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Factors affecting the component community structure of haemoparasites in common voles (*Microtus arvalis*) from the Mazury Lake District region of Poland

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Abstract The prevalence and abundance of infections with haemoparasites were studied over a 4-year period in *Microtus arvalis* (common vole, $n = 321$) sampled from fallow grassland sites in north-eastern Poland. Total species richness was five (prevalence = *Haemobartonella* sp. 63.9%, *Bartonella* spp. 27.7%, *Babesia microti* 9.0%, *Trypanosoma* sp. 8.4% and and *Hepatozoon lavieri* 3.1%) with 76.9% of the voles carrying at least one species and a mean infracommunity species richness of 1.1. Variation in species richness was determined primarily by season and year, the interaction of these factors, and that of year with host age. The observed frequency distribution of infracommunity species richness did not differ from that predicted by a null model, suggesting that there were no marked associations between the species. Analyses of prevalence and abundance of infection with each species in turn, revealed that overall the principal causes of variation were temporal and seasonal, their interaction, and interactions with intrinsic factors (age and sex), the latter playing only a minor role in their own right. However, the relative importance of these combinations varied and was distinct for each of the species in the study. Prevalence data revealed eight sets of two- and three-way associations between species, mostly dependent to some extent on one of the intrinsic and extrinsic factors in the model. Analysis of quantitative associations suggested two sets of positive two-way interactions, none of which remained after controlling for the effect of extrinsic and intrinsic factors on the abundance of each species. These data are discussed in the context of the changing ecological profiles in this region of Eastern Europe and, in a wider context, in relation to current understanding of the factors that shape component community structures of haemoparasites in wild rodents.

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Introduction

Wild rodents play an important role in nature as reservoir hosts for many pathogens, including some that can be transmitted to other animals including humans. Infections with haemoparasites are widespread in wild rodents and are dependent on zoonotic cycles that also involve arthropod vectors. Our understanding of the transmission cycles involved is still incomplete, particularly in respect of the key factors that regulate transmission. In contrast to helminth communities, haemoparasites of small wild rodents have received relatively little attention in Europe and published studies have largely reported on rodent communities in Western and Northern Europe (Baker et al. 1963; Baker 1974; Krampitz 1981; Birtles et al. 1994). In this respect Central and Eastern Europe have been relatively neglected, but there is now growing interest in the occurrence of haemoparasites in wild rodents from Eastern Europe (Karbowiak and Siński 1996a, 1996b; Karbowiak et al. 1999) and our earlier studies in the Mazury Lake District region of Poland have drawn attention to the high prevalence of both haemoparasites and *Cryptosporidium* spp. in the dominant woodland rodents of the region (Paweczyk and Sinski 2000; Bajer et al. 2001, 2002).

Recently we demonstrated that in woodland and meadow habitats of northern Poland, the rodent species *Apodemus flavicollis*, *Clethrionomys glareolus* and *Microtus arvalis* are important hosts for two groups of parasitic arthropods: fleas and mites, especially immature stages of *Ixodes ricinus* (Pawelczyk 2003). The level of infestation in small rodents was very high. Fleas

(*Siphonaptera*), ticks (*Ixodida*) and many mesostigmatic mite species constitute an abundant and frequent component of ectoparasite communities of rodents in Central and Eastern Europe (Siński and Paweczyk 1999, Stanko and Miklisova 2002). They are major vectors of various diseases and infest a wide range of hosts (Balashov 1995) with important consequences for transmission and epidemiology of relevant species of haemoparasites among rodent populations. Our previous investigations in woodland habitats in the Mazury Lake District region of Poland have shown that haemoparasitic infections are frequent among bank voles and we documented the relative importance of intrinsic and extrinsic factors in structuring these communities (Bajer et al. 2001).

In contrast to woodland rodents, those inhabiting the open meadows have been largely ignored. However, in a second phase of our long-term study of haemoparasitic component communities of wild rodents in Poland, we collected data on haemoparasites of the common vole (*M. arvalis*), which is numerically the dominant rodent species inhabiting the open meadows adjoining our woodland study sites. In this paper we evaluate the role of host age and sex (intrinsic factors), and year and season (extrinsic factors), and the interactions between these factors, in structuring the component community of haemoparasites in *M. arvalis* and influencing both prevalence and abundance of each of the species involved.

Materials and methods

Study sites

Our study site was located east of the nature reserve surrounding Lake Luknajno, and north of Lake Sniardwy, near the town of Mikolajki in the Mazury Lake District region of north-eastern Poland. Trapping was conducted in open grassland sites, located close to our woodland study sites (Bajer et al. 2001). The former were intensively cultivated with cereals until 1991, and then abandoned and have lain fallow ever since. The dominant species of plants were: couch grass (*Agropyron repens*), hare's foot (*Trifolium arvense*), daisy fleabane (*Erigeron canadensis*) and several species of grasses. Among taller vegetation were motherworts (*Artemisia vulgaris* and *A. absynthium*) and young silver birches (*Betula verucosa*) (Bajer 2002).

Collection of mice

Rodents were caught live in locally constructed wooden traps, with a small interior metal platform which, when triggered, released a metal door. These were set out at 15-m intervals in parallel lines, 10 m either side of tracks running across the grassland. Trapping sessions comprised a minimum of 4 days, with traps being inspected in the early morning and just before dusk. Fresh traps replaced any traps containing animals and the animals were brought to the University of Warszawa's field station at Urwitalt. Visits to the study sites varied, but generally comprised at least a 4-day duration, at approximately 4-week intervals from late March until mid-November. For both practical and animal welfare reasons, it was not possible to visit the sites in the period between November and March because the ground was mostly frozen hard and deep snow covered our sites for most of the winter period. The months between March and November were divided into three seasons, comprising spring (March–early June), summer (late June–August) and autumn (September–November).

Sampling of hosts

At the field station in Urwitalt, all animals were inspected, identified, sexed, relevant morphometric data were recorded and they were weighed (to the nearest 0.5 g). Ectoparasites were removed from the ears and limbs (mostly larval and nymph ticks and some mites) and the fur was inspected carefully for fleas. Thin blood smears were prepared from drops of blood taken from the tail vein. After inspection most animals were marked and released as near as possible to the original site of capture, whilst others were killed for recovery of endoparasites (data to be published elsewhere).

Blood smears were air-dried, fixed in absolute methanol, stained for 45 min in Giemsa's stain (diluted 1:3) in buffer at pH 7.2 and were brought back to the Department of Parasitology at the University of Warszawa for examination. Each smear was examined under oil immersion (using an Olympus AX70 microscope). Initially sufficient fields of vision were examined to enable up to 50 leukocytes to be inspected for presence of *Hepatozoon lavieri* (approximately 200 fields of vision under a ×100 objective lens). Each field of vision was also examined for the presence of other species, although these were not quantified at this stage. If the blood smear revealed the presence of other species, during this initial phase of examination, an additional 1,000 erythrocytes were inspected and the number of cells infected with *Bartonella* spp., *B. microti* and *Haemobartonella* sp. were recorded and expressed as no. infected cells/100 erythrocytes. The concentration of trypanosomes was also expressed per 100 erythrocytes.

Age classes were established on the basis of weight (Morris 1972) and sexual development as described by Adamczewska-Andrzejewska (1973). Age class 1 comprised immature, juvenile voles < 15 g in weight and approximately < 1.5 months old. Age class 2 comprised young mature voles, 15–19.5 g in weight representing animals approximately 1.5–2.5 months old. Age class 3 comprised adult voles, weighing > 19.5 g, ≥ 2.5 months old.

Statistical analysis

The frequency distribution of infracommunity species richness was tested for goodness-of-fit to the positive binomial distribution (assumption of the null model is a regular distribution), the Poisson distribution (assumption of the null model is a random distribution) and to the null model of Janovy et al. (1995) (assumption of the null model is that, in the absence of associations and interactions between species, the frequency distribution of infracommunity species richness is predicted by prevalence values). Goodness-of-fit in each case was tested by χ^2.

Prevalence (percentage of animals infected) was analysed by maximum likelihood techniques based on log-linear analysis of contingency tables, implemented by the software package, Statgraphics version 7. For each species in turn we entered prevalence of infection as a binary factor (infected = 1, not infected = 0) and then year (four levels: 1997, 1998, 1999 and 2000), season (three levels: spring, summer and autumn), host age (three levels) and host sex (two levels) as factors. Beginning with the most complex model, involving all possible main effects and interactions, those combinations not contributing significantly to an explanation for 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 statistical analysis is presented in the legends, where significant interaction terms are given alongside relevant figures, but it is important to remember that these represent tests of the individual terms in the minimum sufficient model simplified from the full factorial model that initially comprised four factors (year, season, age and sex) and the infection term.

Quantitative data reflecting parasite abundance within hosts were expressed as geometric means because the data were highly overdispersed (Elliott 1977; Dash et al. 1988). In some cases arithmetic means and SEMs are also provided. 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. The degree of aggregation in quantitative data was calculated by the index of dispersion (I; the variance to mean ratio) and the index of discrepancy (D) as described by Poulin (1993; a value of 0 indicates an even distribution of counts across all hosts and a value of 1 indicates all parasites aggregated in a single host). Frequency distributions of individual species were also tested for goodness-of-fit to negative binomial, positive binomial and Poisson models by χ^2 as described by Elliott (1977) and the negative binomial exponent (k) is given as appropriate.

Parasite abundance was analysed by generalized linear interactive modelling (GLIM 4, PC version, Royal Statistical Society 1993) as described previously, using models with normal errors after normalization of the data by log10($x+1$) transformation (Crawley 1993; Wilson and Grenfell 1997; Behnke et al. 1999).Year, season, host age and host sex (see above for levels) were entered as factors. For models with normal errors the change in deviance is divided by the scale parameter and the result divided by the change in *df* following each deletion, to give a variance ratio, F. All models were carefully scrutinised and only the significant terms and relevant F-values are presented in the legends to figures or text. The residuals from all models were checked for approximately normal distribution.

Quantitative associations between parasites were examined by multiple correlation analysis (Spearman rank-order correlation test) of raw parasite data from animals carrying both of two species, in each of the possible two-way combinations (excluding *H. lavieri* because of very low prevalence of this species). To avoid the risk of type I errors we adopted the Dunn-Sidak correction, lowering the cut-off value of P, according to the number of comparisons implemented (Sokal and Rohlf 1981).

We also examined the correlations between the residuals from minimum sufficient three-, two- or one-way ANOVAs, as appropriate for individual species, in order to control for established differences between the years, seasons, host age and sex.

Finally, we carried out a principal components analysis (Statgraphics version 7) on the standardized residuals from each of the five ANOVAs.

Results

M. arvalis

A total of 321 *M. arvalis* were sampled over a period of 4 years. The structure of the sampled host population by year, host sex and age is summarized in Table 1. Host density was estimated to enable some comparison of vole populations between the 4 years of the study (Table 2). Vole numbers were generally low in spring, peaking in most years in August–September and although there was some variation in the density of the peak population in summer–autumn, the general pattern was very similar across all 4 years of the study.

Total species richness in the component community

Five species of blood parasites were recorded (Table 3) and 247 voles (76.9%) carried at least one of these species. *Haemobartonella* sp. was the most common and *H. lavieri* the rarest.

Frequency distribution of infracommunity species richness

The observed distribution of infracommunity species richness, illustrated in Fig. 1, conformed to the negative binomial and did not differ significantly from that predicted by the null model for interactions of parasite species in an assemblage (Janovy et al. 1995).

Table 1 The structure of the sampled *Microtus arvalis* population by year of capture, host sex and age

Year	Sex	Age			Totals by	
		1	2	3	Sex	Year
1997	Male	10	10	13	33	
	Female	12	3	12	27	
	Combined	22	13	25		60
1998	Male	8	8	9	25	
	Female	9	9	19	37	
	Combined	17	17	28		62
1999	Male	13	13	34	60	
	Female	12	14	27	53	
	Combined	25	27	61		113
2000	Male	11	8	16	35	
	Female	12	12	27	51	
	Combined	23	20	43		86
Total by age		87	77	157		
Overall total						321

Table 2 Estimation of population density of *M. arvalis* in the study site. *ND* Not determined

Month	Relative population density[a]			
	1997	1998	1999	2000
March	2.4	ND	ND	ND
April	0.6	1.6	2.5	ND
May	0.5	1.1	7.4	15.1
June	6.4	0	ND	17.8
July	10.0	22.6	19.4	ND
August	14.8	5.4	30.1	39.4
September	10.4	22.6	ND	41.0
October	12.0	13.1	ND	ND
November	12.4	ND	ND	ND

[a]Population density was calculated as the number of voles trapped/trap hours$\times 10^4$

Table 3 Overall prevalence of infection with haemoparasites in male and female common voles

Species	Males	Females	Combined
Haemobartonella sp.	61.4	66.1	63.9
Bartonella spp.	26.8	28.6	27.7
Babesia microti	9.8	8.3	9.0
Trypanosoma sp.	9.8	7.1	8.4
Hepatozoon lavieri	2.0	4.2	3.1

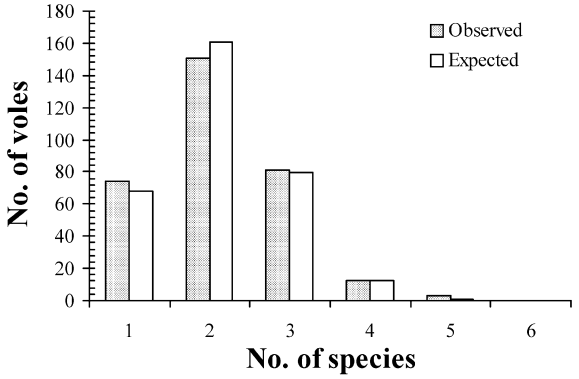

Fig. 1 Frequency distribution of infracommunity species richness. The predicted data are those predicted by the null model of Janovy et al. (1995) (comparison of these distributions gave $\chi^2 = 3.5$, $df = 4$, $P > 0.05$). Test of goodness-of-fit of the observed data to the negative binomial gave $\chi^2 = 4$ ($df = 3$, $P =$ NS), to the Poisson $\chi^2 = 32.1$ ($df = 4$, $P > 0.001$), and to the positive binomial distribution $\chi^2 = 10.66$ ($df = 3$, $P = 0.014$)

Mean infracommunity species richness

The overall mean number of species of blood parasites harboured per host was 1.1 ± 0.05 ($\pm$SEM), with a variance to mean ratio of 0.625 suggesting a positive binomial distribution, although as indicated above (and Fig. 1) a test for goodness-of-fit revealed a significant discrepancy. There was a strong seasonal effect (Fig. 2A) generated by the increase in mean infracommunity species richness from spring, through the summer to autumn, and a weaker year effect, but neither was entirely predictable and the significant interaction with year arose because of the unexpected drop in autumn in mean species richness in 1997. Figure 2B shows that mean species richness declined with increasing age in 1998 and 2000, while in 1997 and 1999 mean species richness increased with host age. Therefore season-dependent changes in mean species richness were apparent and similar in 3 years but not entirely predictable (exception in 1997) and there were changing patterns in respect of host age across the 4 years of the study.

Prevalence of species

Table 3 summarizes the overall prevalence of each of the five species detected in the study by host sex and combined. All prevalence data were analysed further by maximum likelihood methods, testing respectively models for each of the five species in relation to the four quantified factors (year, season, host sex and age). Tests of the significance of the individual effects (interactions with infection), together with overall goodness-of-fit of the complete minimum sufficient models are summarized in the legends to Figs. 3 and 4.

H. lavieri was the least prevalent species in the study, but showed significant variation in relation to year (Fig. 3A) with higher prevalence in 1999 and 1997 compared with the other years. The age×season×infection interaction (data not shown) arose because with the exception of one infected vole in age group 1, none of the other voles sampled in spring ($n = 35$) showed infection. The remaining nine infected voles were sampled in summer ($n = 212$) and autumn ($n = 74$) but in summer five were age cohort-3 animals, whereas in autumn three/four infected voles were in age cohort 1 and the remaining animal in age cohort 3.

The prevalence of *Trypanosoma* sp. was dependent on year: in 1997 prevalence was 20%, more than twice the value recorded in other years (Fig. 3B). To a lesser extent prevalence also varied in relation to host age and season (the season×age×infection interaction, data not shown). No infections were recorded in spring. Among age cohorts 1, 2 and 3 prevalence was 4%, 4.9% and 12.4% in summer and 5.6, 27.3 and 0% respectively in autumn. There was no significant difference in prevalence between the sexes (Table 3).

Bartonella spp. were rare in the spring (prevalence = 5.7%) but more common in the summer and autumn months (prevalence = 30.7 and 29.7%, respectively; the season×infection interaction in the legend to Fig. 3C). Interestingly the relationships with age also varied between years. In 1998 and 2000 prevalence declined with age, whereas in 1997 and 1999 there was no clear pattern to age-related prevalence (Fig. 3C, the year×age×infection interaction).

B. microti infections varied markedly with year and season (Fig. 4A), a pattern generated through the declining prevalence of this species in the summer months with successive years, and the unusually high prevalence in spring and autumn in 1998. The weaker interaction with season and sex arose because in spring and autumn prevalence was higher among females, whereas in the summer months males showed higher prevalence (Fig. 4C, Table 4).

Although *Haemobartonella* sp. generated a highly significant interaction in relation to year and season (Fig. 4b), the only consistent trend apparent was a declining prevalence in summer across the years of the study. In other seasons prevalence varied unpredictably from year to year. The season×sex interaction (Fig. 4D) is mainly attributable to the very low prevalence among male voles in spring (one of 13 voles carried the parasite), but otherwise prevalence showed little variation between the sexes (range 56.8–70.2%).

Frequency distribution of parasite burdens among infracommunities

The extent of aggregation of each of the parasite species was examined by three tests as summarized in Table 5. The three species (*H. lavieri*, *Trypanosoma* sp. and *B. microti*) showing the lowest prevalence, all comprised highly aggregated data sets best described by the negative binomial. *Bartonella* spp. did not conform to the negative binomial, although the discrepancy with other

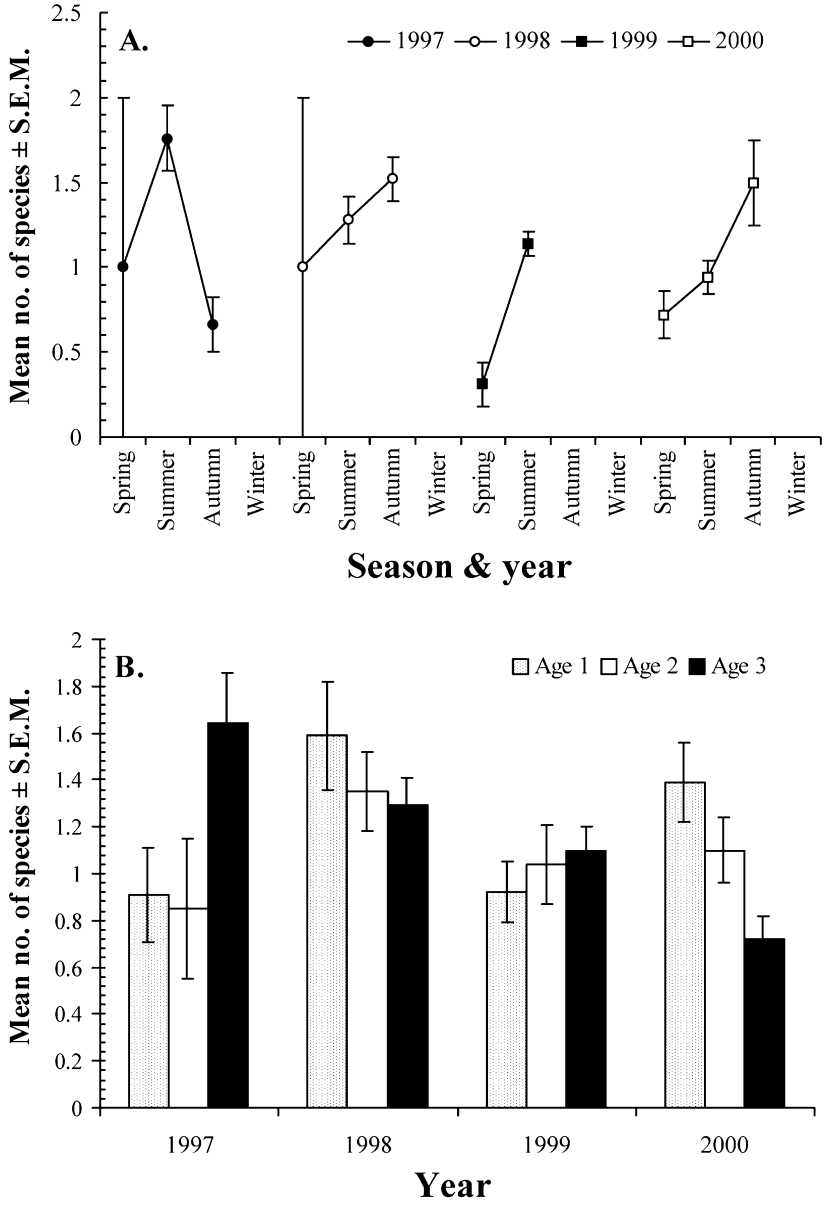

Fig. 2 A Annual and seasonal changes in mean infracommunity species richness. (Four-way ANOVA in generalized linear interactive modelling with normal errors and year, season, host age and sex as factors, main effect of season = $F_{2,314} = 6.72$, $0.005 > P > 0.001$, main effect of year = $F_{3,315} = 2.65$, $0.05 > P > 0.025$; interaction between year and season = $F_{5,295} = 3.9$, $0.005 > P > 0.001$.) **B** Variation in mean infracommunity species richness between age classes and across the 4 years of the study (interaction between age and year = $F_{6,296} = 2.27$, $0.05 > P > 0.025$). Other terms in the model were not significant and the residuals were distributed normally

distributions was greater (Poisson $\chi^2 = 117.3$, $P < 0.0001$; positive binomial $\chi^2 = 123.1$, $P < 0.0001$). Similarly *Haemobartonella* sp. did not conform to any of the distributions tested (Poisson $\chi^2 = 29.6$, $P = 0.001$; positive binomial $\chi^2 = 38.4$, $P = 0.0001$).

Abundance of infection among infracommunities

Too few animals were infected with *H. lavieri* to merit quantitative analysis.

Analysis of *Trypanosoma* sp. revealed much the same as prevalence data with a marked variation between years, the heaviest infections being detected in 1997 (geometric mean = 1.78/1,000 red blood cells), and lowest in 2000 (geometric mean = 0.38/1,000 red blood cells). There was also a significant effect of age, but this was confounded by a season×age interaction (Fig. 5A). No voles were infected in spring, infections were first evident in the summer and by the autumn infections were most intense in the age class 2, when older voles were again without infection.

The overall prevalence of *B. microti* was similar to *Trypanosoma* sp. (Table 3) but quantitative analysis revealed only a complicated picture with variation between all four factors (year, season, sex and age). We have illustrated only the main effects in Fig. 5B. As with prevalence, the abundance of infection was generally higher in 1998, and interestingly the heaviest infections were found in the oldest voles, but clearly this pattern was confounded by the influence of host sex and season of the year (data not shown).

Haemobartonella sp. showed strong seasonal and year-related variation in abundance (Fig. 5C). As with

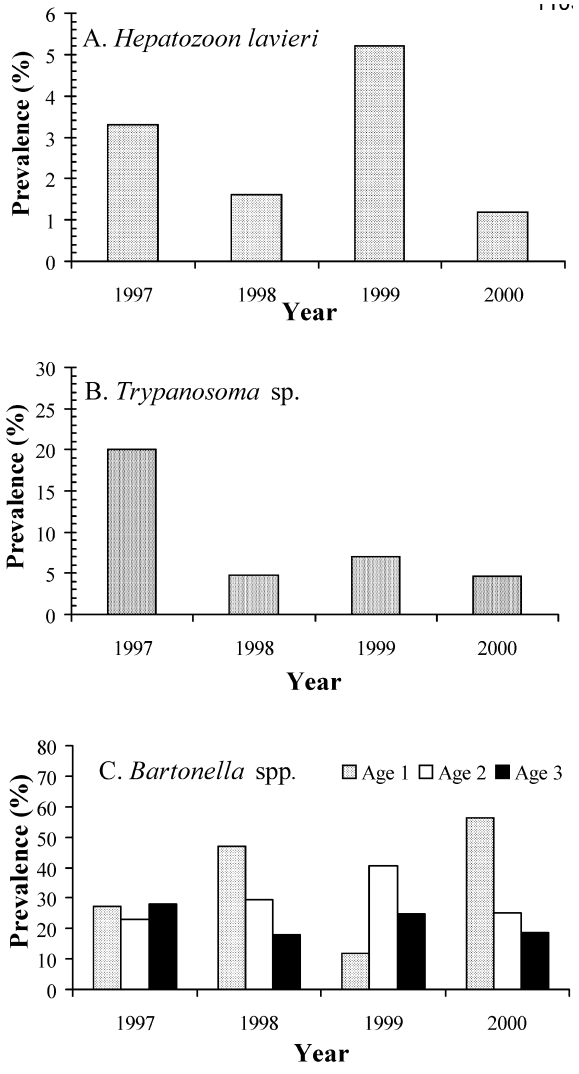

Fig. 3 Prevalence of **A** *Hepatozoon lavieri*, **B** *Trypanosoma* sp. and **C** *Bartonella* spp. across the 4 years of the study and by host age (*Bartonella* spp. only). A, minimum sufficient model included a year×infection interaction ($\chi^2 = 9.73$, $df = 3$, $P = 0.021$) and season×age×infection interaction ($\chi^2 = 11.7$, $df = 4$, $P = 0.02$, data not shown but see text) and the goodness-of-fit of the model gave $\chi^2 = 51.7$ ($df = 114$, $P = 1.0$). **B** Minimum sufficient model included a year×infection interaction ($\chi^2 = 9.5$, $df = 3$, $P = 0.022$) and season×age×infection interaction ($\chi^2 = 9.5$, $df = 4$, $P = 0.05$, data not shown but see text) and the goodness-of-fit of the model gave $\chi^2 = 69.75$ ($df = 114$, $P = 0.9996$). **C** Minimum sufficient model included a year×age×infection interaction ($\chi^2 = 15.5$, $df = 6$, $P = 0.017$) and season×infection interaction ($\chi^2 = 13.06$, $df = 2$, $P = 0.002$, data not shown but see text) and the goodness-of-fit of the model gave $\chi^2 = 83.5$ ($df = 106$, $P = 0.95$)

prevalence, the geometric mean abundance of *Haemobartonella* sp. declined in the summer months with successive years of the study, but varied unpredictably in spring and autumn months (Fig. 5C, and the significant year×season interaction).

Quantitative analysis of *Bartonella* spp. yielded only significant interaction terms and these are illustrated in Fig. 6. In general, abundance of infection increased in most age groups from very low levels in spring, but there was a notable exception in 1997. Later in the season changes were not predictable, with declining or rising abundance in different age groups depending on year of study.

Associations between parasites based on category data

Interactions between parasites should be reflected in co-occurrence or exclusion of species. We carried out a log-linear analysis entering data on the parasites (presence of infection/absence of infection) and three of the other factors (year, host age and sex). A model that included season did not converge satisfactorily. Therefore, our model incorporated eight terms in total and this is summarized in Table 4. Eight of the 12 terms in this model were based on combinations of two or three parasites and seven were context dependent involving various combinations of extrinsic and intrinsic factors. We have illustrated only the two strongest combinations in the model and the only three-way interaction not dependent on either intrinsic or extrinsic factors.

Figure 7A shows that in 1998 and 2000 the prevalence of *Haemobartonella* sp. was very similar among voles irrespective of whether they harboured *B. microti* or not. However, in 1997, *B. microti* -infected animals were more likely to be also infected with *Haemobartonella* sp. whereas in 1999 only voles without *B. microti* carried *Haemobartonella* sp.

The four-way interaction shown in Fig. 7B is based on year, *Trypanosoma* sp., *Bartonella* spp. and *Haemobartonella* sp. and is more complex. The prevalence of *Haemobartonella* sp. across the 4 years of the study remained high in voles infected with *Bartonella* spp. irrespective of whether they carried *Trypanosoma* sp.(66.7–100%) or not (64–83.3%). In contrast among voles without *Bartonella* spp., prevalence varied from 0 to 100%. This variation was greatest among voles with *Trypanosoma* sp. (0–100%) and somewhat more limited among those without *Trypanosoma* sp. (39.5–83.9%).

The three-way interaction between *B. microti*, *H. lavieri* and *Bartonella* spp. (Fig. 7C) arose because voles with *Bartonella* spp. and *B. microti* were more likely to be infected with *H. lavieri* than among the other three combinations. Additionally (data not shown) among voles without *B. microti*, the prevalence of *Bartonella* spp. was higher when *H. lavieri* was also absent (29.0% vs. 11.1%, respectively). In contrast among voles with *B. microti* the prevalence of *Bartonella* spp. was higher when *H. lavieri* was present (100% vs. 17.8%). The remaining interactions incorporating at least two species are weaker and we have not attempted to interpret these further.

Interactions between species based on quantitative data

Interactions between species should be reflected in quantitative associations in hosts carrying both species.

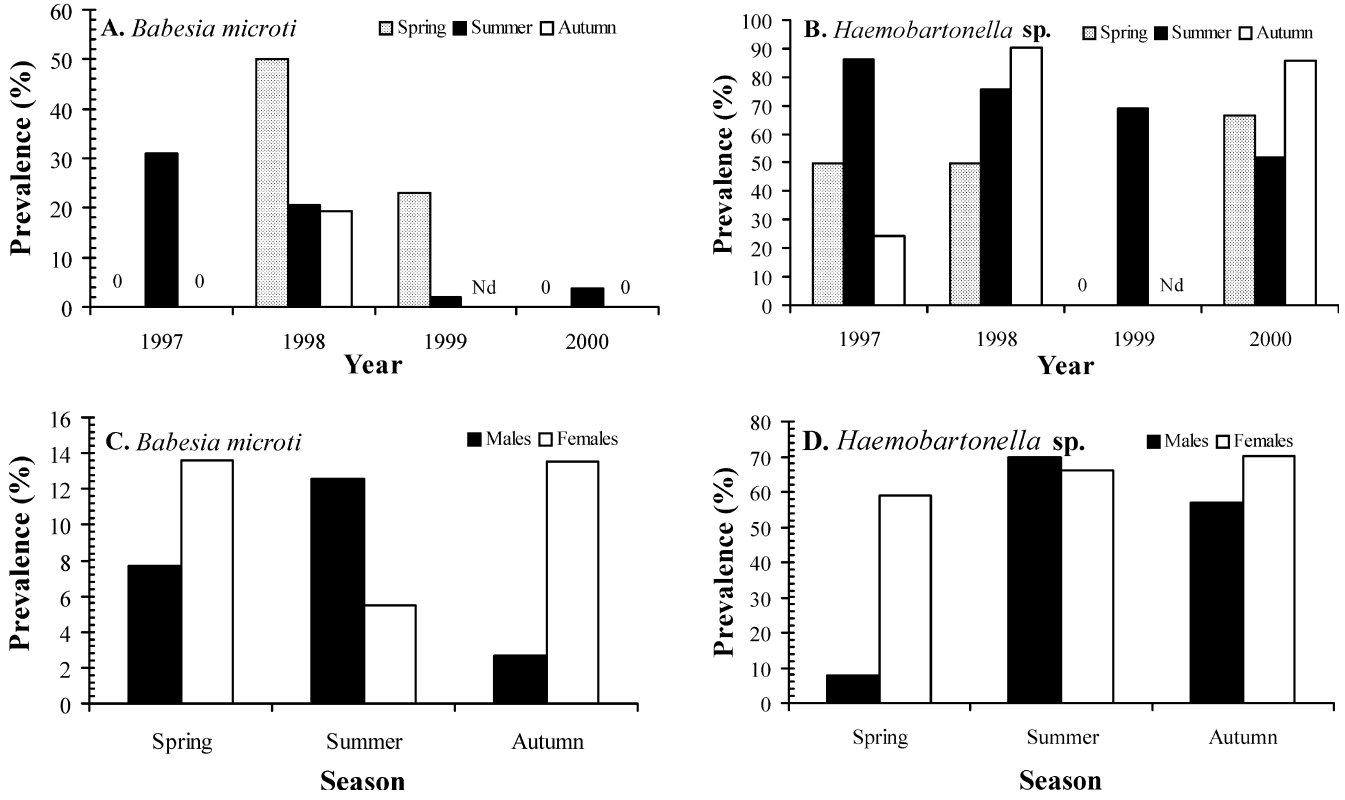

Fig. 4A–D Prevalence of *Babesia microti* and *Haemobartonella* sp. in relation to various combinations of extrinsic and intrinsic factors. **A** Variation in prevalence of *B. microti* across seasons and years of the study. The minimum sufficient model included a year×season×infection interaction ($\chi^2 = 16.9$, $df = 6$, $P = 0.01$). **B** Variation in prevalence of *Haemobartonella* sp. across seasons and years of the study. The minimum sufficient model included a year×season×infection interaction ($\chi^2 = 51.32$, $df = 6$, $P < 0.0001$). **C** Variation in the prevalence of *B. microti* by host sex and across the seasons. The minimum sufficient model included a season×sex×infection interaction ($\chi^2 = 6.54$, $df = 2$, $P = 0.038$). Goodness-of-fit of the whole model gave $\chi^2 = 69.1$ ($df = 108$, $P = 0.9987$). **D** Variation in the prevalence of *Haemobartonella* sp. by host sex and across the seasons. The minimum sufficient model included a season×sex×infection interaction ($\chi^2 = 10.89$, $df = 2$, $P = 0.004$). Goodness-of-fit of the whole model gave $\chi^2 = 79.62$ ($df = 108$, $P = 0.982$)

Table 4 Measures of aggregation for haemoparasites in *Microtus arvalis*. *k* Negative binomial exponent, *I* index of dispersion = variance to mean ratio, *D* index of discrepancy (Poulin 1993)

Species	$k \pm$SEM	*I*	*D*
Haemobartonella sp.	1.78[a] ±0.360	2.383	0.577
Bartonella spp.	0.189[b] ±0.028	8.084	0.865
Babesia microti	0.029[c] ±0.005	50.145	0.970
Trypanosoma sp.	0.170[d] ±0.071	1.592	0.931
Hepatozoon lavieri	0.010[e] ND	45.475	0.987

[a]Goodness-of-fit to the negative binomial distribution $\chi^2 = 20.1$, $df = 5$, $P = 0.005$
[b]Goodness-of-fit to the negative binomial distribution $\chi^2 = 13.8$, $df = 5$, $P < 0.025$
[c]Goodness-of-fit to the negative binomial distribution $\chi^2 = 1.4$, $df = 2$, $P =$ NS
[d]Goodness-of-fit to the negative binomial distribution $\chi^2 = 0.1$, $df = 2$, $P =$ NS
[e]Goodness-of-fit to the negative binomial distribution test—calculation not possible

We first carried out a multiple correlation analysis on abundance data of four of the species in the study using data only from animals that carried both species of each pair (Table 6; excluding infection with *H. lavieri* because only seven animals carried this species and voles with both *B. microti* and *Trypanosoma* sp. because only three animals carried this particular combination). Because of the possible risk of type I errors, we implemented the Dunn-Sidak correction and accordingly this allowed acceptance of just one of the five possible associations as significant, i.e. that between *Haemobartonella* sp. and *Bartonella* spp. (Fig. 8). Interestingly only one other association approached significance.

Some of these relationships could have arisen as a consequence of differences in parasite burdens attributable to year, season, host age and sex effects. To control for possible bias arising out of the factors known to affect abundance of parasites (various combinations of intrinsic and extrinsic factors), we next carried out a multiple correlation analysis on the residuals of each species, following minimum sufficient ANOVAs as described in the preceding section. Two associations had high correlation coefficients, but because of the Dunn-Sidak correction, we could not accept these as significant (Table 6, Fig. 8B).

In a further approach we carried out a principal components analysis (PCA) on the standardized residuals from each of the five ANOVAs. This gave

Table 5 Minimum sufficient maximum likelihood statistical model incorporating three quantified factors and all haemoparasitic species detected in the study. *Each row* represents a significant interaction that therefore forms part of the final model. *Hsp Haemobartonella* sp., *Bd Bartonella* spp., *Bm Babesia microti, Tsp Trypanosoma* sp., *Hl Hepatozoon lavieri*

Principal interactions in explaining variation in data		Parasites	Test of individual effects		
Extrinsic factors	Intrinsic factors		χ^2	*df*	*P*[d]
	Sex[c]	Bm, Tsp, Bd	6.20	1	0.0128
Year[a]		Tsp, Bd, Hsp	13.99	3	0.0029
Year	Sex	Bm, Bl	11.87	3	0.0078
		Bm, Hl, Bd	8.81	1	0.0030
	Age[b]	Tsp, Hsp	7.13	2	0.0282
	Age, sex	Bd	6.95	2	0.0310
Year		Hl, Hsp	11.50	3	0.0093
Year		Hl, Tsp	10.18	3	0.0171
Year		Bm, Hsp	23.04	3	<0.0001
Year	Age	Hsp	24.44	6	0.0004
Year	Age	Bd	20.84	6	0.0020
	Age	Hl	6.50	2	0.0388
Goodness-of-fit of the minimum sufficient model specified by the interactions listed above			116.2	653	1.0[e]

[a]Year—four levels (1997, 1998, 1999, 2000)
[b]Age—three levels (age cohorts 1, 2 and 3)
[c]Sex—two levels (male and female)
[d]Probability that excluding the effect will make a significant change to the model
[e]Probability that the data does not differ significantly from the minimum sufficient model described by the interactions

approximately similar amounts of variation on each axis (PCA 1 = 24.7, PCA 2 = 21.0, PCA 3 = 19.4, PCA 4 = 18.4%, PCA 5 = 16.5). Hence no further reduction of the data was possible.

Discussion

This study focused on the factors that regulate the structure of component communities of haemoparasites in the European common vole, *Microtus arvalis*. Building on earlier, mainly descriptive studies from Central and North-Eastern Europe (Sebek 1978; Karbowiak and Sinski 1996a, 1996b), it follows our comprehensive analysis of haemoparasitic infections in bank vole (*C. glareolus*) populations living in the woodlands adjoining our study sites in the Mazury Lake District region of Poland (Bajer et al. 2001).

The haemoparasites of *M. arvalis* comprised five species of which *Haemobartonella* sp. (more recently on the basis of molecular evidence reclassified as a mycoplasma; Neimark et al. 2001, 2002) was clearly the dominant parasite because prevalence throughout the seasons and across all 4 years of the study was highest with this organism. Although infections with *Haemobartonella* sp. have been reported from *C. glareolus* (Bajer et al. 2001), to our knowledge this parasite has not been reported previously from *Microtus* spp. *H. lavieri* was the least abundant species with just 3.1% of sampled voles infected. Four of the species we recorded are known to infect *Microtus* spp. in other regions of Europe (Krampitz 1964; Sebek et al. 1980; Walter and Liebisch 1980), although the dynamics of infections and their mutual interactions have not been evaluated comprehensively (Healing 1981; Young 1970) especially in the context of quantifiable intrinsic and extrinsic factors likely to influence infection.

Across the 4 years of the study 76.9% of the voles were infected with at least one of the five species that constituted the total species richness of the parasite component community. No animals carried all five species, but 11 voles carried a triple infection and three voles had various combinations of four species. Overall mean species richness was 1.1, a figure similar to but lower than the 1.4 recorded for *C. glareolus* in the adjoining woodlands (Bajer et al. 2001). The species density distribution did not differ statistically from that predicted by a null model and therefore the frequency of detection of two, three and four species co-infections was expected given their respective prevalence rates, as recorded by Bajer et al. (2001) for *C. glareolus*. Thus, at this level of analysis, the data did not support the existence of strong associations between species.

In all 4 years mean species richness increased from spring to summer, and in 2 of the years increased further to peak in the autumn, reflecting exposure to new infections during the summer period, and the onset of infections in young susceptible voles as they entered the population following the start of breeding in March–April. Interestingly, in 1997 there was an unexpected drop of mean species richness in autumn but we could not relate this to any unusual climatic changes in that year such as the earlier onset of winter. Thus, although seasonal differences affected mean species richness, and the precise contribution of the seasonal effect varied between years, overall mean species richness remained relatively stable across the 4 years of our study with annual mean values ranging from 1.0 ± 0.08 (2000) to

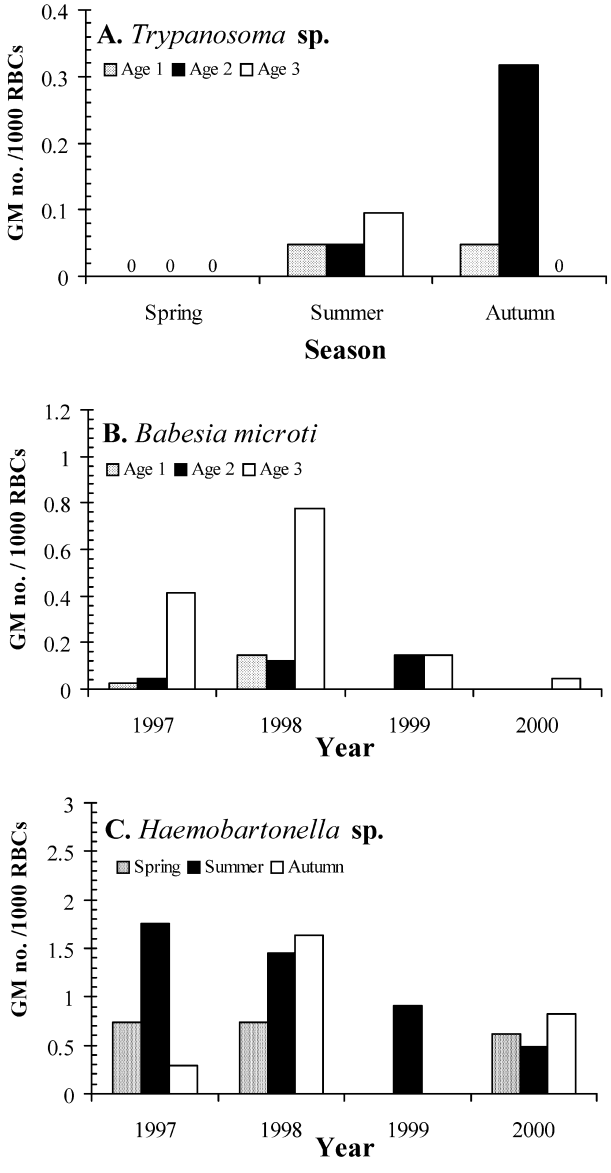

Fig. 5 Abundance of **A** *Trypanosoma* sp. in different seasons and age classes of voles, **B** *B. microti* across the 4 years of the study in the three age classes and *Haemobartonella* sp. across 4 years and by season. **A** *Trypanosoma* sp. Main effects of year = $F_{3,315} = 3.38$, $0.025 > P > 0.01$ (data not shown, but see text) and age ($F_{2,314} = 3.07$, $0.05 > P > 0.025$), and the interaction between season and age $F_{4,294} = 2.45$, $0.05 > P > 0.025$. **B** Analysis of *B. microti* by four-way ANOVA revealed only a complicated picture with a significant four-way interaction ($F_{3,271} = 3.0$, $0.05 > P > 0.025$), one significant three-way interaction (year×season×sex $F_{3,274} = 3.18$, $0.025 > P > 0.01$) and two significant main effects, those of year ($F_{3,315} = 5.34$, $0.005 > P > 0.001$) and age ($F_{2,314} = 3.27$, $0.05 > P > 0.025$). **C** *Haemobartonella*, main effects of season $F_{2,314} = 6.78$ ($0.005 > P > 0.001$) and year $F_{3,315} = 9.68$ ($P < 0.001$) and interaction between season and year $F_{5,295} = 4.83$ ($P < 0.001$). *GM* Geometric mean, *RBC* red blood cells

1.4 ± 0.01 (1998). However, this pattern was further confounded by host age, independently of seasonal changes, because in 2 of the years mean species richness increased with host age, and in the alternate 2 years it declined, contrasting with our observations in *C. glareolus* (Bajer et al. 2001) where intrinsic factors did not affect mean species richness and the only significant effects were from season and between-year variation.

In contrast to variation in mean species richness, fluctuations in prevalence and abundance of individual species of parasites were considerably more marked. However, infections with *Trypanosoma* sp. showed the least variation between years, although 1997 was a peak year in both prevalence and abundance. The only trypanosome species recorded from common voles in this region of Europe is *T. microti* (Walter and Liebisch 1980) and our specimens conformed to the morphological descriptions of this species, but recent molecular analyses of trypanosome isolated from *A. sylvaticus* have indicated that rodents may carry more than one species indistinguishable by conventional microscopy, and that host-specificity is not as rigid in these host-parasite systems as previously thought (Noyes et al. 2002).

Overall, prevalence of *Trypanosoma* sp. in our study was 8.4% but rose to as high as 27.3% in some subsets of voles (e.g. age class 2 in autumn). Prevalence rates of this order concur with those reported for *M. agrestis* in Germany and Norway (16.2%, Walter and Liebisch 1980; 20.8%, Wiger 1979) and in the UK (20.4%, Young 1970; 7–14%, Healing 1981) and further afield in Alaska in other *Microtus* spp. (Laakkonen et al. 2002). Both prevalence and abundance of *Trypanosoma* sp. were highly seasonal and host-age dependent. Across all 4 years of the study no voles sampled in the spring period carried infection but later infections increased in prevalence and abundance climbing to peak either in the summer or autumn in older age classes (2 or 3). These observations concur with earlier work on *M. agrestis* (Young 1970; Wiger 1979; Walter and Liebisch 1980) and with our earlier observations on bank voles, where seasonal fluctuations were also strong, although a small proportion of bank voles was already infected in the spring period in most years (Bajer et al. 2001, 2–12%). Rodent *Trypanosoma* spp. belong to the *lewisi* group of trypanosomes and are known to be transmitted by fleas (Molyneux 1969), but theoretically transmission by ticks may be possible also because live trypanosomes have been reported in *Ixodes ricinus* collected from forests in Europe (Aeschliemann et al. 1979; Karbowiak and Wita 2000, Petko, pers. com.). Experimental infections with *T. evotomys* have shown that parasitaemia can last for 3 weeks, with circulating trypomastigotes arising from intracellular amastigotes in the spleen and lymphoid tissues (Molyneux 1969). Larval *I. ricinus* increase in density on vegetation and commence host-finding and feeding activities in late spring (beginning of June; Pawelczyk 2003), and this, together with an increase in flea activity in the warm months, may explain the summer or autumnal peaks in *Trypanosoma* sp. infection in *M. arvalis*.

It is likely that as with other related trypanosome species, there is strong acquired immunity, once the primary infection has been controlled (Albright and Albright 1991), hence we expected to see this species

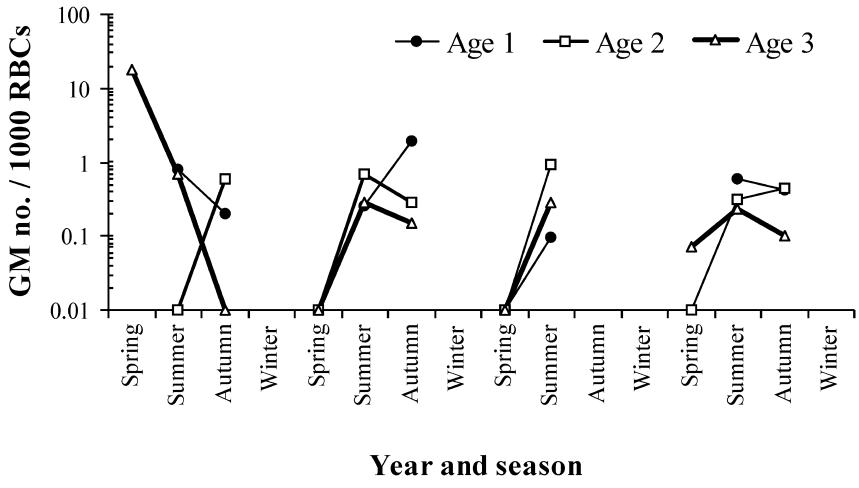

Fig. 6 Abundance of *Bartonella* spp. in three age classes, and by season and year of the study. Analysis by four-way ANOVA revealed only one significant three-way interaction (year×season×age, $F_{7,278} = 3.2$, $0.005 > P > 0.001$) and three, two-way interactions (sex×age, $F_{2,292} = 3.1$, $0.05 > P > 0.025$; year×age, $F_{6,296} = 3.13$, $0.005 > P > 0.001$; year×season, $F_{5,295} = 2.72$, $0.025 > P > 0.01$). For abbreviations, see Fig. 6

mainly in young animals, as was found by Healing (1981). In the autumn period this indeed proved to be the case, but in summer, when both prevalence and abundance were still quite low, infections were marginally higher among the oldest animals, perhaps suggesting that at this stage older voles are more exposed to infection (Young 1970) but manage to control parasitaemia better than younger animals in due course. In contrast to studies on *M. agrestis* (Walter and Liebisch 1980; Young 1970), but consistent with our study on *C. glareolus* (Bajer et al. 2001), we did not find any sex bias in either prevalence or abundance of infection.

B. microti was the most prevalent protozoan among the haemoparasites recorded in *M. arvalis*. The prevalence rate of 9% is much higher than recorded in other studies from Central/North-Eastern Europe (Mahnert 1972; Sebek et al. 1977; Karbowiak et al. 2002) but lower compared to studies in the UK, Austria and Germany (Young 1970; Sebek et al. 1980; Walter and Liebisch 1980; Turner 1986; Turner and Cox 1985). *B. microti* was described for the first time by Franca (1912) in Portugal as *Smithia microti* from the vole *Microtus incertus* (*M. arvalis incertus*) and voles from the genus *Microtus* are still considered to be the main reservoir of this human-infectious pathogen worldwide (Watkins et al. 1991; Karbowiak et al. 1999, 2002). Although there are many records of infections in humans in the USA (Grunwaldt 1977; Parry et al. 1977; Steketee et al. 1985) and also in Europe (Sebek et al. 1977; Krampitz et al. 1986), thus far only one case of human babesiosis has been reported in Poland (Humiczewska and Kuzna-Grygiel 1997).

Infections with *B. microti* were very variable and subject to both extrinsic and intrinsic factors with no consistent pattern over the 4 years of the study. There was considerable variation between years with prevalence down to 2.3% in 2000, but as high as 21% in 1998, and this was also reflected in abundance. However this between-year variation was complicated by seasonal changes. Summer months constituted the only season when some infections were detected in all 4 years. By the autumn infections were rare and only detected in 1998, and similarly in spring no infections were detected in 2 years, but the highest prevalence on record (50%) was during spring in 1998. It may be relevant that in April of 1998 the mean intensity of tick infestation in woodland rodents, which constitute the main reservoir of ticks locally, was markedly higher than in other years of study (Siński and Paweczyk 1999, Paweczyk 2003). Both the larvae and nymphs of the tick *I. ricinus* are vectors for this parasite (Walter and Liebisch 1980) and both feeding larvae and nymphs were found in greatest numbers on *M. arvalis* during late spring and early summer months in our study sites, so this is when normally transmission would have been most intense (Pawelczyk 2003). Similar seasonal trends were observed by Young (1970) and Walter and Liebisch (1980) in *M. agrestis*. Infections were most common in males in summer, but in female voles there was a curious bimodal distribution with peaks in the spring and autumn. Although prevalence did not vary significantly with age, abundance was clearly highest among the oldest (age class 3) voles across all 4 years of the study. This suggests that voles acquire infection with age or are more exposed to infection with increasing age and indeed, higher tick infestation rates were observed among heavier i.e. older voles (Pawelczyk 2003) in our study site. This concurs with Young (1970) and Healing (1981) working on *M. agresitis*, and Turner (1986) on *C. glareolus* and *A. sylvaticus*, all of whom found *B. microti* infections to be more frequent among older hosts.

H. lavieri is a parasite for which only the sexual phases, the gametocytes, are quantifiable from blood smears, since the ex-erythrocytic stages reside in the lungs where they can last for several months (Krampitz and Haberkorn 1988). Hence the real prevalence was probably much higher than the 3.1% based on the presence of gametocytes in peripheral blood and this was well within the range of prevalence rates (0.2–17.4%) reported by others (Laakkonen et al. 2002; Healing 1981; Young 1970, Krampitz 1964; Mahnert 1972). In *C. glareolus* sampled from the forest adjoining the

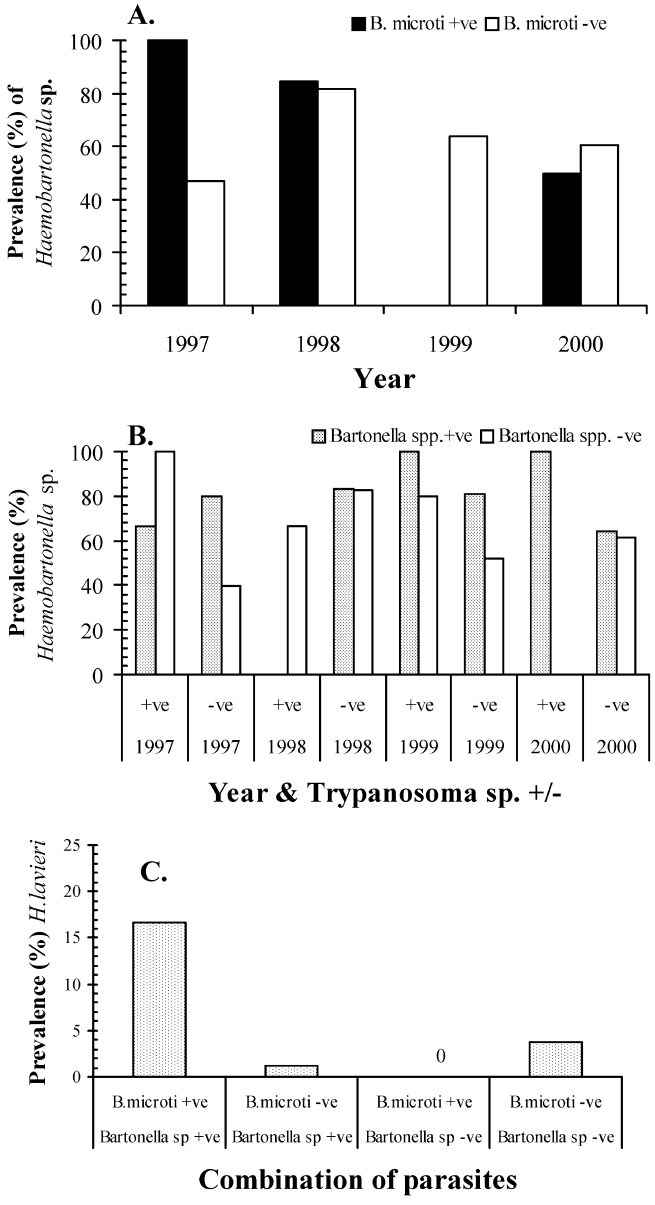

Fig. 7A–C Associations between species based on prevalence. **A** Prevalence of *Haemobartonella* sp. among voles either infected or not infected with *B. microti* by year of the study. **B** Prevalence of *Heamobartonella* sp. in voles with either *Bartonella* spp. or *Trypanosoma* sp, both or none of these species, by year of study. **C** Prevalence of *Hepatozoon lavieri* in voles with and without *B. microti* and *Bartonella* spp.

grassland meadows where *M. arvalis* were trapped, and during the same years, prevalence of *H. erhardovae* was 10 times higher (Bajer et al. 2001). Laakkonen et al. (2001) reported very similar results in their study from northern Europe. As with *Trypanosoma* sp., infections with *H. lavieri* were rarest in the spring (only one young vole infected) and more common in summer and autumn, and among the oldest age class. In some respects this pattern is similar to that reported by Young (1970) who found very marked seasonal patterns in *M. agrestis* in the UK characterised by a peak in prevalence in the summer months (18%). The youngest voles in our study showed the highest prevalence (4.6%), although prevalence was similar in age class 3 (3.8%), but infections were surprisingly absent from age class 2, a pattern that contrasts with that found by Young (1970) who reported prevalence of *Hepatozoon* sp. as increasing sequentially with age of *M. agrestis* (0%, 5.6%, 10% and 20%, respectively for age classes 1, 2, 3 and 4). Healing (1981) found little variation between age classes in *M. agrestis* but prevalence in his study was very low (1.2–1.4%). We were not able to sample rodents in the winter period because of climatic restrictions, but nevertheless the three seasons generated a complex picture in which the between-year effect was stronger than any underlying seasonal effect as in our parallel study in *C. glareolus* (Bajer et al. 2001).

Bartonella spp. (previously known as *Grahamella* spp., see Birtles et al. 1995) have in the past been designated as separate species mainly by host of origin (Krampitz and Kleinschmidt 1960). However, this approach was questioned early on (Hoyte 1954) and concern is supported by recent evidence, based on molecular techniques, which demonstrated that *M. agrestis* can harbour up to three genotypes some of which are shared with other rodent hosts (Birtles et al. 1994). Although the species affecting *Microtus* sp. has been referred to as *B. doshiae* in the past and more recently (Birtles et al. 1995), with more than a single genotype now recognized from *M. agrestis*, this may no longer be appropriate without molecular confirmation. *Bartonella* spp. usually show a high prevalence in rodent populations and in our study this was 28% which is towards the top end of the range reported in *Microtus* spp. by Laakkonen et al. (2002), Healing (1981), Young (1970), and that found by Mahnert (1972) and Sebek et al. (1980) (0%, 15.4%, 16.6%, 9–22% and 8–28%, respectively).

Bartonella spp. are transmitted by fleas (Krampitz 1962) and some species, e.g. *B. grahamii*, are known to last as long as 120 days in rodents (Tyzzer 1942). Species of *Bartonella* are also known to be infective to humans (Birtles et al. 1995, Anderson and Neuman 1997) although there is currently no evidence to indicate that the species harboured by *M. arvalis* fall into this category. In our study the prevalence of *Bartonella* spp. varied significantly between seasons, rising from a low value in spring, as in the case of some of the preceding species discussed above, and peaking in summer and autumn months, a pattern similar to that seen by Young (1970) in *M. agrestis* and by Turner (1986) and Bajer et al. (2001) in *C. glareolus*, and most likely explained by the increase in flea densities from winter to summer (Krampitz 1962).

There was no clear effect of age on either prevalence or abundance of *Bartonella* spp. in this and our previous study on bank voles (Bajer et al. 2001), but age-related variation in both parameters was dependent on year of study and host sex. However, despite these interactions, generally the infections were more common and more intense in the two youngest age classes (1 and 2)

Table 6 Analysis of quantitative associations between species. Each test was carried out on voles carrying both species in the pair specified. r_s Spearman's correlation coefficient, *NS* not significant

Combination	n[a]	Raw data		Residuals[b]	
		r_s	P[c]	r_s	P
Babesia microti + *Bartonella* spp.	6	0.426	NS	0.371	NS
B. microti + *Haemobartonella* sp.	21	0.444	0.047	0.455	0.042
Trypanosoma sp. + *Bartonella* spp.	10	0.073	NS	−0.402	NS
Trypanosoma sp. + *Haemobartonella* sp.	20	0.357	NS	0.223	NS
Bartonella spp. + *Haemobartonella* sp.	68	0.496	0.0001	0.265	0.030

[a]Number of voles carrying both species
[b]Residuals from minimum sufficient ANOVAs in generalized linear interactive modelling as explained in the text
[c]Probability (two-tailed). Applying the Dunn-Sidak correction for multiple comparisons, we accept $P = 0.0102$ as the cut-off for rejecting the null hypothesis (Sokal and Rohlf 1981)

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) but not by Young (1970).

It is not possible to reliably distinguish between some of the Anaplasmataceae and mycoplasma species on Giemsa-stained blood smears and this can only be done reliably by sequencing the 16rDNA genes (Neimark et al. 2001, 2002). Moreover, since infections due to mycoplasma species have not been found to date in *Microtus* spp. we treated the organisms infecting our animals as *Haemobartonella* sp. However, we cannot be totally confident about this nor about the species involved. *H. microti* has been described from *M. pennsylvaniucus* in the USA (Tyzzer and Weinman 1939) and *H. arvicolae* from common vole (*M. arvalis)*, (Yakimoff 1928 after Weinman 1944). The Anaplasmataceae are transmitted by arthropod vectors, and likely candidates in this region are fleas and lice (Weinman 1944; Gothe and Kreier 1977). If both arthropods are involved, inevitably cycles of transmission will be complex because of the different seasonal occurrence of these vectors. As in our recent study of *C. glareolus* (prevalence = 63%, Bajer et al. 2001), prevalence in *M. arvalis* was high (64%) and our data are the first to report such a high prevalence of *Haemobartonella* sp. in *Microtus* sp. in Europe. Young (1970) failed to detect this parasite in the 210 field voles that he examined. The season of maximum prevalence and abundance was not entirely predictable, depending on the year of study, although it was always either the summer or the autumn. A priority for future work on this taxon of parasites, must be to characterise the genotypes and their host specificities (Neimark et al. 2001, 2002).

With five species of parasites affecting the host population, a large number of two-way, three-way and four-way associations between species were possible, but our prevalence data revealed only eight sets of two- and three-way associations between species, and these were

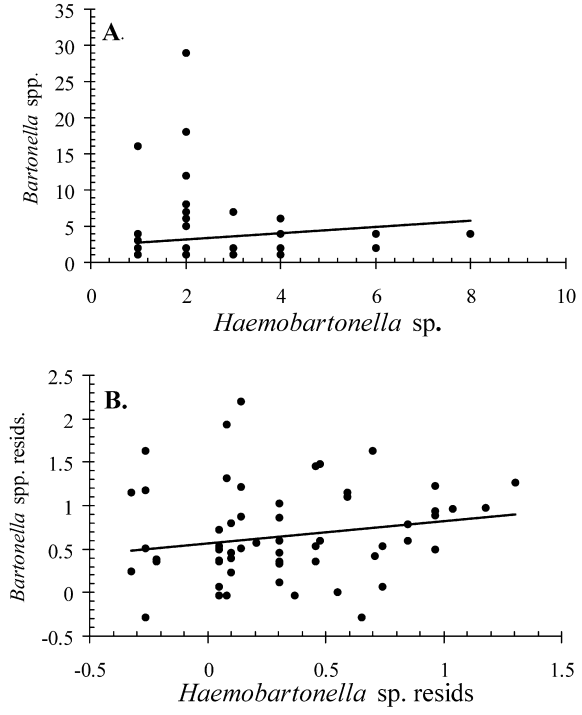

Fig. 8 Correlation between the abundance of *Bartonella* spp. and *Haemobartonella* sp. based on **A** raw data or **B** residuals of minimum sufficient ANOVAs

mostly dependent to some extent on one of the intrinsic and extrinsic factors in the model. There were no clear patterns of co-occurrence or exclusion by any two species. In the significant interaction between *Bartonella* spp. and *B. microti* the occurrence of co-infections depended on the year of study: in 1997 voles carrying *B. microti* were more likely to be infected with *Bartonella* spp.; in 1998 there was no difference; in 1999 *Bartonella* spp. were found only among voles without *B. microti* but prevalence of *B. microti* was low in that year; in 2000, as

in 1998, infection with *B. microti* made little difference to the prevalence of *Bartonella* spp. The second significant instance of co-infection between parasites involved three species — *Haemobartonella* sp., *Bartonella* spp. and *Trypanosoma* sp. but was also dependent on year of the study. Generally, prevalence of *Haemobartonella* sp. was high in *Bartonella* spp.-infected voles, and even higher if they were additionally infected with *Trypanosoma* sp., although there were exceptions in 1997 and 1998. The only case of an interaction that was not context dependent was the three-way interaction between *B. microti*, *H. lavieri* and *Bartonella* spp., and in which voles with *B. microti* and *Bartonella* sp. were more likely to be infected also with *H. lavieri*. This has some similarity to the association between *Bartonella* spp. and *B. microti* in bank voles (Bajer et al. 2001). The only one case of a clearly positive quantitative interaction was observed in the association of *Bartonella* spp. and *Haemobartonella* sp. but even this did not retain significance after controlling for the effects of extrinsic and intrinsic factors on the abundance of each species and multiple comparisons. Interestingly, this association was the only significant quantitative association that we found in the case of the haemoparasite component community in *C. glareolus* (Bajer et al. 2001) where significance was retained after controlling for extrinsic and intrinsic factors. Associations between *Hepatozoon* spp., *Bartonella* spp. and *Trypanosoma* species have also been reported previously (Healing 1981; Young 1970).

Finally, this study represents the first comprehensive analysis of the haemoparasitic component community structure of *M. arvalis* from North-Eastern Europe. Our data established that this component community, like that of *C. glareolus* (Bajer et al. 2001) is highly dynamic, with marked variation between years and seasons among individual species of haemoparasites, but greater consistency with respect to overall mean species richness. The extrinsic factors (year and season), and their interaction, were responsible for the greatest proportion of the variation in our data. Our analyses did not attribute marked roles to host intrinsic factors (sex and age) in shaping the structure of this component community and although host age influenced infections to varying degrees, in no case was host age on its own a major determinant of variation in data. Rather, host intrinsic factors contributed to the two- and three-way interactions that we detected. It is also interesting to note that after the failure and widespread abandonment of the collective agricultural system in this part of Poland in the early 1990s, natural succession on the fallow grasslands on which our studies were conducted has led to increasing colonisation by birch forming forests of young trees, and the populations of common voles are rapidly losing their habitats (Rogozinska 1997). It will be interesting to monitor the effect of these ecological changes on the haemoparasites component community because in the years ahead transmission potential is likely to decline as the habitat becomes increasingly fragmented. The haemoparasite component communities may therefore alter radically as *M. arvalis* sub-populations become increasingly isolated.

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