August–September (Fig. 6). In localities II and IV, the activity of ticks during the spring and summer–autumn peaks were comparable. However, localities I and II were characterised by markedly higher activity of ticks in September than in the spring months.

The temperature between April–October 2000 ranged from 9–27°C. The statistical test indicated a low correlation between the temperature and total number of *I. ricinus* specimens collected ($r=0.1832$). The correlation between the temperature and the number of collected females ($r=0.2240$) and males ($r=0.2076$) was also low. No cor-

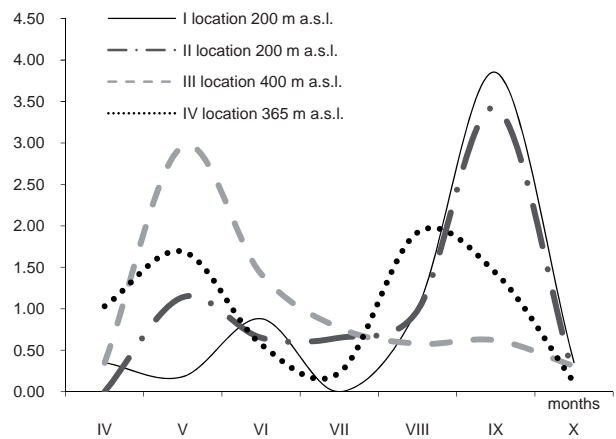


Figure 6. Seasonal dynamics of activity of *Ixodes ricinus* ticks in 4 localities studied.

relation between the number of larvae ($r=-0.1333$) and nymphs ($r=0.0496$) and temperature was found.

DISCUSSION

Our study demonstrated the emergence of new infectious TBE foci in the Świętokrzyskie province, a region that has been classified as a TBE non-endemic area. In recent years, there has been a marked increase in the number of TBEV virus infections in this region. The disease prevalence in the Świętokrzyskie province is perhaps much higher than the number of the registered cases, due to the abundant occurrence of numerous *I. ricinus* ticks, which are the vectors and reservoirs of TBEV in this area. Moreover, the uncharacteristic influenza-like symptoms of the initial phase of TBE [21, 29, 71] are not often associated with viral infections by the patients and, therefore, are not reported to medical care institutions. As indicated in our study, the symptoms which necessitated hospitalisation were only notified by patients; they usually accompanied relapses of the disease or acute tick-borne encephalitis. The number of registered cases is undoubtedly affected by organisation of medical care services in various regions. Better prevention, diagnostics and treatment are facilitated by a larger number of medical institutions and doctors. Moreover, in recent years, actions promoting TBE prophylaxis and prevention of threats posed by ticks have been undertaken; nevertheless, knowledge about this issue is still insufficient [2].

The increased tick-borne disease prevalence rate in the Świętokrzyskie province is undoubtedly related to human behaviour (more frequent recreational exposure to ticks in their habitats) and to deteriorating socio-economic conditions in the region [5], where the inhabitants find sources of income in forest work (e.g. sorting wood, collecting groundcover fruit and wood), and thus are at the highest risk of tick exposure. As shown in the study conducted by Stefanoff *et al.* [59], 54% of TBE cases in Poland affect rural inhabitants and 45.5% of urban dwellers. People whose

professional activities are conducted in tick habitats are the most susceptible to tick-borne diseases [50]. Anti-TBEV antibodies were detected in as many as 19.8% of forestry workers and 32.0% of farmers in eastern Poland [9]. TBEV viruses were found in 8.8% of *I. ricinus* ticks feeding on humans in Germany (2002) [61] and in 13.2–40.9% in Latvia [3]. TBEV prevalence in unfed ticks collected from vegetation in various European countries has a wide range, from 0.34–28% [3, 23, 30, 61, 69]. In some habitats in eastern Poland, 4.2% of ticks are infected with TBEV; the mean number of specimens for the region amounts to 1.8% [8]. TBEV infection usually takes place during the tick feeding period [31, 41, 48], but also during consumption of milk from infected cows or goats, and its products (e.g., cheese) [17]. Most TBE cases reported by the Busko Regional Sanitary-Epidemiological Station involve infections through consumption of uncooked goat's milk purchased from a local breeder. Anti-TBEV antibodies were detected in the goat and in the producer [45].

Increased TBEV infection prevalence is observed also in other regions, especially in TBE endemic areas in western and north-western Poland. In the years 1999–2006, the TBE incidence proportion in the Podlaskie province increased from 3.43 to 12.94 [1]. A similar tendency has been observed in other European countries: in Italy [10, 70], Germany [63], Russia [34], Estonia [68], Latvia [16], the Czech Republic [13, 14], Slovenia [55] and Slovakia [55], with the exception of Austria, where the long-term decline in the disease incidence is closely related to the increasing number of TBE-vaccinated patients [39]. Similar to the results of our studies, in other countries, e.g., Finland [23], Sweden, Norway [22], Italy [7], Switzerland [37] and Germany [62, 63, 64, 65], there are reports of higher TBE prevalence, even in regions of low risk of tick-borne disease, and of reactivation of previously active TBE endemic areas [38, 63].

The prevalence of TBE and other tick-borne diseases is related to tick density and their activity dynamics [26, 60] and is modified by microclimatic conditions [54]. Attention has been paid to the effect of the global warming resulting in prolongation of the tick activity period [43, 52, 66] and affecting population density [3, 43, 55], and to the influence of anthropopressure on distribution of ticks [12, 57, 58]. The results of our study conducted in south-eastern Poland indicate a general tendency towards increased tick activity in May, and at the turn of August and September, and its decline in July. Simultaneously, differences were demonstrated in the density and activity of various developmental stages and the total number of ticks depending on the biotope. We obtained convergent data in the localities placed at similar altitudes, i.e., 200 m a.s.l. (localities I and III) and 365–400 m a.s.l. (localities III and IV). The highest *I. ricinus* tick density and activity were observed in the localities situated on hills overgrown with heterogeneous vegetation (localities III and IV), which ensure high humidity levels even during summer months characterised

by low precipitation. Such a terrain shape offers favourable conditions for vertebrates-tick hosts and pathogen reservoirs [51, 53]. The effect of saturation deficit on seasonal variation of density of questing *I. ricinus* tick, defined as tick phenology, was also observed in Central Europe by other authors [4, 20, 28, 47]. Tick density and seasonal questing tick activity [4, 20, 28, 47], as well as the prevalence of *B. burgdorferi* in *I. ricinus* ticks [6, 47], were lower in localities situated at higher altitudes above sea level.

The differences in the density and activity of *I. ricinus* larvae, nymphs and adults in the study localities may be conditioned by the occurrence of hosts of the particular stages and by different conditions in microhabitats, e.g. the type of the biotope, insolation and humidity conditions. The effect of the microclimate on adult activity [11, 15, 18, 44, 49] and nymph activity [18, 67] of *Ixodes* ticks was observed also in other field studies. Since the phenology of *I. ricinus* depends on numerous biotic (host species, host abundance and behaviour, vegetation structure) and abiotic (climate) factors, the TBE threat prediction requires continuous monitoring of the abundance and activity of ticks in various environments.

In the present study, the TBE cases were registered in the summer-autumn period, with increased intensity in the autumn season, i.e., after the period of the highest seasonal activity of *I. ricinus* nymphs and females (different in the particular study habitats) which most frequently attack humans. Seasonality of tick-borne diseases is also noted in other regions of the world [36, 48].

The occurrence of abundant tick population and the increasing tendency in tick-borne disease prevalence in the study area and other parts of Europe necessitates multidirectional activity for improvement of medical services, promotion of prophylaxis methods and continuous environmental monitoring.

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