The ecology of *Bartonella* spp. infections in two rodent communities in the Mazury Lake District region of Poland

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SUMMARY

Prevalence and abundance of *Bartonella* spp. infections were studied over a 3-year period in woodland and grassland rodents in North-Eastern Poland. Prevalence of bacterial infections was similar in the two rodent communities, with one leading host species in each habitat (46·3% in *Apodemus flavicollis* versus 29·1% in *Myodes glareolus* in forest, or 36·9% in *Microtus arvalis* versus 13·7% in *Mi. oeconomus* in grassland). Prevalence/abundance of infections varied markedly across the 3 years with 2006 being the year of highest prevalence and abundance. Infections were more common during autumn months in *My. glareolus* and *A. flavicollis*, and in juvenile and young adult (age classes 1 and 2) *My. glareolus* and *Mi. oeconomus* than in adults (age class 3). Higher prevalence and abundance of *Bartonella* infections were found in male *A. flavicollis* in comparison to females. These data are discussed in relation to the parasite genotypes identified in this region and with respect to the role of various ecological factors influencing *Bartonella* spp. infections in naturally infected host populations.

Key words: *Bartonella*, ecology, *Myodes glareolus*, bank vole, *Apodemus flavicollis*, yellow-necked mouse, *Microtus arvalis*, common vole, *Microtus oeconomus*, root vole, Poland.

INTRODUCTION

Bartonella spp. are vector-borne bacteria associated with numerous emerging infections in humans and animals (Breitschwerdt and Kordick, 2000). *Bartonella* spp. typically parasitize the erythrocytes of mammalian hosts, resulting in long-lasting infections (Seubert *et al*. 2002). Diverse *Bartonella* species/strains have been isolated recently from a wide range of wild mammals, including rodents (Kosoy *et al*. 1997; Heller *et al*. 1998; Hofmeister *et al*. 1998; Birtles *et al*. 2001; Holmberg *et al*. 2003; Tea *et al*. 2004; Jardine *et al*. 2005; Knap *et al*. 2007). In Europe, the most common small mammal species in rural environments are bank voles (*Myodes glareolus*), field voles (*Microtus agrestis*), common voles (*Mi. arvalis*), wood mice (*Apodemus sylvaticus*), yellow-necked mice (*A. flavicollis*) and common shrews (*Sorex araneus*) and these have been identified as reservoirs of many microparasites, including *Bartonella* spp. but also *Anaplasma phagocytophilum*, *Borrelia burgdorferi* and *Babesia microti*, all of which are also important pathogens of humans and domesticated animals (Healing, 1981; Birtles *et al*. 1994; Bown *et al*. 2004; Pawełczyk *et al*. 2004; Siński *et al*. 2006; Bray *et al*. 2007; Welc-Falęciak *et al*. 2008*b*). Different groups of arthropods (fleas, lice, sandflies) act as vectors for *Bartonella* spp. Among rodent *Bartonella* spp., fleas are well-recognized vectors (Bown *et al*. 2004) but transmission by the tick *Ixodes ricinus* has also been suggested recently (Cotté *et al*. 2008).

There are relatively few studies on the ecology of *Bartonella* spp. in naturally infected hosts (Birtles *et al*. 2001; Kosoy *et al*. 2004; Telfer *et al*. 2007*a*). Rodents constitute very good models for such studies because of the high heterogeneity and dynamics of their populations, facilitating investigations on the contribution of a range of quantifiable intrinsic and extrinsic factors to the cause of the patterns of variation observed in the field. Each rodent community is subdivided into different functional subgroups including for example settled, territorial adults of both sexes and mobile juveniles. The role of these subgroups as hosts for *Bartonella* spp. is not known, nor has the precise contribution of unpredictable external factors (i.e. temperature, humidity) that create complex unique temporary combinations of environmental effects been quantified comprehensively. Little is currently known about the long-term dynamics of *Bartonella* in host populations/communities, because of the limited number of systematic long-term ecological studies in naturally infected hosts (Kosoy *et al*. 2004). In our preliminary studies in N.E. Poland, we demonstrated that here wild rodents harbour 2 *Bartonella* species, including *B. grahamii* – a species associated with human illness (Welc-Falęciak *et al*. 2008*a*), and were generally

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heavily infested with parasitic arthropods, especially fleas and immature stages of the tick *I. ricinus* (Pawełczyk, 2003; Welc-Falęciak *et al.* 2008*b*).

The aims of the present study were (1) to evaluate the stability of *Bartonella* infections in 2 rodent communities of 4 species by monitoring infections over a 3-year time period and (2) to evaluate the influence of selected ecological factors (host sex and age; season and year of study) on the infection levels in these rodent populations. Improved knowledge of the ecology of these parasites in naturally infected rodent populations will result in a better understanding of their epidemiology and transmission opportunities in nature.

MATERIALS AND METHODS

Field studies

Small rodents were live-trapped during, at minimum, 5 consecutive nights in the breeding season (May–September) in the years 2004–2006 in 2 habitats: bank voles (*Myodes glareolus*) and yellow-necked mice (*Apodemus flavicollis*) in forest sites, common voles (*Microtus arvalis*) and root voles (*Microtus oeconomus*) in abandoned fallow agricultural land. Our 3 forest sites were located in Mazury in the North-Eastern corner of Poland, close to the towns of Mikołajki, Ryn and Pisz – named 'Urwitałt', 'Tałty' and 'Pilchy', respectively. The study sites, including a map, were fully described by Behnke *et al.* (2001). These 3 sites were chosen on the basis of their similar habitat quality (managed mixed forests). As no significant differences in prevalence/abundance of *Bartonella* were found among the sites, all the sampled animals were treated as a 'woodland community'. Fallow land was located close to the field station at Urwitałt, on the east side of Lake Łuknajno and near one of our forest sites. This grassland habitat consists of small dry hillocks (up to 5 m of elevation) and wet lower terrain with small temporary ponds, creating 2 kinds of microhabitats. The dry territory is inhabited mainly by common voles and the damp surroundings of the ponds, by the root voles. Forest species were sampled during 3 seasons (spring [May and June], summer [July/August] and autumn [September]) in the year 2004 and during 2 seasons (spring [May and June] and autumn [September]) in the years 2005–2006. *Microtus* spp. were caught only during the summer period (July/August) in each year. Rodents were live trapped in wooden traps that were inspected twice daily, and processed according to the procedures described in detail by Bajer *et al.* (2001).

Sampling of hosts

At the field station in Urwitałt, all the animals were inspected, identified, sexed, relevant morphometric data were recorded and they were weighed (to the nearest 0·5 g). Ectoparasites (ticks) were removed mostly from ears and limbs and the fur was inspected carefully for fleas. After inspection most animals were live-sampled, marked and released as near as possible to the original site of capture, whilst others (approx. 35%) were bled by cardiac puncture under terminal (ether) anaesthesia.

Ageing of rodents

Three age classes were established on the basis of body weight (Morris, 1972) and sexual development, supported with dry lens weight (when available), corresponding to immature juveniles (age class 1), young mature animals (age class 2) and adults (age class 3) (see Behnke *et al.* 2001; Bajer *et al.* 2002).

Blood collection and DNA extraction

Thin blood smears were prepared from drops of blood taken from the tail vein or heart. Blood smears were air-dried, fixed in absolute methanol and stained for 1 h in Giemsa stain in buffer at pH 7·2. Each smear was examined under oil immersion. From the sacrificed animals, 200 μl of whole blood were collected into 0·001 M EDTA and frozen at a temperature of −20 °C. From the live-sampled individuals a few drops of blood were collected from the tail vein into 200 μl of 0·001 M EDTA and frozen. From individuals that were found dead in the trap, the whole heart was isolated and homogenized in 400 μl of 0·001 M EDTA. For this group of animals (12%), blood smears were not obtained and diagnosis for *Bartonella* infection was done exclusively on the basis of PCR. For the remaining 88% of samples both microscopical analysis of stained blood smears and DNA amplification were used. A strong correlation between the results of these two methods was obtained by the Fisher exact test (only 3% of discrepancy, in favour of PCR). Genomic DNA was extracted from whole blood or heart homogenates using AxyPrep MiniPrep Blood kit (AxyGen, USA) and stored at a temperature of −20 °C. The extracted DNA was subjected to a PCR with the primers CS140f (Birtles and Raoult, 1996) and BhCS1137n (Norman *et al.* 1995), targeting a specific fragment of the gene encoding the enzyme citrate synthase (*gltA*) (Birtles and Raoult, 1996). The PCR was incubated at 95 °C for 2 min to denature genomic DNA and the thermal cycle reaction programmed for 40 cycles of 1 min at 95 °C, 1 min at 54 °C and 2 min at 72 °C, with a final extension step of 7 min at 72 °C. PCR products were subjected to electrophoresis on a 1·5% agarose gel and stained with ethidium bromide.

Prevalence (percentage of animals infected) was estimated based on microscopical observations and

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the PCR, and values are reported with the 95% confidence limits (given in brackets in the text), calculated by bespoke software based on the tables of Rohlf and Sokal (1995). Abundance of infection was estimated as the number of *Bartonella* spp. infected erythrocytes/200 fields of vision at × 1000 magnification. In cases when the samples were only positive by PCR (7% of all cases), an intensity of 0·001 infected erythrocytes/200 fields of vision was implemented into quantitative statistical analysis. Quantitative data are reported as geometric means (GM means) of all animal in a given subset (infected and non-infected, as defined Margolis *et al.* 1982), with 95% confidence limits calculated as described by Elliott (1977).

Statistical analysis

Prevalence of infection was analysed by maximum likelihood techniques based on log linear analysis of contingency tables, implemented by the software package, SPSS v. 14. Prevalence of infection as a binary factor (infected = 1, not infected = 0) and then habitat (2 levels), host species (4 levels, *My. glareolus*, *A. flavicollis*, *Mi. arvalis* and *Mi. oeconomus*), year (3 levels), host age (3 levels), host sex (2 levels), season (3 levels, only for forest species) were entered as factors. Beginning with the most complex model, involving all possible main effects and interactions, those combinations not contributing significantly to explaining variation in the data were eliminated stepwise, beginning with the highest-level interaction.

A minimum sufficient model was then obtained, for which the likelihood ratio of χ^2 was not significant, indicating that the model was sufficient in explaining the data. The analysis of prevalence of infection was first conducted for all 4 host species together, and when host species appeared as a significant main effect, the analysis was repeated for each host species separately.

In an additional set of analyses, flea and tick prevalence (as a binary factor: infested = 1, not infested = 0) were entered as factors. However, as no significant correlations resulted from that analysis, these data are not presented in this paper.

Quantitative data reflecting parasite abundance within hosts were expressed as geometric means (GM) because the data were highly overdispersed (Elliott, 1977; Dash *et al.* 1988). These means reflect the abundance of infection as defined by Margolis *et al.* (1982) and include all subjects within the specified group, infected and not infected, for which relevant data were available. Parasite abundance was analysed by multifactorial GLM using models with normal errors after normalization of the data by log10 (x + 1) transformation (Crawley, 1993; Wilson and Grenfell, 1997). The same factors as those used for analysis of prevalence were employed for analysis of abundance, implementing the approach of step-wise backward simplification of models by removal of non-significant terms.

RESULTS

Rodent communities

Overall a total of 1100 rodents were sampled over the 3-year period and of these 74·5% were woodland rodents (of which 80·2% were bank voles, 19·8% were yellow-necked mice) and 25·5% were grassland rodents (of which 53·2% were common voles, 46·8% were root voles). The structure of the sampled host communities/population by year, season, host species and sex is summarized in Table 1. The dynamics of relative densities of hosts are presented in Table 2. During the period of study, prevalence of bacterial infections was similar in the two rodent communities (woodland and grassland rodents – 32·5% and 26·1%, respectively) with 1 leading host species in each habitat (46·3% in *A. flavicollis* versus 29·1% in *My. glareolus*, or 36·9% in *Mi. arvalis* versus 13·7% in *Mi. oeconomus*) (Table 3).

Prevalence and abundance of Bartonella *spp. in four host species*

Overall prevalence and abundance values, across the 3 years of the study by host species and by host sex, are presented in Table 3. The effect of host species, year, sex and age was analysed in a dataset comprising 1085 animals. In the resulting minimal sufficient model, the influence of year of study on *Bartonella* spp. prevalence was evident (year × *Bartonella* prevalence: $\chi^2_2 = 10{\cdot}2$, $P = 0{\cdot}006$): 2006 was the year of highest prevalence (33·6% [27·6%–40·3%]) and the lowest number of infected rodents was observed in 2005 (27·3% [22·5%–32·7%]).

Also the influence of interaction of host species and sex on prevalence was marked (Table 3) (host species × sex × *Bartonella* prevalence: $\chi^2_3 = 9{\cdot}3$, $P = 0{\cdot}025$; goodness of fit for model: $\chi^2_{92} = 110{\cdot}9$, $P = 0{\cdot}088$). Prevalence of *Bartonella* spp. was highest in *A. flavicollis* (46·3% [37·7%–55·2%) and lowest in *Mi. oeconomus* (13·7% [9·0%–20·1%]). The multifactorial GLM revealed a strong main effect of host species on the abundance of *Bartonella* spp. ($F_{3, 951} = 11{\cdot}5$, $P < 0{\cdot}001$). As earlier in the analysis of prevalence, abundance was much higher in mice (geometric mean [GM] = 4·66 [3·33–6·41]) in comparison to other host species (Table 3) and lowest in root voles (GM = 0·37 [0·00–0·87]). Because of the strong influence of host species on both infection parameters, the analysis was repeated for each host species separately, beginning with full factorial models incorporating year, and season of study (for forest species), host sex and age as factors.

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Table 1. The structure of the sampled host populations by year, season, host species and sex in the 3-year period of study

Host species	Year of study/and season	2004			2005			2006			Total		
		M	F	Σ	M	F	Σ	M	F	Σ	M	F	Σ
Myodes glareolus	Spring	2	4	6	12	15	27	8	20	28	22	39	61
	Summer	42	31	73	nd	nd	nd	nd	nd	nd	42	31	73
	Autumn	38	24	62	73	70	143	168	151	319	279	245	524
	Total	82	59	141	85	85	170	176	171	347	343	315	658
Apodemus flavicollis	Spring	0	2	2	4	3	7	6	3	9	10	8	18
	Summer	24	19	43	nd	nd	nd	nd	nd	nd	24	19	43
	Autumn	21	12	33	28	18	46	15	7	22	64	37	101
	Total	45	33	78	32	21	53	21	9	31	98	63	162
Microtus arvalis	Summer	23	30	53	26	28	54	16	26	42	65	84	149
Microtus oeconomus	Summer	1	1	2	31	36	67	37	25	62	69	62	131

nd, Not done.
M – males; F – females.
Σ – total.

Table 2. Dynamics of relative densities of hosts

Host species	Year of study/ and season	2004	2005	2006
Myodes glareolus	Spring	2·4	18·5	20·7
	Summer	34·5	nd	nd
	Autumn	95·7	181·6	50·7
Apodemus flavicollis	Spring	0·8	6·1	7·5
	Summer	11·0	nd	nd
	Autumn	37·5	77·4	37·9
Microtus arvalis	Summer	34·8	26·4	17·1
Microtus oeconomus	Summer	1·2	33·5	26·3

nd, Not done.

Dynamics of Bartonella *spp. infection in* My. glareolus

This dataset consisted of 643 bank voles sampled during the 3-year period. There was only 1 significant term in the minimal sufficient model (goodness of fit: $\chi^2_{77} = 58{\cdot}7$, $P = 0{\cdot}928$). Infection rates with *Bartonella* spp. varied markedly in relation to year (year × *Bartonella* prevalence: $\chi^2_2 = 20{\cdot}2$, $P < 0{\cdot}001$) with the highest noted prevalence in 2006 (36·4% [31·2%–42·0%]) compared to the previous years of study (17·7% [12·2%–24·9%] and 24·1% [17·1%–32·7%] in 2004 and 2005, respectively).

Season was not a significant component of the minimum sufficient model but nevertheless some variation was noted. *Bartonella* spp. infections were identified in 21·3% [13·7%–31·7%] voles in spring; in 15·1% [7·8%–25·5%] voles in summer and in 32% [29·2%–35·5%] of individuals in autumn. Likewise, host age did not achieve statistical significance and was not a component of the minimum sufficient model, but some pertinent trends were evident. The highest prevalence of *Bartonella* spp. was noted in juveniles (35·8% [27·7%–44·6%] in age class 1 voles) in comparison to 2 older age classes (29·7% [25·7%–33·9%] and 24·6% [20·6%–29·2%] in age class 2 and 3, respectively).

Multifactorial GLM generated a simple model for the abundance of *Bartonella* spp. with, again, the only significant factor being the main effect of year ($F_{2,551} = 5{\cdot}87$, $P = 0{\cdot}003$). The highest GM was noted in 2006 (GM = 1·38 [0·77–2·22]) compared to the previous years of study (2004 – GM = 0·60 [0·07–1·38]; 2005 – 0·80 [0·26–1·58]).

Dynamics of Bartonella *spp. infection in* A. flavicollis

This dataset consisted of 162 yellow-necked mice sampled over the 3-year period. In the analysis of the prevalence of *Bartonella* spp., sex contributed to 2 significant terms in the minimal sufficient model (goodness of fit: $\chi^2_{74} = 41{\cdot}0$, $P = 0{\cdot}999$). A higher infection rate was found in male mice compared with females during each season but the extent of the difference between the sexes varied from season to season (Fig. 1A; season × sex × *Bartonella* prevalence: $\chi^2_2 = 6{\cdot}26$, $P = 0{\cdot}044$). Overall, prevalence of *Bartonella* spp. was 5 times higher in autumn in comparison to spring (54·5% [47·4%–61·2%] *vs* 11·8% [2·1%–35.0%]). In each of the 3 years prevalence was higher in male mice (Fig. 1B) and also in the pooled dataset (54·5% [40·6%–67·7%] *vs* 33·3% [23·7%–44·3%] but the discrepancy between the sexes varied and was least in 2006 (year × sex × *Bartonella* prevalence: $\chi^2_2 = 6{\cdot}77$, $P = 0{\cdot}034$).

Multifactorial GLM generated a simpler model for the abundance of *Bartonella* spp. with 2 main

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Table 3. Overall prevalence and abundance of *Bartonella* spp. in rodent host (all host species and all years combined)

		Prevalence			Abundance		
Species	Host sex	% Infected	95% CL	*N*	Geometric Mean	95% CL	*N*
Woodland		***32·5***	***29·0–36·3***	***805***	***1·62***	***1·30–1·98***	***703***
Myodes glareolus	Males	28·5	23·7–33·9	340	0·96	0·74–1·21	292
	Females	29·7	25·1–34·8	303	0·93	0·70–1·19	259
	Combined	29·1	26·0–36·4	643	1·12	0·84–1·44	551
Apodemus flavicollis	Males	54·5	40·6–67·7	99	7·55	4·12–13·29	91
	Females	33·3	23·7–44·3	63	2·05	0·63–7·71	61
	Combined	46·3	37·7–55·3	162	4·66	3·33–6·41	152
Grassland		***26·1***	***21·8–30·9***	***280***	***1·06***	***0·66–1·56***	***246***
Microtus arvalis	Males	33·8	24·1–45·0	65	2·35	1·22–4·05	60
	Females	39·3	27·3–52·0	84	1·52	0·76–2·61	78
	Combined	36·9	29·1–45·4	149	1·86	1·15–2·79	138
Microtus oeconomus	Males	10·3	4·6–18·5	69	0·30	0·03–0·63	54
	Females	18·0	10·6–27·6	62	0·46	0·16–0·84	54
	Combined	13·7	9·0–20·1	131	0·37	0·00–0·87	108

N, number of animals examined.

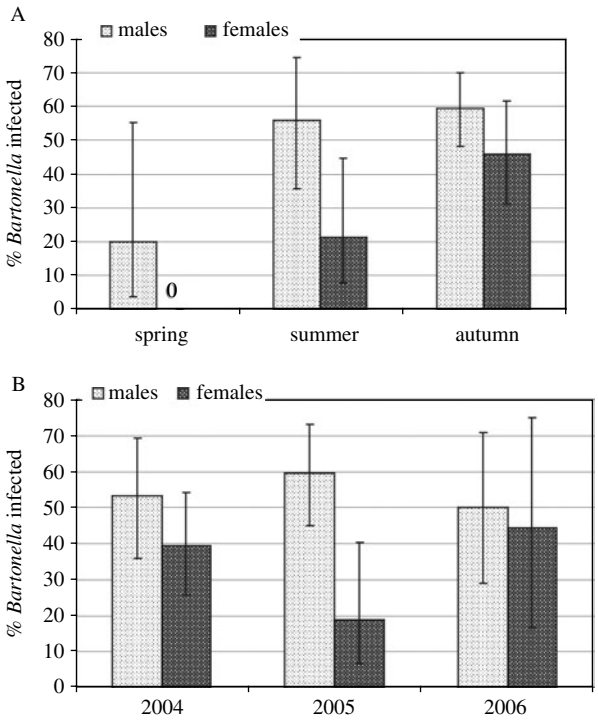

Fig. 1. (A) The effect of host sex and season of study on *Bartonella* spp. prevalence in yellow-necked mouse (*Apodemus flavicollis*). (B) The effect of host sex and year of study on *Bartonella* spp. prevalence in yellow-necked mouse (*A. flavicollis*).

effects (year of study and host sex). The geometric mean number of *Bartonella*-infected red blood cells was higher in 2006 (GM = 47·2 [12·8–175·60] *vs* 2·90 [0·53–8·95] and 1·33 [0·09–3·97] than in 2004 and 2005, respectively, as for prevalence (main effect of year on *Bartonella* abundance: $F_{2,152} = 8{\cdot}64$, $P < 0{\cdot}001$). Markedly higher abundance was found in males (GM = 5·98 [3·51–17·11]) compared to females (GM = 2·30 [0·00–3·68]) (main effect of sex on *Bartonella* abundance: $F_{1,152} = 8{\cdot}87$, $P = 0{\cdot}003$). Host age did not affect *Bartonella* infection in the yellow-necked mouse population. Prevalence of *Bartonella* spp. infection varied in the range 27·3% [12·6%–50·0%] – 50% [37·4%–62·6%] among the 3 age classes.

Dynamics of Bartonella *spp. infection in* Mi. arvalis

This dataset consisted of 149 common voles trapped during the 3-year period. In this host species, neither the analysis of prevalence nor the analysis of abundance revealed any significant terms. The prevalence remained similar in consecutive years of study (2004 – 41·5% [31·7%–51·8%]; 2005 – 37·0% [27·7%–47·3%]; 2006 – 31·0% [17·0%–47·5%]). There was little difference between the two sexes (Table 3) and 3 age classes (age class1 – 25·0% [9·0%–50·0%]; age class 2 – 34·4% [21·0%–52·8%]; age class 3 – 35·2% [23·8%–48·3%]). However, a reversed trend was observed with respect to abundance. The overall highest GM number of *Bartonella*-infected erythrocytes was noted in age class 1 during the 3 years of study but this was not significant.

Dynamics of Bartonella *spp. infection in* Mi. oeconomus

This dataset consisted of 129 root voles trapped during the 2-year period (only 2 years of study were analysed because only 2 root voles were trapped in

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Fig. 2. The effect of host age and year of study on *Bartonella* spp. prevalence in the root vole (*Microtus oeconomus*).

year 2004; Table 1). In the analysis of prevalence of *Bartonella* spp., none of the initially fitted extrinsic or intrinsic factors significantly affected this infection parameter. Prevalence across age classes varied slightly with the prevalence being higher in the youngest animals (age class 1, 2 and 3 – 27·3% [7·9%–59·6%], 13·0% [6·4%–21·6%] and 12·5% [7·1%–21·2%], respectively) and between sexes (10·3% [4·7%–19·6%] in males, 18·0% [10·9%–27·9%] in females).

Multifactorial GLM generated a simple model for the abundance of *Bartonella* spp. with 2 main effects (year of study and host age) and an interaction of year of study with host age. A higher geometric mean number of *Bartonella* infected erythrocytes was noted in 2006 (1·41 [0·57–2·71]) than in 2005 (0·36 [0·03–0·82]) (main effect of year on *Bartonella* abundance: $F_{1,108}=4{\cdot}75$, $P=0{\cdot}032$). Abundance of infection in juvenile root voles was nearly 8 times higher than in the 2 older age classes (age class 1 – 2·55 [0·77–6·10]; age class 2 – 0·33 [0·06–0·68] and age class 3 – 0·26 [0·00–0·63]) (main effect of age on *Bartonella* abundance: $F_{2,108}=3{\cdot}94$, $P=0{\cdot}023$). The same pattern of age-related variation was found during the 2 years of study (year × host age × *Bartonella* abundance: $F_{2,108}=3{\cdot}36$, $P=0{\cdot}039$) although in 2006 in age class 1 mean abundance was up to 9 times higher in comparison to similar values in age class 2 and 3 (Fig. 2).

Host sex did not affect *Bartonella* infection in root voles (Table 3).

DISCUSSION

The results reported in this paper, based on a 3-year ecological study in the Mazury Lake District region of Poland, are consistent with earlier work (Bajer *et al*. 2001) and extend that study by establishing the relative importance of the 4 host species as reservoirs of *Bartonella* spp. The hosts were considered as falling into 2 ecological communities, one associated with the forests and one with open fallow grassland and, in each of these 2 communities, the prevalence and abundance of *Bartonella* infection was high in relation to many other published reports (Engbaek and Lawson, 2004; Tea *et al*. 2004). Moreover, in each habitat one host species dominated as the more heavily infected; yellow-necked mice in the forest habitat and common voles on fallow land. Both parameters (prevalence and abundance) were the lowest in root voles, and to our knowledge this is a new host record for *Bartonella*.

Overall, prevalence of *Bartonella* spp. in the Mazury Lake District estimated both on the basis of blood-smear examination or DNA amplification was remarkably high (45%). Prevalence in each rodent community was higher than reported from central Sweden for the same and related rodent species (17% in *A. flavicollis*; 24% in *A. sylvaticus*; 15% in *My. glareolus* and 1 infected of 3 tested for *Mi. agrestis;* Holmberg *et al*. 2003). Prevalence in our study was also higher than reported from small mammal communities in Denmark (28% in *Mi. agrestis*, *A. flavicollis*, *A. sylvaticus* and *Sorex vulgaris*; Engbaek and Lawson, 2004), Greece (31% in 7 species, including *A. flavicollis*; Tea *et al*. 2004), and comparable to that reported in Slovenia (40% in *A. flavicollis*, *A. sylvaticus*, *A. agrarius* and *My. glareolus*; Knap *et al*. 2007) and the UK (64%; Birtles *et al*. 2001), where molecular methods were used to assess the presence of infection. High prevalence of *Bartonella* infection in naturally infected rodent populations is caused most likely by persistent or long-term bacteraemia, and/or by high incidence rates of infection (Kosoy *et al*. 1997; Birtles *et al*. 2001). Our microscopy-based epizootiological studies in rodents revealed that the vast majority of *Bartonella* infections occur with low bacteraemia, thus suggesting that these infections are in the chronic and persistent phase. In an experimental study, injection of *B. birtlesii* into BALB/c mice induced a long-lasting bacteraemia of 5 to 8 weeks duration p.i. (Boulouis *et al*. 2001). Given the results presented in this report and the fact that the average life span of wild rodents is about 3–4 months in nature (Pucek *et al*. 1970), it is likely that once acquired, infections persist until the end of the rodent's life. However, there are also strong indications that re-infection can take place under natural conditions and there is evidence for exchange of different species/genotypes in a single individual (Birtles *et al*. 2001).

Between-year variation was well marked for *Bartonella* spp. in bank and root voles as well as in yellow-necked mice but was less evident in common voles. Between-year dynamics of infection had been observed in bank voles during our earlier study (Bajer *et al*. 2001). In contrast, in a recent study in the UK no effect of year on *Bartonella* spp. prevalence was found in common shrews (Bray *et al*. 2007). These contrasting patterns are probably caused by fluctuations in relative population densities of both

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hosts and vectors (fleas) and resulting alterations in transmission efficiency. The relatively high prevalence of *Bartonella* infections in bank and common voles and in yellow-necked mice in 2006 may be linked to the higher flea infestations of rodents in 2006 (84%) compared with 2004 (34%) (own data, not published). On the other hand, the positive association between host densities and parasite distribution has been noted in other ecological studies on rodent microparasites (Begon *et al*. 1999; Bajer, 2008).

In our study the prevalence of *Bartonella* infections in bank voles and yellow-necked mice varied among seasons, increasing from spring to peak in autumn. Under the climatic conditions in N.E. Poland, only a small proportion of animals survive the winter period, and these serve as a source of infection for newly-born cohorts later in the season, among which infections spread in the naïve young animals. A similar pattern was found by Turner (1986), Bajer *et al*. (2001) and Telfer (2007*a*, *b*) and most likely explained by the increase in flea densities from winter to summer (Bown *et al*. 2004). Here, the spring to autumn increase in prevalence of *Bartonella* spp. was paralleled by flea infestations with the highest level of infestation noted in autumn (68%) in comparison to spring (56%) and summer (33%) (Bajer *et al*., unpublished data).

Host age played a more important role in the ecology of *Bartonella* infections than host sex. There was an indication of an age effect on prevalence and abundance of *Bartonella* spp. in bank voles and a similar tendency for such an effect in root voles. In both cases, the infections were more common and more intense in the 2 youngest age classes (1 and 2) compared with age class 3 suggesting that age-dependent immunity may be a feature of this host-parasite relationship. A similar age-dependent pattern was found by Healing (1981), Pawełczyk *et al*. (2004) and Holmberg *et al*. (2003) but not by Young (1970), Bajer *et al*. (2001) and Bray *et al*. (2007). As juvenile rodents are more mobile than territorial adults, they may spread the infections between different populations in the local ecosystem.

Fichet-Calvet *et al*. (2000) suggested that *Bartonella* infection may be self-limiting and immunizing, such that older animals that have cleared their earlier infections, remain immune to further infections. On the other hand there is evidence for re-infection in individual rodents (Birtles *et al*. 2001), and this may be a consequence of infection by different species of *Bartonella* in sequence. The high *Bartonella* prevalence in young rodents may be a result of more efficient horizontal transmission among naive youngsters or it may be a result of vertical transmission between females and their litters. Isolations of these bacteria from the fetuses of rodents (white-footed mouse and cotton rats) suggest that transmission of *Bartonella* spp. *in utero* may occur among natural hosts (Kosoy *et al*. 1998).

The significance of rodents as a reservoir of pathogens of public health interest depends on the parasite genotypes that actually occur in naturally infected populations. Based on earlier comparative sequence analysis of the citrate synthase gene (*gltA*) fragment, which is commonly used for the genotyping of *Bartonella*, a considerable heterogeneity exists among *Bartonella* isolates derived from these rodent populations (Welc-Falęciak *et al*. 2008*a*). During our preliminary genotyping studies 2 *Bartonella* species were found – *B. taylorii* and *B. grahamii*. *B. grahamii* has been reported earlier from the ocular fluids of a patient with neuroretinitis, suggesting that these bacteria can be a cause of pathological changes in humans (Kerkohoff *et al*. 1999) but the infectivity and pathogenic potential of *B. taylorii* in humans remains unknown.

This study represents the first comprehensive analysis of the *Bartonella* infection in 2 rodent communities comprising 4 species of hosts in N.E. Europe, and for the first time including *Mi. oeconomus*. To our knowledge, this is also the first ecological study on the dynamics of natural infections in wildlife that took account of the range of intrinsic and extrinsic factors known to influence *Bartonella* infection, and the dynamics of relative host densities. This 3-year field study conducted in 2 contrasting habitats confirmed that, overall, *Bartonella* spp. are prevalent in rodent hosts in N.E. Poland, despite between-year, spatial and seasonal variation. In consequence, there is likely to be widespread dissemination of these bacteria in the local ecosystem, where human encroachment is currently increasing rapidly because of the expanding local populations and increasing numbers of summer visitors that are attracted to this part of Europe by the opportunities for aquatic and outdoor leisure activities.

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