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ABUNDANCE OF WILD RODENTS, TICKS AND ENVIRONMENTAL RISK OF LYME BORRELIOSIS: A LONGITUDINAL STUDY IN AN AREA OF MAZURY LAKES DISTRICT OF POLAND

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Abstract: The results of a longitudinal epidemiological survey in two contrasting habitats in an area of the Mazury Lakes district of Poland indicate that both host and vector (*Ixodes ricinus*) densities, may be the most important risk factors for the tick-transmitted spirochetes of *Borrelia burgdirferi* s.l. However, the results also highlight that even related host species, such as the wild rodents *Apodemus flavicollis* and *Clethrionomys glareolus* that share the same habitat, can show quite different dynamics of tick infestation. We provide evidence that the woodland populations of *A. flavicollis* and *C. glareolus* are more frequently infested with larvae than nymphs, and more frequently with both stages than *M. arvalis* in the neighbouring open fallow lands. The prevalence of infestation with larvae varied from 92% for *A. flavicollis*, and 76% for *C. glareolus* to 37% for *M. arvalis*. Other factors, such as population age structure and sex, were also shown to impact on tick densities on hosts at particular times of the year and hence on the zoonotic risk. Moreover, particular species of rodents from different habitats, *A. flavicollis* (woodlands) and *Microtus arvalis* (fallow lands) carry infected immature *I. ricinus* ticks more frequently than *C. glareolus* voles (woodlands). Thus, the relative contribution of each species to the cumulative reservoir competence differs among species living in the woodland habitats and in relation to voles living in the fallow lands. It follows, therefore, that any factor which reduces the relative density of *A. flavicollis* in comparison to other hosts in the wild rodent community, will reduce also the risk of human exposure to Lyme borreliosis spirochetes.

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INTRODUCTION

The spirochetes *Borrelia burgdorferi* sensu lato (s.l.) are agents of Lyme borreliosis, the most common tick-borne zoonosis throughout the world [22]. However, the role of host species composition and wildlife diversity in the ecology and dynamics of Lyme borreliosis, especially in ecosystems where there is a high risk of human infection, are incompletely understood. Nevertheless, it is universally accepted that *B. burgdorferi* s.l., throughout its endemic range, is vectored between hosts by members of the *Ixodes persulcatus* "species complex", and in Europe is transmitted predominantly from animal hosts to humans by nymphs of *I. ricinus* [2]. The immature stages

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(larvae and nymphs) of *I. ricinus* are generalists and feed on a wide range (dozens or hundreds) of vertebrate hosts, including mammals, birds and lizards. However, while these hosts may provide a blood meal for the ticks, they are not all competent hosts for the transmission of the Lyme borreliosis agent. Previous studies have shown that in the woodland and open fallow land habitats of central Europe, infection with *B. burgdorferi* s.l. is carried by natural populations of small rodents, especially the yellow-necked mouse (*Apodemus flavicollis*) (Schreber, 1780), bank vole (*Clethrionomys glareolus*) (Melchior, 1834) and common vole (*Microtus arvalis*) (Pallas, 1779). These rodents serve as reservoirs of the Lyme borreliosis agent, and since their relative and absolute population densities vary, this has consequences for tick infestations and for the dynamics of transmission of tick-borne agents of disease [4, 6, 8, 10, 12, 16, 19]. It has also been established recently that non-rodent hosts are relatively poor reservoirs for Lyme borreliosis and dilute the disease agent in the environment by feeding ticks without infecting them with spirochetes. The dilution effect model [14] predicts that high species diversity in the community of tick hosts will reduce the prevalence of infection in vectors by diluting the effects of the most competent disease reservoir. In other words, with increasing host diversity, there should be a corresponding reduction in the prevalence of infection in the tick population [11]. It follows, then, that changing patterns of natural infection in rodents, the most competent disease reservoir, and in ticks are likely to have important consequences for the risk of human infection. In the Polish ecosystems, the importance of these interactions is still poorly understood.

The present longitudinal studies, conducted in an area where Lyme borreliosis is highly endemic, aimed to investigate: (1) the seasonal abundance of wild life rodents in their habitat, (2) the ecology and dynamics of the transmission of *B. burgdorferi* s.l. in woodland and fallow land habitats, (3) seasonal patterns of abundance of the immature stages of *I. ricinus* from wild rodents, (4) the prevalence of *B. burgdorferi* s.l. infections in population of ticks collected from infested rodents as well as other tick hosts, and (5) the relative importance of the observed intrinsic (species, age, sex) and extrinsic (season, year) factors in explaining changes in diversity and the dynamics of the Lyme borreliosis reservoir in nature.

MATERIALS AND METHODS

Field studies. The studies were carried out in heterogenous deciduous woodlands and neighbouring fallow lands at Urwitałt near Mikołajki (53º47.745' N, 21º39.640' E) in the Mazury Lakes district of north-eastern Poland, as part of longitudinal epizootiologic surveys conducted at monthly intervals between April and October from 1998 to 2001. The yellow-necked mice (*A. flavicollis*), bank vole (*C. glareolus*) and common vole (*M. arvalis*) were trapped with live traps in each habitat, monthly for 5 days and nights consecutively. Two traps were placed at each of 20 points along 2 transects. In the laboratory at the field station in Urwitałt, each trapped rodent was marked, identified to species level, sexed, weighed, and examined for feeding tick larvae and nymphs. Tissue samples were taken by ear punch from each ear and placed in 70% ethanol for the detection of DNA of *B. burgdorferi* s.l. The age class was established on the basis of weight and sexual development, corresponding to immature juveniles (GW1), young mature animals (GW2) and adults (GW3) [1]. For *A. flavicollis* age classes GW1, GW2, GW3 comprised respectively, mice < 20 g (less than 3.5 months old), 20-30 g (3.5-7 months), > 30 g (7 months and older). For *C. glareolus* the age classes corresponded to voles < 15 g in weight (less than 1.5 month old), 15-19.5 g (1.5-2.5 months old), > 19.5 g (2.5 months and older). For *M. arvalis* these were respectively, voles <14 g (less than 1.5 month old), 14.5-19 g (1.5-2.5 months), > 19 g (2.5 months and older). The population density in each studied habitat was estimated according to the number of mice/ voles per 1 trapping hour $\times 10^{-4}$.

Detection of *B. burgdorferi* s.l. in ticks. The genomic DNA of ticks collected from rodents was extracted by lysis in amonium hydroxide [18]. Polymerase chain reaction (PCR) was performed according to Picken *et al.* [17] using the oligonucleotide primers:

FL6b (5' TTC AGG GTC TCA AGC GTC TTG GAC T 3') and FL7b (5'GCA TTT TCA ATT TTA GCA AGT GAT G 3') in conserved regions of the *fla* gene of *B. burgdorferi* s.l. The DNA samples were initially denatured for 3 min. at 95ºC and 35 cycles performed (94ºC for 30 s, 54ºC for 45 s, 72ºC for 45 s). In each PCR assay, DNA of *B. burgdorferi* s.l. strain Bo-148 c/2 (kindly provided by dr. Joanna Stańczak from Institute of Maritime and Tropical Medicine, Gdynia, Poland) was used as the positive control, and distilled water as the negative control. All DNA samples from larvae and nymphs of *I. ricinus* were examined in pools of maximum 10 or 5 specimens, respectively.

Detection of *B. bugrdorferi* s.l. in rodent tissues. From 251 ear-tissue samples DNA was extracted using the DNA Purification Kit Wizard® Genomic for DNA. The prevalence of *B. burgdorferi* s.l. infection among sample of rodents (207 *A. flavicollis* and 44 *C. glareolus*) was determined by PCR assay using Fl6b and Fl7b primers. All samples of DNA from ear-tissues were examined separately.

Electrophoresis and imaging. The PCR products were resolved by 1.5% agarose gel electrophoresis in 1x TRIS-buffer, and visualized under UV light following ethidium bromide staining, and gels were analysed with GelDoc programme. The achieved specific amplification products of 276 base pairs (bp), both of ticks collected from rodents and rodent ear-tissue samples were considered a positive result (Fig. 1).

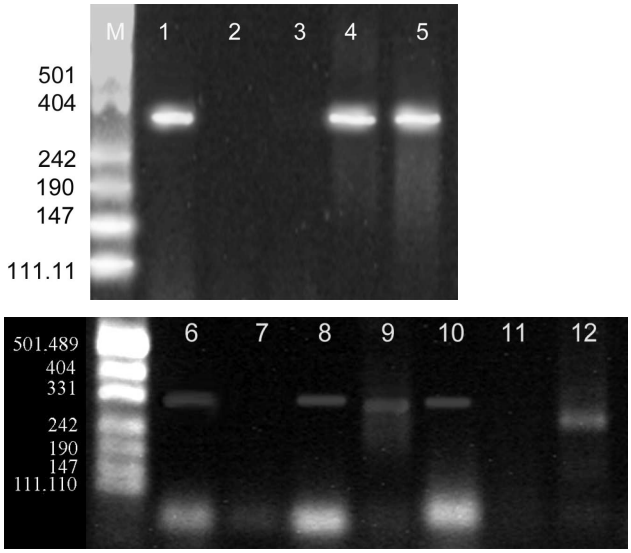

Figure 1. Ethidium bromide-stained agarose gels, showing presence of PCR-amplified *Borrelia burgdorferi* s.l. DNA extracted from larvae, nymphs of *Ixodes ricinus* and from rodent hosts. M – marker; 1, 6 – positive control; 2, 7 – negative control; 4, 5 – DNA of *Borrelia burgdorferi* s.l. in larvae *Ixodes ricinus* collected from rodents; 8, 9 – DNA of *Borrelia burgdorferi* s.l. isolated from *Apodemus flavicollis*; 10 - DNA of *Borrelia burgdorferi* s.l. isolated from *Clethrionomys glareolus*; 12 – DNA of *Borrelia burgdorferi* s.l. isolated from *Microtus arvalis*; 3, 11 – negative probes.

Data analysis and statistics. Estimates of host infestation with *I. ricinus* ticks were obtained using the following parasitological indices [5]: prevalence of infestation (percentage of hosts carrying ticks), abundance of infestation (average number of ticks per host considering the entire host population sampled). Estimates of *B. burgdorferi* s.l. infections were measured as infection prevalence (percentage of ticks or hosts infected). Prevalence of infested rodents by ticks was analysed by maximum likelihood techniques based on log linear analysis of contingency tables, implemented by the software package using Statgraphics version 7. The abundance of infestation by ticks in populations of rodents was assessed by GLIM (a statistical system for generalized linear interactive modelling) [1] with species, host age, sex season, and year taken into account.

RESULTS

Population density of rodents. A total of 1,304 wild small rodents comprising 3 species, and including 281 (21.5%), 663 (50.8%) and 360 (27.6%) of *A. flavicollis*, *C. glareolus* and *M. arvalis*, respectively, were captured between April 1998-October 2001. Table 1 summarizes the relative population densities across the 4 years of the study in 2 habitats by host species and by season of study. Relative population density varied between the 3 host species, and in relation to season of the year. Rodent numbers were generally low in spring, and although there was some variation in the density of the peak population in summer-autumn, the general pattern was quite similar across all 4 years of the study. However, *M. arvalis* from the fallow land habitat generally showed lower population densities compared to the 2 species (*A. flavicollis* and *C. glareolus*) from the woodland habitat. Bank voles, *C. glareolus*, especially showed very high population densities, 68.6 and 188.3 in the summer and autumn of 1999, respectively (Tab. 1).

Table 1. Changes in rodent population density in each studied habitat during 1998-2001.

Species/ habitat	Season	Relative population density*			
		1998	1999	2000	2001
A. flavicollis/ woodland	Spring	1.1	1.7	4.1	6.4
	Summer	2.6	11.3	11.2	35
	Autumn	5.9	78.5	5	12.5
C. glareolus/ woodland	Spring	8.6	6	9.7	6.6
	Summer	23.7	68.6	27	23.6
	Autumn	39	188.3	66	41
M. arvalis/ fallow land	Spring	1.3	5	16.5	4.8
	Summer	14	23	39	ND
	Autumn	18	ND	41	ND

* calculated as the number of rodents trapped divided by trap hours × 10^4; ND – not detected.

Table 2. Prevalence of infestation with *Ixodes ricinus* small wild rodents.

Species	No. collected	Percent of infestation	Abundance
Larvae			
A. flavicollis	2,808	92%	10 (± 1.0)
C. glareolus	2,832	76%	4 (± 0.5)
M. arvalis	734	37%	2 (± 0.1)
Nymphs			
A. flavicollis	155	10%	0.2 (± 0.10)
C. glareolus	50	6%	0.1 (± 0.01)
M. arvalis	96	20%	0.2 (± 0.02)

± = standard error of the mean.

Infestation with ticks. A total of 6,675 ticks comprising 6,374 (95.4%) larvae and 301 (4.5%) nymphs of *I. ricinus* were collected during the 4-year period. Only a few larvae and nymphs of *Dermacentor reticulates* were also found, but were excluded from the following analysis. The overall prevalence of infestation with *I. ricinus* larvae was 92%, 76% and 37% for *A. flavicollis*, *C. glareolus* and *M. arvalis*, respectively (Tab. 2). Abundance of larval infestation was higher on *A. flavicollis* than on *C. glareolus* and *M. arvalis*, but not so for nymphs. In addition, some engorged nymphs were collected from rodents that were mostly infested with larvae (Tab. 2), and where nymph infestations were detected they were generally confined to a single nymph per host (data not shown).

However, prevalence rates with the immature stages of *I. ricinus* on rodents from the woodland (*A. flavicollis* and

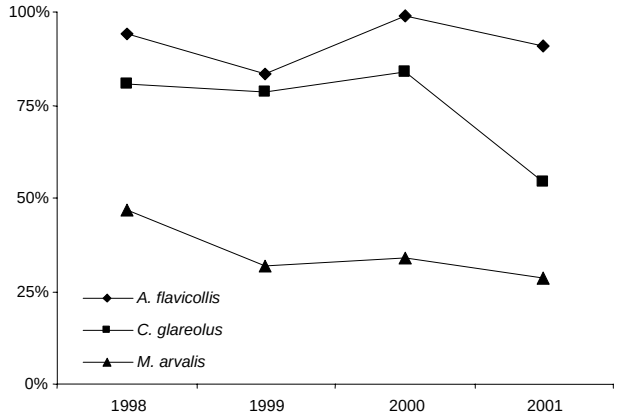

Figure 2. Prevalence of infestation rates with immature stages of *Ixodes ricinus* in *Apodemus flavicollis*, *Clethrionomys glareolus* and *Microtus arvalis*.

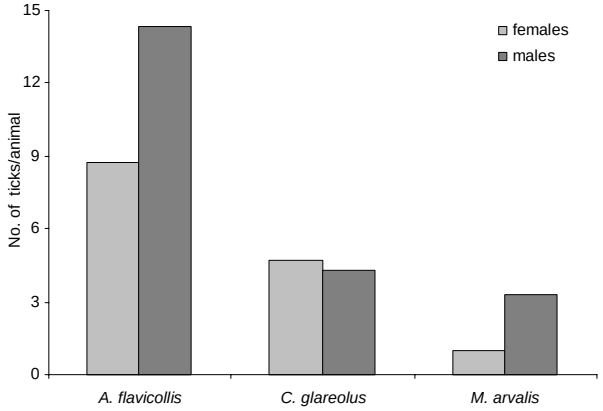

Figure 3. Abundance of infestation in *Apodemus flavicollis*, *Clethrionomys glareolus* and *Microtus arvalis* with immature stages of *Ixodes ricinus* in different sex categories.

C. glareolus) over the 4-year period of study were consistently higher than in voles from fallow land habitat (year x species: n = 1305, χ^2 =1 6.08, df = 6, p = 0.001) (Fig. 2). The mean abundance values of infestation with immature stages of *I. ricinus* varied significantly with host species and host sex. There was no sex difference in *C. glareolus*, but in both *A. flavicollis* and *M. arvalis* from the 2 contrasting habitats, abundance was higher among males (2-way interaction between host species and sex: n = 1305, $F_{4,1220}$ =1.29, p<0.001) (Fig. 3). The abundance of infestation with immature stages of *I. ricinus* also varied significantly between species of hosts in relation to host age and season of the year. The interaction arose primarily because of the very high abundance of infestation in the oldest age class of *A. flavicollis*; however for all age groups of this host species, maxima of tick infestation levels were observed in spring. For *C. glareolus*, clearly high, age dependent levels of infestation were observed both in spring and summer. The abundance of immature stages of *I. ricinus* on *M. arvalis* also varied significantly between combinations of extrinsic and intrinsic factors (3-way interaction, season x age x species; $F_{8,1154}$ = 2.54, p<0.01) and in the youngest as well as the oldest age class maximum abundance was evident in the summer, rather than spring season (Fig. 4).

Table 3. *Borrelia burgdorferi* s.l. DNA in immature stages of ticks collected from rodent hosts*.

Host species	Larvae		Nymphs	
	Positive/ examined	Percent infected	Positive/ examined	Percent infected
A. flavicollis	19/330	5.7%	2/36	5.5%
C. glareolus	20/440	4.5%	4/52	7.6%
M. arvalis	3/35	8.6%	0/0	0

* Variation in prevalence of *B. burgdorferi* s.l. across host species was not significantly different.

Table 4. *Borrelia burgdorferi* s.l. DNA of examined rodent hosts.

Host species	Host of ticks*	
	Positive/examined	Percent infected
A. flavicollis	4/163	4.5%
C. glareolus	2/59	1.2 %

* Variation in prevalence of *B. burgdorferi* s.l. across host species was not significantly different.

Prevalence of infected ticks collected from rodents and of infection in the rodent hosts. Altogether, 19 (5.7%) out of 330, 20 (4.5%) out of 440 and 3 (8.6%) out of 35 samples of DNA from larvae collected from *A. flavicollis*, *C. glareolus* and *M. arvalis*, respectively, were found to be positive for DNA of *B. burgdorferi* s.l. The infection rates in nymphs, based on DNA samples examined, were 5.5% and 7.6%, collected from *A. flavicollis* and *C. glareolus*, respectively (Tab. 3). Four (4.5%) out of 163 *A. flavicollis* and 2 (1.2%) out of 59 *C. glareolus* examined were positive for the DNA of *B. burgdorferi* s.l. (Tab. 4). Generally, it can be concluded that prevalence of infection with *B. burgdorferi* s.l., both in immature stages of ticks and in their hosts, was rather low and statistically there was no significant difference between hosts.

DISCUSSION

The primary purpose of this study was to evaluate the ecology and dynamics of *B. burgdorferi* s.l. infection, of its tick vectors, and to assess the relative importance of different species of small wild rodents as potential reservoir hosts for both the spirochetes and ticks in areas previously described as being endemic for Lyme borreliosis in north-eastern Poland [16]. Subsidiary aims included determining the seasonal abundance of wild rodents in their habitats and eventual influence of intrinsic (species, age, sex) and of extrinsic (season, year) factors on changes in the dynamics of the ticks and Lyme borreliosis reservoir in nature.

To understand fully the dynamics of tick-transmitted *B. burgdorferi* s.l. the interactions among pathogen, vectors

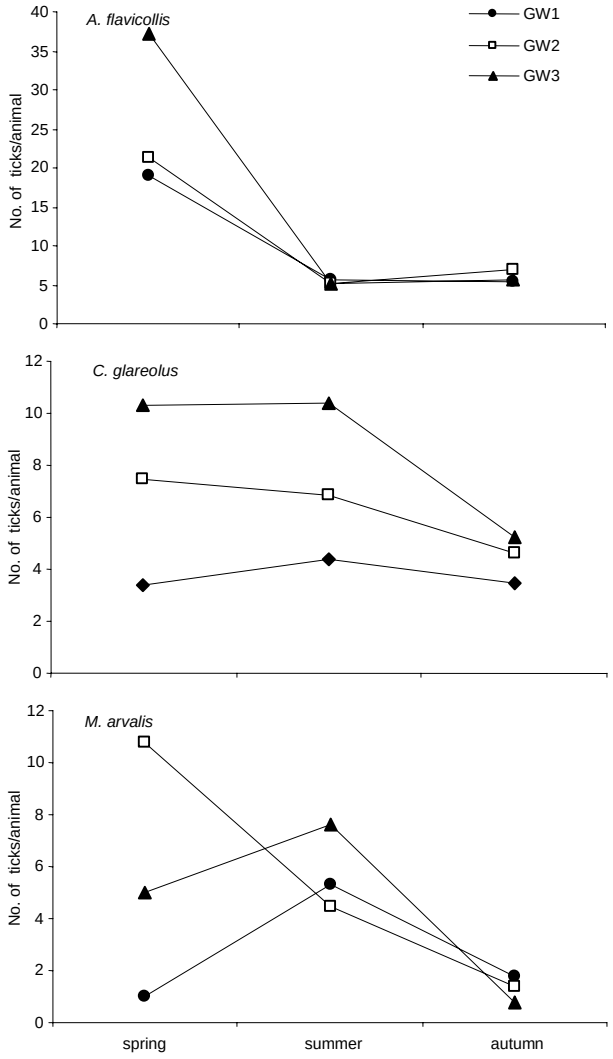

Figure 4. Abundance of infestation in *Apodemus flavicollis*, *Clethrionomys glareolus* and *Microtus arvalis* with immature stages of *Ixodes ricinus* in different seasons and ages of hosts.

and vertebrate hosts must first be explored on an ecological level, and to this end comprehensive longitudinal surveys of prevalence in wild animals are prerequisites for the implementation of appropriate prevention strategies and control of tick-borne zoonosis. However, in Poland, studies on the wild rodents involved in the epidemiology of *B. burgdorferi* s.l. and its principal vector, the *I. ricinus* ticks are rare [19, 15, 13]. The question of which species among the small wild rodents acts as the most important competent reservoir host, and is associated with the greatest local risk to humans, still remains open. This is an important consideration because the Mazury Lake district of Poland, is a popular recreational area. The wild rodents that we sampled showed high prevalence of immature ticks and thus constitute important vertebrate hosts for perpetuating the cycle of *I. ricinus* ticks, and for contaminating the very locations where visitors are likely to relax without awareness of the risk of picking up ticks. This role of rodents as a natural reservoir for larvae and nymphs is well established [7, 9, 3, 20]. It is also well known that nymphs of *I. ricinus* are responsible for transmission of spirochetes to reservoir animals, as well as to humans [21].

The data generated by this study are likely to reflect realistically the fluctuating patterns of immature *I. ricinus* tick infestations on rodents that are generated by seasonal influences, changing host species diversity, and relative host population densities in the woodland and fallow lands where we sampled. In the woodland habitat, the most frequently captured rodent species and the most abundant was *A. flavicollis*, and this was associated with a 2-fold higher abundance of immature ticks. Our results clearly revealed that larvae more frequently than nymphs infested rodent populations of *A. flavicollis* and *C. glareolus* in the woodlands, and both were comparatively rare on *A. microtus* in the fallow lands. Prevalence of infestation varied from 92% for *A. flavicollis* mice, and 76% for *C. glareolus* voles to just 37% for *M. arvalis* voles. However, the dynamics of infestation with immature stages of *I. ricinus* were highly dependent not only on the host species, but also on host sex, age and season of investigation. Moreover, the season of highest risk was spring in the case of the woodlands where yellow-necked mice and bank voles live (Fig. 4), and extended much later into the summer in the open fallow lands where common voles are encountered.

The immature stages of *I. ricinus* ticks are generalists and feed on a wide rang of vertebrates hosts, but not all of these hosts are competent for the transmission of the Lyme borreliosis spirochetes. However, the community composition of hosts of *I. ricinus* ticks is an important factor determining tick abundance and prevalence of infection with Lyme borreliosis bacteria. Some recent studies [14, 11] suggest that preservation of vertebrate biodiversity and community composition can reduce the incidence of Lyme borreliosis. The various wild rodent species, hosts of immature *I. ricinus*, may contribute to the perpetuation of Lyme borreliosis; however, the relative contribution of each species to the overall reservoir competence is likely to differ. Contrasting patterns of infestation with larvae and nymphs were observed in the woodland habitat and out in the open fallow lands. Notably, whilst *A. flavicollis* mice carried an almost 2-fold greater load of immature *I. ricinus* compared with *C. glareolus*, and 5-fold greater than those on *M. arvalis*, the percentage of *Borrelia* positive DNA samples prepared from larvae feeding on these former 2 species was half that of DNA samples prepared from larvae feeding on *M. arvalis* from the fallow land habitat. Pertinently, an earlier study [5] reported that the prevalence of infestation with immature ticks and with *B. burgdorferi* s.l. in *A. flavicollis* and *C. glareolus* was similar to the results presented in this paper, therefore our values for prevalence on these rodent hosts concur with other studies.

Rodent populations in woodland habitats are characterized by high tick burdens but low reservoir competence,

and by relatively high population densities. It is possible that *C. glareolus* acts as a species that is able to reduce the risk of exposure to the *Borrelia* bacteria by a dilution effect of the more competent reservoir host, *A. flavicollis*, which shares the same habitat, and that this facet of the ecology and community composition of rodent hosts that live sympatrically in similar habitats may have an important bearing on the dynamics of transmission of *B. burgdorferi* s.l. spirochetes to feeding *I. ricinus* ticks. Any reduction in the relative density of *A. flavicollis* in relation to other hosts, therefore, should reduce the risk of human exposure to immature ticks and hence to *B. burgdorferi* s.l. spirochetes.

CONCLUSION

The abundance of the immature *I. ricinus* vector, and spirochete-carrying rodent host species inhabiting woodland or open fallow lands in the Mazury Lake district of Poland varies markedly, with the consequence that each rodent species makes its own characteristic contribution to the natural reservoir of *B. burgdorferi*: *A. flavicollis* in the woodland habitats is the most important host for the larvae *per se*, while *M. arvalis* from the open fallow lands carries the highest percentage of infected ticks.

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