ORIGINAL ARTICLE

Annals of Agricultural and Environmental Medicine 2012, Vol 19, No 3, 465-468
www.aaem.pl

Risk of Lyme disease at various sites and workplaces of forestry workers in eastern Poland

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Cisak E, Wójcik-Fatla A, Zając V, Sroka J, Dutkiewicz J. Risk of Lyme disease at various sites and workplaces of forestry workers in eastern Poland. Ann Agric Environ Med. 2012; 19(3): 465-468.

Abstract

Knowledge about the seasonal activity of various stages of the *Ixodes ricinus* tick is of great importance while developing models of the circulation of pathogens transmitted by ticks in a given environment, as well as while evaluating the risk of infection with these pathogens among individuals performing work in this environment. The objectives of the study was determination of the relative activity of *Ixodes ricinus* ticks occurring at 4 different workplaces of workers employed in one randomly selected forestry inspectorate, and the comparison of this activity to the *Borrelia burgdorferi* prevalence in ticks collected from the above-mentioned working stands. Ticks were collected by dragging a woollen flag over lower vegetation and litter along the paths and edges of a forest in July and September 2011 at the following sites and workplaces: acquisition of timber, growing of forest, forest cultivation and forest protection. The relative activity (density) of ticks was determined by means of a combined method of single sample and area sampling. A forest area of approximately 100 m^2 was brushed with a flag. The isolates obtained from *Ixodes ricinus* ticks were examined for the presence of *Borrelia burgdorferi* sensu lato DNA by polymerase chain reaction, using primers FLA1 and FLA2 specific for the fragment of *fla* gene sequence. In the statistical analysis, the Mann-Whitney test, χ^2 test and Spearman test were applied. The differences between *Ixodes ricinus* activity at individual places of work where various biotopes were observed, were not statistically significant. A statistically significant variation in the tick infection rate, depending on work stands, was found for all the tick stages, i.e. for nymphs ($\chi^2 = 76.516$, $p<0.000001$), females ($\chi^2 = 18.832$, $p<0.000292$) and males ($\chi^2 = 69.257$, $p=0.000001$). Nevertheless, the statistical analysis showed the significant difference in total infection rates only between timber acquisition and growing of forest sites ($p=0.049$), and between growing of forest and forest cultivation sites ($p=0.049$). The study showed also the lack of relationship between the ticks' activity and *Borrelia burgdorferi* infection of ticks at individual places and sites of work of forestry workers. In conclusion, forestry workers employed at such stands of work as timber acquisition, growing of forest, forest cultivation and forest protection are nearly at the same risk of *Borrelia burgdorferi* infection.

Key words

Ixodes ricinus, activity, *Borrelia burgdorferi* infection, forestry environment, various working stands, eastern Poland

INTRODUCTION

Diseases transmitted by ticks, called transmissive diseases, occur as natural foci, and their range is equivalent to the geographical spread of certain species of ticks [1, 2, 3, 4, 5, 6, 7, 8, 9]. In Europe, the main vector of pathogenic microorganisms causing transmissive diseases, such as Lyme borreliosis, tick-borne encephalitis and meningitis, human granulocytic anaplasmosis or babesiosis (Lyme disease), is a common tick – *Ixodes ricinus,* which occurs widely in deciduous and mixed forests (more rarely in coniferous forests), also in municipal parks, on lawns and in allotment gardens [5, 10, 11, 12, 13, 14, 15, 16, 17].

Those who perform work in the forest environment, farmers and undergrowth pickers, are especially exposed to infection with tick-borne pathogens [5, 18, 19, 20, 21, 22].

Among the diseases transmitted by ticks, borreliosis (Lyme disease) is especially dangerous. At present, it is the most frequent occupational disease registered in Poland. In 2009, a total number of 3,146 occupational diseases were registered in Poland, including 888 cases of contagious diseases, among which borreliosis – 664 cases – constituted 74.8% [23].

Knowledge concerning the seasonal activity of various stages of the *Ixodes ricinus* tick is of great importance while developing models of the circulation of pathogens transmitted by ticks in a given environment, as well as while evaluating the risk of infection with these pathogens among individuals performing work in this environment [10, 18, 24].

Studies on the activity of *Ixodes ricinus* ticks in forestry environment in eastern Poland were conducted as early as 2001 and again in 2009 [18, 25]. However, no studies have been carried out so far concerning the activity of ticks at individual workplaces in the forestry sector.

Objectives. The objectives of the study were determination of the relative activity of *Ixodes ricinus* ticks occurring at 4 different workplaces assigned to workers employed in the environment of forest exploitation, and comparison of this activity with the *Borrelia burgdorferi* prevalence in ticks collected from these workplaces.

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Received: 5 June 2012; accepted: 30 July 2012

MATERIAL AND METHODS

Area of the study and ticks collection. After recognition of the workplaces among people employed in one randomly selected forestry inspectorate subordinated to the Regional Board for National Forests in Lublin, ticks were collected in order to determine their activity at individual workplaces.

Ticks were collected by dragging a woollen flag over lower vegetation and litter along the paths and edges of a forest in July and September 2011 at the following sites and workplaces:

- Acquisition of timber, i.e. felling trees, timber trimming, measurement, classification, transport; workplaces: lumberjack, saw operator, forest worker, forester, sub-forester, supervising engineer. Acquisition of timber took place in the mid-part of a mixed forest with the prevalence of deciduous trees, comprising: oak, hornbeam, maple, ash, elm and pine. The forest was characterized by rich undergrowth and light insolation.
- Growing of forest (work activities in forest tree nurseries), i.e. watering of nursery plants, misting, spraying, weeding, fertilizing with compost; workplaces of a forest worker, forester, sub-forester. The site was located in an isolated glade within a mixed forest, with the prevalence of coniferous trees, comprising pine, hornbeam and birch. The area was characterized by very poor undergrowth.
- Forest cultivation, i.e. work activities associated with the care of young pine forests: i.e. weeding, thinning, replanting, elimination of undesirable species; workplaces of a forest worker, forester, sub-forester and forest inspector. The young pine forest was located at the boundary of a mixed forest and farm area, and characterized by rich undergrowth and abundant insolation.
- Forest protection (pest control, counting of forest stands, patrolling of stands); workplaces of a forest ranger, forester, sub-forester, and forest service employee. The area was located within a mixed forest with a prevalence of oak and fir. The area was characterized by poor undergrowth compared to areas B and C, and by light insolation.

Relative ticks' activity (mean density) was determined by means of a combined method of single sample and area sampling. A forest area of approximately 100 m^2 was brushed with a flag. The number of ticks collected by 1 person during 1 hour on such a delineated area was considered as the relative activity of ticks at a given site/workplace of a forestry employee [18, 26, 27].

Examination of ticks

DNA isolation. All *Ixodes ricinus* ticks were investigated individually. Bacterial DNA was isolated by boiling in 0.7 M ammonium hydroxide and stored in -20° C for further investigation.

Detection of *Borrelia burgdorferi* sensu lato DNA by polymerase chain reaction (PCR). The isolates obtained from *Ixodes ricinus* ticks were examined for the presence of *Borrelia burgdorferi* sensu lato DNA by polymerase chain reaction using primers FLA1 and FLA2 specific for fragment of the *fla* gene sequence. The amplification was carried out in C1000 Thermal Cycler (BioRad, USA) according to Wójcik-Fatla *et al.* [28].

Statistical analysis. In the statistical analysis the Mann-Whitney test and χ^2 test was applied. The correlation between the ticks' activity and ticks' infection was carried out by the Spearman test.

RESULTS

Activity of ticks. The highest activity of the *Ixodes ricinus* tick was observed at the site of timber acquisition (42.0). At sites related to the growing and protection of forests the activity of ticks was similar, 35.0 and 37.0, respectively. At the site of forest cultivation the activity of *I. ricinus* ticks was the lowest – 8.0 (Tab. 1).

The differences between *Ixodes ricinus* activity at individual places of work where various biotopes were observed, evaluated by Mann-Whitney test, were not statistically significant. Considering the stages of development of the *I. ricinus* tick, the activity of nymphs was considerably higher than that of adult forms at the sites of timber acquisition and forest protection: 35.0 and 29.0, respectively, whereas adult forms (mainly females) were highly dominant at the site of forest cultivation and growing: 29.5 and 4.33, respectively.

By χ^2 test, a statistically significant variation in the tick activity, depending on working stands, was found for nymphs ($\chi^2 = 49.085$, $p<0.000001$) and females ($\chi^2 = 46.229$, $p<0.000001$), but not for males ($\chi^2 = 2.247$, $p=0.5227$).

Infection by ticks. The highest *Borrelia burgdorferi* s. l. infection rate was observed in ticks collected from workplaces at growing of forest (55.0%). The infection rates in the workplaces at forest cultivation and forest protection were comparable and amounted to 24.8% and 20.3%, respectively (Tab. 1). At the place of acquisition of timber the percentage of ticks infected with *B. burgdorferi* s. l. amounted to 14.8%.

Table 1. Prevalence of *Borrelia burgdorferi* in *Ixodes ricinus* ticks and activity of *I. ricinus* at individual sits and places of work of forestry workers

Sit/place of work	Stage of tick			Total
	Females Ni/Ne (%), a	Males Ni/Ne (%), a	Nymphs Ni/Ne (%), a	
Acquisition of timber	14/50 (28.0%), a =2.5	7/24 (29.2%), a= 4.5	6/108 (5.5%), a= 35.0	27/182 (14.8%), a=42
Growing of forest	2/5 (40.0%), a= 4.33	5/7 (71.4%), a =1.17	4/8 (50.0%) a= 2.5	11/20 (55.0%), a=8.0
Forest cultivation	25/87 (28.7%), a =29.5	29/112 (25.9%), a= 2.5	27/127 (21.2%), a= 3.0	81/326 (24.8%), a= 35
Forest protection	1/2 (50.0%), a= 4.0	2/10 (20.0%), a= 4.0	20/101 (19.8%), a= 29.0	23/113 (20.3%), a=37
Total	42/144 (29.2%), a=40.3	43/153 (28.1%), a=12.2	57/344 (16.6%). a=69.5	142/641 (22.1%), a=122.0

Ni – No. infected; Ne – No. examined; a – activity.

By χ^2 test, a statistically significant variation in the tick infection rate, depending on working stands, was found for all the tick stages; i.e. for nymphs ($\chi^2 = 76.516$, $p<0.000001$), females ($\chi^2 = 18.832$, $p<0.000292$) and males ($\chi^2 = 69.257$, $p=0.000001$).The statistical analysis, assessing differences between individual sites by Mann–Whitney test, showed a significant difference in total infection rate only between timber acquisition and growing of forest sites ($p=0.049$),

and between growing of forest and forest cultivation sites (p=0.049).

Results of the Spearman test for correlation showed a lack of significant relationship between the ticks' activity and the infection of ticks with *Borrelia burgdorferi* at individual places and sites of work of forestry workers.

DISCUSSION

The status, distribution and abundance of the main tick vector species are an implicit requirements for the risk assessment of tick-borne pathogens (especially *Borrelia burgdorferi* spirochetes) infection in humans [10, 18, 24, 26, 27].

In 2009, a scale for the threat in the areas of municipal parks according to ticks' activity was developed by Supergan and Karbowiak [27]. Into the areas of the highest activity of ticks they classified the sites where ticks' activity was over 50, followed by areas of high risk – within the range 26-50, mediocre risk – 11-25, limited risk – 4-10, and low risk – 1-3. The researchers defined as safe the areas were the presence of ticks was not observed.

Referring the results of own studies to the above-mentioned scale, it may be presumed that in the environment of forest exploitation a high risk of exposure to potentially infected *Ixodes ricinus* ticks occurred at the place of timber acquisition, cultivation and protection of forest.

The ticks' activity evaluated in the presented study was comparable with that observed by Bartosik *et al.* [22] in 9 different regions of Świętokrzyskie province (south-central Poland), where the mean ticks' density varied from 2.0-42.0. The cited authors did not confirm any correlation between the mean number of ticks collected and borreliosis incidence in a particular area [22].

Although the relative ticks' density in the presented study differed at individual sites and workplaces where various biotopes were observed, the differences between these sites were not statistically significant, although a general variability for all sites was found by χ^2 test.

Studies of the activity of *I. ricinus* ticks with relation to the biotope have been conducted in many scientific centres [1, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39]. In the Czech Republic, Hubalek *et al.* [30] indicated that the activity of adult forms of these ticks in a forest with rich undergrowth was close to the activity of ticks at the site of timber acquisition, as observed in own studies, and was: 45.0 and 42.0, respectively. For nymphs, these values were, respectively, 26.0 in the Czech studies, and 35.0 in own studies. In turn, the above-mentioned researchers evaluated the scope of activity of ticks in the areas with poor undergrowth as 19.6 – 34.5 for nymphs, and 0.9-27 for adult ticks. These scopes were significantly higher than the values obtained in own studies on the activity of ticks at the site of forest breeding (poor undergrowth) which were, respectively, 2.5 for nymphs and 4.33 for females. The differences which occurred in the activity of ticks in the forest environment without undergrowth, and in poor undergrowth recorded in own and in Czech studies, may be explained, among other things, by the very high humidity in this environment in own studies (once ticks were collected after rainfall).

The percentage of ticks infected with *Borrelia burgdorferi* sensu lato (collected at 4 workplaces in the presented study) was 22.1%, and remained within the scope of the results of studies conducted in the last decade in various European countries [2, 20, 28, 34, 35, 37, 40, 41].

In 1997, studies of the relationship between the activity of. *I. ricinus* and their infection rate with *B. burgdorferi* spirochetes were conducted in Poland by Petko *et al.* [26]. In these studies it was found that ticks collected at 9 different sites from south-eastern Poland did not show any correlation between infection with spirochetes *Borrelia burgdorferi* and their activity. The lack of relationship between the relative activity of ticks from eastern Poland and the degree of their infection with *Borrelia burgdorferi* and *Anaplasma phagocytophilum* was also showed by Wójcik-Fatla *et al.* [28].

Similarly, Nazzi et al. [3] did not find any correlation between the prevalence of. *B. burgdorferi* in nymphal *I. ricinus* and their density, and between the incidences of Lyme borreliosis and *Borrelia* prevalence in nymphal stages of *Ixodes ricinus* in northeast Italy.

On the contrary, Estrada–Pena *et al.* found a correlation between climatic traits, such as temperature, vegetation stress and their seasonal changes, and *Borrelia burgdorferi* prevalence in questing ticks in the Western Palearctic region [35].

The own studies conducted in 2001 showed that there is a relationship between seroprevalence of specific antibodies against *Borrelia burgdorferi* among the employees of forestry and farmers, and the activity of *Ixodes ricinus* ticks occurring at workplaces of these workers and, moreover, based on the activity of *I. ricinus* tick in a given environment, it is also possible to estimate the degree of infection with *B. burgdorferi* among people performing work in this environment [18].

Taking into account the results of ticks' activity and their infection with *B. burgdorferi* spirochetes obtained in the present study, it can be concluded that forestry workers performing work on such working stands as timber acquisition, growing of forest, forest cultivation and forest protection are nearly at the same risk of *Borrelia burgdorferi* infections and contracting Lyme disease.

Acknowledgements

The presented study is based on the results of Stage 2 of a many-year project: 'Improvement of occupational safety and work conditions', financed in the years 2011-2013 within scientific studies and projects for practical implementation, financed by the Ministry of Science and Higher Education, and the National Centre for Research and Development in Warsaw, Poland. The Project Coordinator was the Central Institute of Labour Protection in Warsaw.

REFERENCES

1. Danielová V, Rudenko N, Daniel M, Holubová J, Materna J, Golovchenko M, Schwarzová L. Extension of *Ixodes ricinus* ticks and agents of tick-borne diseases to mountain areas in the Czech Republic. Int J Med Microbiol. 2006; 296 (Suppl 40): 48-53.
2. Daniel M, Materna J, Honig V, Metelka L, Danielová V, Harcarik J, et al. Vertical distribution of the tick Ixodes ricinus and tick-borne pathogens in the northern Moravian mountains correlated with climate warming (Jeseníky Mts., Czech Republic). Cent Eur J Public Health. 2009; 17(3): 139-145.
3. Nazzi F, Martinelli E, Del Fabbro S, Bernardinelli I, Milani N, Iob A, et al. Ticks and Lyme borreliosis in an alpine area in northeast Italy. Med Vet Entomol. 2010; 24(3): 220-226.
4. Franke J, Hildebrandt A, Meier F, Straube E, Dorn W. Prevalence of Lyme disease agents and several emerging pathogens in questing ticks from the German Baltic coast. J Med Entomol. 2011; 48(2): 441-444.

5. Dutkiewicz J, Cisak E, Sroka J, Wójcik-Fatla A, Zając V. Biological agents as occupational hazards – selected issues. Ann Agric Environ Med. 2011; 18(2): 286-93.
6. Ruiz-Fons F, Fernández-de-Mera IG, Acevedo P, Gortázar C, de la Fuente J. Factors driving the abundance of *Ixodes ricinus* ticks and the prevalence of zoonotic *I. ricinus*-borne pathogens in natural foci. Appl Environ Microbiol. 2012; 78(8): 2669-2676.
7. Capelli G, Ravagnan S, Montarsi F, Ciocchetta S, Cazzin S, Porcellato E, et al.Occurrence and identification of risk areas of *Ixodes ricinus*-borne pathogens: a cost-effectiveness analysis in north-eastern Italy. Parasit Vectors. 2012; 27(5): 61-70.
8. Huegli D, Moret J, Rais O, Moosmann Y, Erard P, Malinverni R, Gern L. Prospective study on the incidence of infection by Borrelia burgdorferi sensu lato after a tick bite in a highly endemic area of Switzerland. Ticks Tick Borne Dis. 2011; 2(3): 129-136.
9. Stanek G, Wormser GP, Gray J, Strle F. Lyme borreliosis. Lancet 2012; 379(9814): 461-473.
10. Jameson LJ, Medlock JM. Tick surveillance in Great Britain. Vector Borne Zoonotic Dis. 2011; 11(4): 403-412.
11. Kempf F, De Meeûs T, Vaumourin E, Noel V, Taragel'ová V, Plantard O, et al. Host races in *Ixodes ricinus*, the European vector of Lyme borreliosis. Infect Genet Evol. 2011; 11(8): 2043-2048.
12. Jaenson TG, Lindgren E. The range of *Ixodes ricinus* and the risk of contracting Lyme borreliosis will increase northwards when the vegetation period becomes longer. Ticks Tick Borne Dis. 2011; 2(1): 44-49.
13. Estrada-Peña A, Santos-Silva MM. The distribution of ticks (Acari: Ixodidae) of domestic livestock in Portugal. Exp Appl Acarol. 2005; 36(3): 233-246.
14. Kesteman T, Rossi C, Bastien P, Brouillard J, Avesani V, Olive N, et al. Prevalence and genetic heterogeneity of *Borrelia burgdorferi* sensu lato in *Ixodes ticks* in Belgium. Acta Clin Belg. 2010; 65(5): 319-322.
15. Richter D, Matuschka FR. "Candidatus Neoehrlichia mikurensis" Anaplasma phagocytophilum, and lyme disease spirochetes in questing european vector ticks and in feeding ticks removed from people. J Clin Microbiol. 2012; 50(3): 943-947.
16. Santos-Silva MM, Beati L, Santos AS, De Sousa R, Núncio MS, Melo P, et al. The hard-tick fauna of mainland Portugal (Acari: Ixodidae): an update on geographical distribution and known associations with hosts and pathogens. Exp Appl Acarol. 2011; 55(1): 85-121.
17. Schicht S, Junge S, Schnieder T, Strube C. Prevalence of *Anaplasma phagocytophilum* and coinfection with *Borrelia burgdorferi* sensu lato in the hard tick *Ixodes ricinus* in the city of Hanover (Germany). Vector Borne Zoonotic Dis. 2011; 11(12): 1595-1597.
18. Cisak E, Chmielewska-Badora J, Dutkiewicz J, Zwoliński J. Preliminary studies on the relationship between *Ixodes ricinus* activity and tick-borne infection among occupationally-exposed inhabitants of eastern Poland. Ann Agric Environ Med. 2001; 8(2): 293-295.
19. Cisak E, Chmielewska-Badora J, Zwoliński J, Wójcik-Fatla A, Polak J, Dutkiewicz J. Risk of tick-borne bacterial diseases among workers of Roztocze National Park (south-eastern Poland). Ann Agric Environ Med. 2005; 12(1): 127-32.
20. Oehme R, Hartelt K, Backe H, Brockmann S, Kimmig P. Foci of tick-borne diseases in southwest Germany. Int J Med Microbiol. 2002; 291 (Suppl 33): 22-29.
21. Buczek A, Rudek A, Bartosik K, Szymańska J, Wojcik-Fatla A. Seroepidemiological study of Lyme borreliosis among forestry workers in southern Poland. Ann Agric_Environ Med. 2009; 16(2): 257-261.
22. Bartosik K, Lachowska-Kotowska P, Szymańska J, Pabis A, Buczek A. Lyme borreliosis in south-eastern Poland: relationships with environmental factors and medical attention standards. Ann Agric Environ Med. 2011; 18(1): 131-137.
23. Wilczyńska U, Szeszenia-Dąbrowska N, Sobala W. [Occupational diseases in Poland, 2009]. Med Pr. 2010; 61: 369-79. Polish.
24. Siński E, Pawełczyk A, Bajer A, Behnke J. Abundance of wild rodents, ticks and environmental risk of Lyme borreliosis: a longitudinal study in an area of Mazury Lakes district of Poland. Ann Agric Environ Med. 2006; 13(2): 295-300.
25. Wójcik-Fatla A, Szymańska J, Zając V. [Relative activity of *Ixodes ricinus* ticks in forests of the Lublin makroregion]. Med Og. 2009; 3(3): 414-420. Polish.
26. Petko B, Siuda K, Stanko M, Tresová G, Karbowiak G, Fricova J. *Borrelia burgdorferi* sensu lato in the *Ixodes ricinus* ticks in southern Poland. Ann Agric Environ Med. 1997; 4(2): 263–269.
27. Supergan M, Karbowiak G. The estimation scale of endangerment with tick attacks on recreational towns areas. Przeg Epidemiol. 2009; 63(1): 67-71.
28. Wójcik-Fatla A, Szymańska J, Wdowiak L, Buczek A, Dutkiewicz J. Coincidence of three pathogens (*Borrelia burgdorferi, Anaplasma phagocytophilum* and *Babesia microti*) in *Ixodes ricinus* ticks in the Lublin macroregion. Ann Agric Environ Med. 2009; 16(1): 151-158.
29. Boyard C, Barnouin J, Bord S, Gasqui P, Vourc'h G. Reproducibility of local environmental factors for the abundance of questing *Ixodes ricinus* nymphs on pastures. Ticks Tick Borne Dis. 2011; 2(2): 104-110.
30. Hubalek Z, Halouzka J, Juricova Z, Sikutova S, Rudolf I. Effect of forest clearing on the abundance of *Ixodes ricinus* ticks and the prevalence of *Borrelia burgdorferi* s.l. Med Vet Enomol. 2006; 20(2): 166-172.
31. Dautel H, Dippel K, Kämmer D, Werkhausen A, Kahl O. Winter activity of *Ixodes ricinus* in a Berlin forest. Int J Med Microbiol. 2008; 298 (Suppl1): 50-54.
32. Dobson AD, Taylor JL, Randolph SE. Tick (*Ixodes ricinus*) abundance and seasonality at recreational sites in the UK: hazards in relation to fine-scale habitat types revealed by complementary sampling methods. Ticks Tick Borne Dis. 2011; 2(2): 67-74.
33. Egyed L, Elő P, Sréter-Lancz Z, Széll Z, Balogh Z, Sréter T. Seasonal activity and tick-borne pathogen infection rates of Ixodes ricinus ticks in Hungary. Ticks Tick Borne Dis. 2012; (3): 90-94.
34. Tack W, Madder M, De Frenne P, Vanhellemont M, Gruwez R, Verheyen K. The effects of sampling method and vegetation type on the estimated abundance of *Ixodes ricinus* ticks in forests. Exp Appl Acarol. 2011; 54(3): 285-292.
35. Estrada-Peña A, Ortega C, Sánchez N, Desimone L, Sudre B, Suk JE, et al. Correlation of *Borrelia burgdorferi* sensu lato prevalence in questing *Ixodes ricinus* ticks with specific abiotic traits in the western palearctic. Appl Environ Microbiol. 2011; 77(11): 3838-3845.
36. Hancock PA, Brackley R, Palmer SC. Modelling the effect of temperature variation on the seasonal dynamics of *Ixodes ricinus* tick populations. Int J Parasitol. 2011; 41(5): 513-522.
37. Herrmann C, Gern L. Survival of *Ixodes ricinus* (Acari: Ixodidae) under challenging conditions of temperature and humidity is influenced by *Borrelia burgdorferi* sensu lato infection. J Med Entomol. 2010; 47(6): 1196-1204.
38. Hornok S, Farkas R. Influence of biotope on the distribution and peak activity of questing ixodid ticks in Hungary. Med Vet Entomol. 2009; 23(1): 414-6.
39. Knap N, Durmisi E, Saksida A, Korva M, Petrovec M, Avsic-Zupanc T. Influence of climatic factors on dynamics of questing *Ixodes ricinus* ticks in Slovenia. Vet Parasitol. 2009; 64(2-4): 275-281.
40. Tijsse-Klasen E, Jacobs JJ, Swart A, Fonville M, Reimerink JH, Brandenburg AH, et al. Small risk of developing symptomatic tick-borne diseases following a tick bite in The Netherlands. Parasit Vectors. 2011; 10(4): 17.
41. Reis C, Cote M, Paul RE, Bonnet S. Questing ticks in suburban forest are infected by at least six tick-borne pathogens. Vector Borne Zoonotic Dis. 2011; 11(7): 907-916.