Prevalence and abundance of *Cryptosporidium parvum* and *Giardia* spp. in wild rural rodents from the Mazury Lake District region of Poland

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SUMMARY

Prevalence and abundance of *Cryptosporodium parvum* and *Giardia* spp. were studied in 3 species of rodents from forests and abandoned agricultural fields in N.E. Poland (*Clethrionomys glareolus* $n = 459$; *Microtus arvalis* $n = 274$; *Apodemus flavicollis* $n = 209$). Overall prevalence was consistently higher in the voles compared with *A. flavicollis* (70·6, 73·0 and 27·8% respectively for *C. parvum* and 93·9, 96·3 and 48·3% respectively for *Giardia* spp.). Prevalence and abundance of infection also varied markedly across 3 years with 1998 being a year of higher prevalence and abundance with both species. Fewer older animals (especially *C. glareolus* and *M. arvalis*) carried infection with *C. parvum* and infections in these animals were relatively milder. Although seasonal differences were significant, no consistent pattern of changes was apparent. Host sex did not influence prevalence or abundance of infection with *C. parvum*, but made a small contribution to a 4-way interaction (in 5-way ANOVA) with other factors in the case of *Giardia* spp. The 2 species co-occurred significantly and in animals carrying both parasites there was a highly significant positive correlation between abundance of infection with each, even with between-year, seasonal, host age, sex and species differences taken into account. Quantitative associations were confined to the 2 vole species in the study. These results are discussed in relation to the importance of wild rodents as reservoir hosts and sources of infection for local human communities.

Key words: *Clethrionomys glareolus*, *Apodemus flavicollis*, *Microtus arvalis*, *Cryptosporidium parvum*, *Giardia* spp., ecology, interactions, co-occurrence.

INTRODUCTION

The intestinal protozoan parasite, *Cryptosporidium parvum*, Tyzzer, 1912, is considered to be an important cause of diarrhoea in humans and in domestic livestock (Griffiths, 1998; de Graaf *et al.* 1999). A wide range of natural reservoir hosts has been reported for *C. parvum* (Sturdee, Chalmers & Bull, 1999) and experimental transmission studies have established that some strains of this organism lack host specificity and can be transmitted between mammalian hosts (O'Donoghue, 1995; Okhuysen *et al.* 1999). However, the existence of distinct host-specific genotypes, and the extent of their abilities to infect other hosts, are both subjects of intensive current investigation. The complexity involved is reflected in a recent study, employing molecular techniques, which provided evidence for 2 new genotypes of the parasite, both infective for humans and identical to feline and canine isolates (Pieniazek *et al.* 1999).

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Giardia spp. are also very common intestinal parasites responsible for acute and chronic infections in humans and other animals (Novotny *et al.* 1990; Tonks, Brown & Ionas, 1991). For *G. intestinalis* (*G. duodenalis*), the commonest species infecting mammals and the principal cause of human giardiasis, a wide range of animal reservoir hosts, including rodents, is believed to exist (Marino *et al.* 1992; Karanis *et al.* 1996*a*, *b*).

Environmentally resistant and long-term infective oocysts of *C. parvum* and cysts of *G. intestinalis* are often found in surface water (Wallis *et al.* 1996; Chalmers *et al.* 1997*b*) and are responsible for waterborne outbreaks of cryptosporidiosis and giardiasis affecting human communities (Majewska & Kasprzak, 1995; Cicirello *et al.* 1997). However, for the most part, sources of environmental contamination remain unresolved and hence detailed field surveys of potential wildlife reservoirs are required to fully elucidate the diversity of transmission routes to human communities and to domestic livestock. Wild rodents are known to act as reservoir hosts, especially those species with commensal relationships with humans e.g. wild house mice, *Mus* spp. and rats *Rattus* spp. (Webster & MacDonald, 1995;

Marino *et al.* 1992; Chilvers *et al.* 1998; Abd el-Wahed *et al.* 1999). Less is known about the transmission potential from wild voles and non-commensal mice, although both groups of rodents often live at high density on the outskirts of human communities and are known to carry both *C. parvum* and *Giardia* spp. (Sinski, Hlebowicz & Bednarska, 1993; Bajer, Bednarska & Sinski, 1997; Sinski, Bednarska & Bajer, 1998). In N.E. Europe, bank voles *Clethrionomys glareolus*, yellow-necked mice *Apodemus flavicollis*, and wood mice *A. sylvaticus* are the dominant rodents in woodland habitats and common voles *Microtus arvalis* in the open grasslands. Earlier studies on forest rodents in Central Europe (Karanis *et al.* 1996*a*) suggested that these animals also could be an important source of *Giardia* spp. cysts for human communities and data on the distribution of *C. parvum* in rodents in agricultural sites in the United Kingdom (Chalmers *et al.* 1995, 1997*a*; Webster & MacDonald, 1995) support the idea that rodents also act as reservoir hosts further afield.

However, there is still a paucity of information on variation in patterns of infection throughout seasons and across years in rural wild rodents, and on the relative importance of hosts of different species, sex and age as reservoirs of infection. In this paper we report on the prevalence and abundance of these 2 intestinal parasites in 3 populations of small rodents inhabiting 2 neighbouring habitats in the Mazury Lake District region in north-eastern Poland. We test the hypothesis that prevalence and abundance of infection differ between species of rodents in this region and we evaluate the extent of annual and seasonal variation, as well as the influence of the intrinsic factors, host age and sex.

MATERIALS AND METHODS

Study sites

Our study site was located east of the nature reserve surrounding Lake Łuknajno, and north of Lake Śniardwy, near the town of Mikołajki in the Mazury Lake District region of N.E. Poland. Trapping was conducted in mature woodland with Scots pine (Bajer *et al.* 2001) and in fields used for growing cereals until 1991 and essentially lying fallow since then.

Trapping and sampling wild rodents

Rodents were caught live in locally constructed wooden traps and processed according to the procedures described in detail by Bajer *et al.* (2001). Visits to the study sites comprised at least a 4-day duration, at approximately 4-week intervals from late March until mid-November over a 3 year period (1997–1999). 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 hard frozen and deep snow covered our sites for most of the winter period. The months between March and November were divided into 3 seasons, comprising spring (March to early June), summer (late June to August) and autumn (September to November).

At the field station in Urwitałt, all animals were inspected, identified, sexed, relevant morphometric data were recorded and they were weighed (to the nearest 0·5 g). After the inspection most animals were marked by standard toe clipping (Fullagar & Jewell, 1965) and released as near as possible to the original site of capture, whilst others (approximately 35 %) were killed for recovery of endoparasites (data to be published elsewhere).

Ageing rodents

Three age classes were established on the basis of weight (Morris, 1972) and sexual development, corresponding to immature juveniles (age class 1), young mature animals (age class 2) and adults (age class 3). For *C. glareolus* age classes 1, 2 and 3 comprised respectively voles < 15 g in weight (approximately less than 1·5 month old), 15–19·5 g in weight (1·5 to 2·5 months old) and > 19·5 g (2·5 months and older; Mazurkiewicz, 1972; Kozakiewicz, 1976). For *M. arvalis* these were respectively voles < 14 g (less than 1·5 month old), 14·5–19 g (1·5 to 2·5 month old) and > 19 g (2·5 months and older; Adamczewska-Andrzejewska, 1973). For *A. flavicollis* the 3 age classes corresponded to mice < 20 g (less than 3·5 month old), 20–30 g (3·5 to 7 month old) and > 30 g (7 months and older; Swierczewska, 1981), respectively.

Faecal analysis

Individual faecal samples were collected from traps immediately after retrieval of the captured rodent. A few pellets were used to prepare thin faecal smears which were stained according to modified Ziehl-Neelsen technique (Henriksen & Pohlenz, 1981), after drying and fixation in methanol. Then, at least 200 fields of vision under 400 × magnification were carefully examined on each slide for presence or absence of *C. parvum* oocysts. These were identified on the basis of their characteristic size (4–5 × 3·5–4·5 μm), general morphology and bright red/pink colour. For some animals only this method provided evidence of infection and in such cases we recorded a minimum detectable intensity, entered into the quantitative analysis as 400 oocysts/ml of concentrated sample (see below).

For identification of genus and quantification of infection we used a commercially available immuno-fluorescence assay (IFA) capable of labelling oocysts and cysts of the protozoa (MerIFluor Cryptospori-

Table 1. The structure of the sampled rodents by host species, year of capture, host sex and age

Year	Sex	Age 1	Age 2	Age 3	Totals by Sex	Totals by Year
Clethrionomys glareolus						
1997	Male	44	43	9	96	
	Female	29	29	18	76	
	Combined	73	72	27		172
1998	Male	22	27	25	74	
	Female	27	10	24	61	
	Combined	49	37	49		135
1999	Male	27	30	15	72	
	Female	33	25	22	80	
	Combined	60	55	37		152
Total by age		182	164	113	Overall total 459	
Apodemus flavicollis						
1997	Male	12	15	27	54	
	Female	5	26	24	55	
	Combined	17	41	51		109
1998	Male	1	4	5	10	
	Female	0	3	3	6	
	Combined	1	7	8		16
1999	Male	3	15	25	43	
	Female	7	15	19	41	
	Combined	10	30	44		84
Total by age		28	78	103	Overall total 209	
Microtus arvalis						
1997	Male	10	11	14	35	
	Female	14	5	14	33	
	Combined	24	16	28		68
1998	Male	9	8	12	29	
	Female	10	9	20	39	
	Combined	19	17	32		68
1999	Male	17	14	43	74	
	Female	12	21	31	64	
	Combined	29	35	74		138
Total by age		72	68	134	Overall total 274	

dium/Giardia (Meridian Diagnostics Inc., Cincinnati, Ohio, USA). For IFA 1 faecal sample (weighing between 0·3 and 1 g) from each animal was concentrated using a modified Sheather's sucrose flotation method (Garcia & Bruckner, 1988). The volume of concentrated material was estimated by comparison to calibrated Eppendorfs and the pellet was resuspended (1:3, v/v, pellet:10% formalin; dilution factor = 4). Ten μl of suspension were used for the IFA test, which was carried out according to the manufacturer's instructions. Identification was aided by comparison with positive control samples provided in the kit. Wells were examined under $400\times$ magnification, and the numbers of *C. parvum* oocysts and *Giardia* spp. cysts were recorded. For estimation of abundance of infection the total numbers of oocysts/cysts detected were multiplied by 400 (dilution factor $\times$ 100 = oocysts/cysts per ml) to give the number/ml of concentrated sediment. Thus the lowest limit of detection was 400 oocysts/cysts/ml of concentrated sediment.

Statistical analysis

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 parasite species in turn we entered prevalence of infection as a binary factor (infected = 1, not infected = 0) and then host species (3 levels, *C. glareolus*, *A. flavicollis* and *M. arvalis*), year (3 levels, 1997, 1998 and 1999), season (3 levels, spring, summer and autumn), host age (3 levels) and host sex (2 levels) 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 interaction

Table 2. *Changes in rodent population density in the study site during 1997–1999*

Month	Relative population density*		
	1997	1998	1999
Clethrionomys glareolus			
March	15·6	N.D.	N.D.
April	8·3	10·2	7·6
May	4·0	1·1	4·1
June	7·2	14·4	N.D.
July	18·0	13·4	52·3
August	31·5	34·1	85·0
September	34·5	44·5	188·3
October	20·0	33·5	N.D.
November	35·4	N.D.	N.D.
Apodemus flavicollis			
March	2·1	N.D.	N.D.
April	3·8	1·6	3·8
May	9·1	1·1	1·4
June	13·6	0·7	N.D.
July	6·7	1·7	9·3
August	16·6	3·6	13·3
September	20·8	5·9	78·5
October	11·4	0	N.D.
November	20·3	N.D.	N.D.
Microtus arvalis			
March	2·4	N.D.	N.D.
April	0·6	1·6	2·5
May	0·5	1·1	7·4
June	6·4	0	N.D.
July	10·0	22·6	19·4
August	14·8	5·4	30·1
September	10·4	22·6	N.D.
October	12·0	13·1	N.D.
November	12·4	N.D.	N.D.

* Population density was calculated as the number of rodents trapped divided by trap hours × trap number × 10^{-4}.
N.D., Not done.

terms that did not include the infection term reflect differences in numbers of animals sampled in particular categories between years, seasons, species, age and sex categories.

Quantitative data reflecting parasite abundance within hosts were expressed as geometric means (GM) because the data were highly overdispersed (Elliott, 1977; Dash, Hall & Barger, 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. 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 GLIM (a statistical system for generalized linear interactive modeling; GLIM 4, PC version, Royal Statistical Society, 1993) as described previously, using models with normal errors after normalization of the data by $\text{Log}_{10}(x+1)$ transformation (Crawley, 1993; Wilson & Grenfell, 1997; Behnke *et al.* 1999). Year, season, host species, age and host sex (see above for levels) were entered as factors. For models with normal errors the change in deviance is used to calculate the variance ratio, F. Significant main effects and interactions from minimum sufficient models are given in the legends to the figures. The residuals from all models were checked for approximately normal distribution.

Quantitative associations between parasites were

Table 3. *Summary statistics for prevalence and abundance of infection with Cryptosporidium parvum and Giardia spp. in three rodent host species*

| Host	Sex	*Cryptosporidium parvum*				*Giardia* spp.			
				Abundance				Abundance	
		Prevalence	*n*	Geometric mean ±	95 % CL	Prevalence	*n*	Geometric mean ±	95 % CL
Clethrionomys glareolus	Males	69·8	242	275·6	165·4–458·9	92·3	233	17837·9	11350·7–28032·5
	Females	71·4	217	281·4	166·4–475·6	95·8	212	32238·5	21361·2–48654·4
	Combined	70·6	459	278·3	193·2–400·7	93·9	445	23648·1	17389·4–32159·2
Apodemus flavicollis	Males	29·0	107	6·6	3·1–13·4	47·1	104	35·8	16·0–78·5
	Females	26·5	102	5·4	2·4–11·0	49·5	99	40·5	17·9–89·9
	Combined	27·8	209	6·0	3·5–9·9	48·3	203	38·0	21·6–66·3
Microtus arvalis	Males	71·0	138	239·8	127·1–451·9	94·8	134	54352·3	31212·1–94647·8
	Females	75·0	136	373·3	197·3–705·6	97·7	133	42270·9	27420·7–65163·3
	Combined	73·0	274	298·8	190·9–467·2	96·3	267	47955·0	33788·1–68061·7
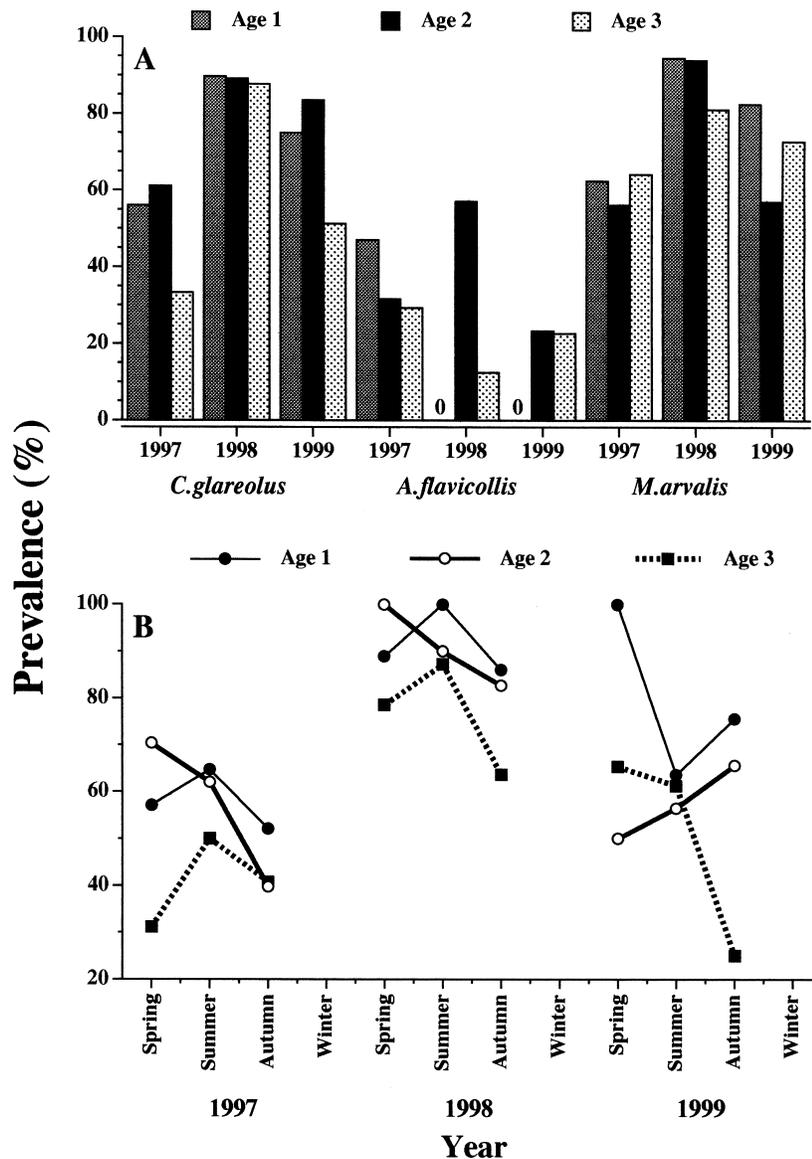
Fig. 1. Prevalence of *Cryptosporidium parvum* in *Clethrionomys glareolus*, *Apodemus flavicollis* and *Microtus arvalis*. (A) Annual changes in relation to age classes among the 3 host species (year × species × age × infection $\chi^2_8 = 20 \cdot 43$, $P = 0 \cdot 009$). (B) Annual and seasonal variation among age classes with hosts combined (year × season × age × infection $\chi^2_8 = 20 \cdot 90$, $P = 0 \cdot 007$). Additional significant terms in the minimum sufficient model included four 3-way interactions; species × sex × age $\chi^2_4 = 11 \cdot 8$, $P = 0 \cdot 02$), season × sex × age ($\chi^2_4 = 10 \cdot 85$, $P = 0 \cdot 03$), season × species × age ($\chi^2_8 = 17 \cdot 1$, $P = 0 \cdot 03$) and year × season × species ($\chi^2_8 = 136 \cdot 1$, $P < 0 \cdot 0001$), but none of these incorporated infection. Test of the goodness of fit of the minimum sufficient model gave $\chi^2_{197} = 167 \cdot 4$, $P = 0 \cdot 94$). See text for full explanation.

examined by correlation analysis (Spearman Rank-order correlation test) of raw parasite data from animals carrying both species. We also examined the correlations between the residuals from minimum sufficient 5-way ANOVAs, in order to control for established differences between the host species, years, seasons, host age and sex.

RESULTS

Rodents sampled

A total of 942 rodents were sampled in the 3 year period and of these 459 (48·7 %) were *C. glareolus*,

209 (22·2 %) were *A. flavicollis* and 274 (29·1 %) were *M. arvalis*. The structure of the sampled host population by year, host species, sex and age is summarized in Table 1. Host density was estimated to enable comparison of each of the host populations between the 3 years of the study and this was calculated as the number of trapped animals divided by the product of the number of traps set and the duration of trapping hours (Table 2).

Overall summary statistics

Table 3 summarizes the overall prevalence rates across the 3 years of the study by host species and by
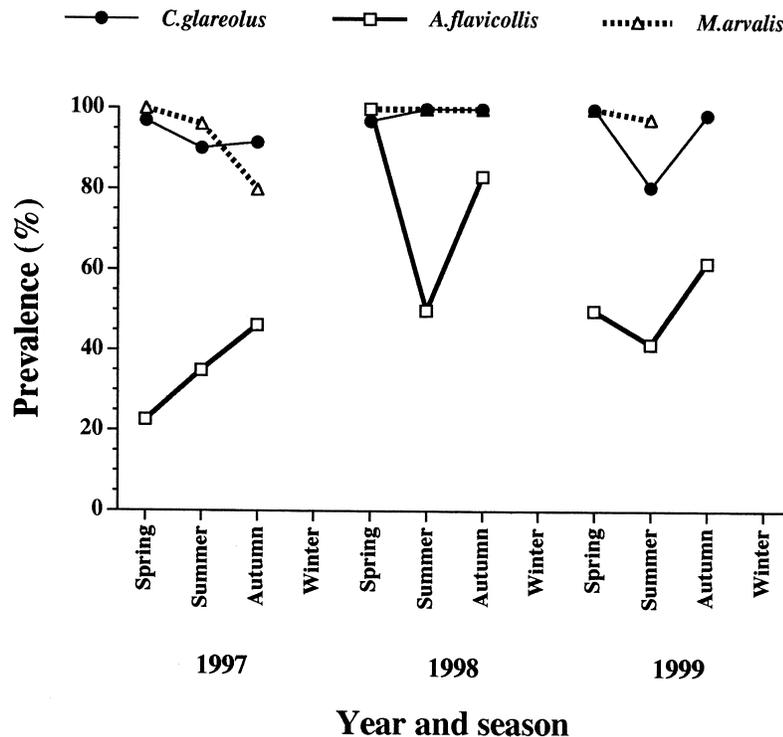
Fig. 2. Annual and seasonal variation in the prevalence of *Giardia* spp. among *Clethrionomys glareolus*, *Apodemus flavicollis* and *Microtus arvalis*. The minimum sufficient model incorporated 6 interactions, 2 of which included infection (season × species × infection, $\chi^2_4 = 18.9$, $P = 0.0008$ and year × infection, $\chi^2_2 = 29.5$, $P < 0.0001$) and 4 did not (year × season × species × sex, $\chi^2_8 = 16.8$, $P = 0.03$; year × season × sex × age, $\chi^2_8 = 15.3$, $P = 0.05$; year × species × sex × age, $\chi^2_8 = 23.6$, $P = 0.003$; season × species × sex × age, $\chi^2_8 = 16.1$, $P = 0.04$. Test of the goodness of fit of the minimum sufficient model gave $\chi^2_{183} = 107.1$, $P = 1.0$. See text for full explanation.

host sex. *C. parvum* showed a slightly higher prevalence and overall abundance in *M. arvalis* compared with *C. glareolus*, but both parameters were considerably lower in *A. flavicollis*. Much the same picture emerged for *Giardia* spp. except that prevalence was relatively higher across all species and abundance more intense. The data suggest that *A. flavicollis* was the least important reservoir host for both species of intestinal protozoa. However, in addition to variation arising from between host differences and from sexes, we sampled animals across 3 seasons and 3 years and samples comprised animals of different age. The analysis that follows quantifies the relative contribution of each factor and their interactions to variation in our data-set.

Prevalence

For *C. parvum* prevalence varied between the 3 host species and, as can be seen from Fig. 1A, *A. flavicollis* showed lower prevalence compared to the 2 vole species throughout. However, this difference between the species was confounded by some differences between years and among age classes (the year × species × age × infection interaction). Maximum prevalence was observed in 1998 in both *C. glareolus* and in *M. arvalis* and there was even a peak among *A. flavicollis* in age class 2 mice in that year.

The age effect is primarily attributable to the lower prevalence in age class 3 animals in 5 of the 9 sets of age blocks illustrated in Fig. 1A, as well as some of the fluctuations among the remaining classes.

The overall effect of year on prevalence of *C. parvum* can also be seen in Fig. 1B in which the species have been combined to illustrate the second interaction (year × season × age × infection). Here again the age effect is clearly apparent, with age class 3 generally showing lower prevalence, and overall little difference between age classes 1 and 2 except in the spring of 1999 (when prevalence for age class 1 = 100 % and 2 = 50 %). Otherwise the seasonal effect showed no consistent pattern (4 peaks in the summer, 4 in the spring and 1 in the autumn) and with the exception of spring 1997 and 1999 and the autumn of 1999, prevalence among age cohorts 1 and 2 was similar across seasons within years.

The prevalence of *Giardia* spp. also differed markedly between the species (Fig. 2), with *A. flavicollis* showing considerably lower prevalence than the voles, at all times except in the spring of 1998. However, prevalence also varied significantly with season (the season × species × infection interaction) and independently with year (the year × infection interaction). Among voles prevalence remained high throughout (Table 3), not dropping below 80 % in any season (Fig. 2) although there were minor

Table 4. *Measures of aggregation for* Cryptosporidium parvum *and for* Giardia *spp. in the combined samples of the three host species*

Species	k*	$+/-$	S.E.M†	I‡	D§
Cryptosporidium parvum	0·202	$+/-$	0·010	424491·9	0·833
Giardia spp.	0·495	$+/-$	0·023	911573·5	0·605

* Negative binomial exponent.
† Standard error of the mean.
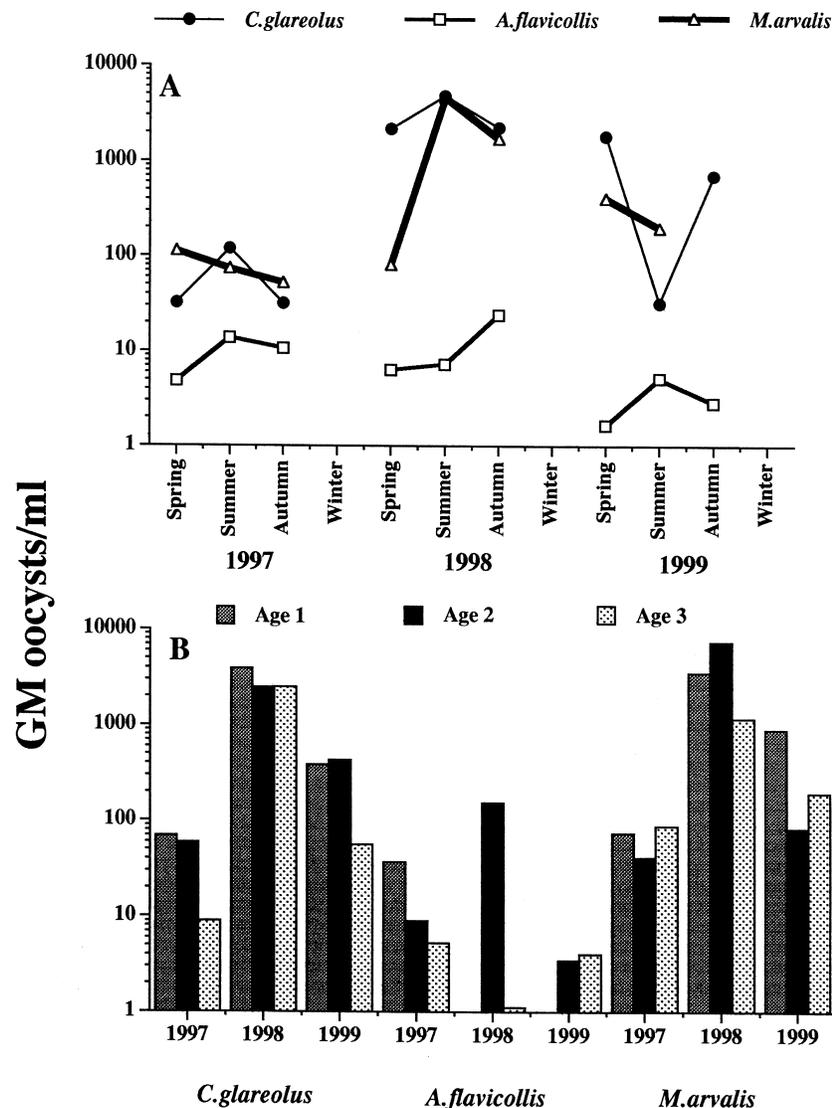‡ Index of dispersion = variance to mean ratio.
§ Index of discrepancy (Poulin, 1993).

Fig. 3. Variation in the abundance of *Cryptosporidium parvum* in *Clethrionomys glareolus*, *Apodemus flavicollis* and *Microtus arvalis* (GM = geometric mean). (A) Annual and seasonal variation among the 3 host species. (B) Annual changes in relation to age classes among the 3 host species. The minimum sufficient statistical model incorporated 3 main effects (age $F_{2,934} = 6\cdot2$, $P < 0\cdot005$, species $F_{2,934} = 40\cdot6$, $P < 0\cdot001$ and year $F_{2,934} = 56\cdot1$, $P < 0\cdot001$), two 2-way interactions (year × species $F_{4,904} = 8\cdot8$, $P < 0\cdot001$; year × season $F_{4,904} = 6\cdot4$, $P < 0\cdot001$) and two 3-way interactions (year × species × age $F_{8,853} = 2\cdot3$, $P < 0\cdot025$; year × season × species $F_{7,852} = 2\cdot5$, $P < 0\cdot025$).

seasonal fluctuations as for example in the autumn of 1997 when prevalence dropped in both vole species compared to spring. However, the major contribution to seasonal and year effects was derived from *A. flavicollis* in which prevalence rose from summer to autumn in all 3 years and in which there was considerable variation among spring animals (varying from 22·5 % in 1997 to 100 % in 1998).
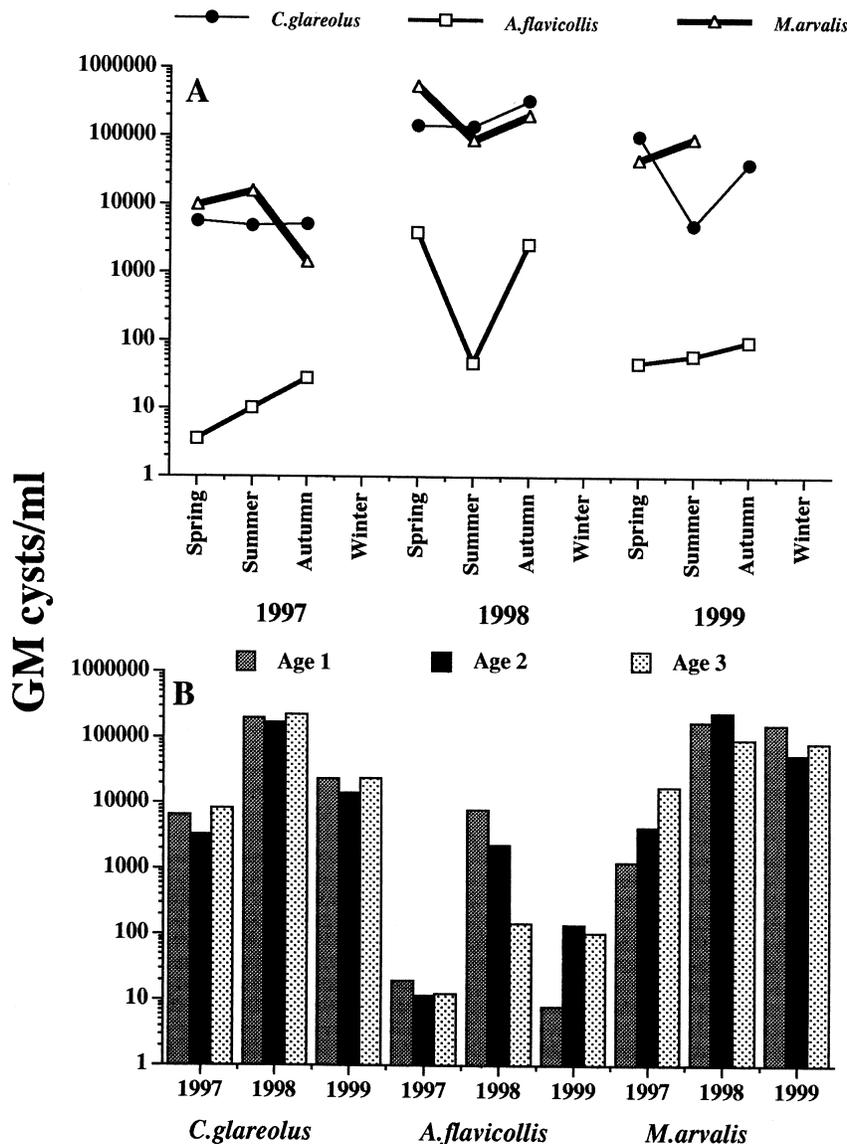
Fig. 4. Variation in the abundance of *Giardia* spp. in *Clethrionomys glareolus*, *Apodemus flavicollis* and *Microtus arvalis* (GM = geometric mean). (A) Annual and seasonal variation among the 3 host species. (B) Annual changes in relation to age classes among the 3 host species. The minimum sufficient statistical model incorporated 2 main effects (species $F_{2,907} = 169{\cdot}5$, $P < 0{\cdot}001$ and year $F_{2,907} = 69{\cdot}9$, $P < 0{\cdot}001$), one 2-way interaction (season × species $F_{4,877} = 3{\cdot}2$, $P < 0{\cdot}025$) and one 4-way interaction (year × species × age × sex $F_{7,790} = 2{\cdot}1$, $P < 0{\cdot}05$).

Abundance of infection

Both species were aggregated in the 3 host populations as shown in Table 4.

There was a considerable difference in the abundance of *C. parvum* between the host species (Fig. 3). On the whole, the geometric means were an order of magnitude higher in voles, sometimes 2–3 orders higher (Table 3; Fig. 3A, spring 1999), compared with *A. flavicollis*. Quite clearly *A. flavicollis* harboured much milder infections than the 2 voles. However, there was also a strong influence of year, season and age and these relationships are shown in Fig. 3A and B. As with prevalence the year effect arose because in 1998 infections were markedly more intense than in either 1997 or 1999, particularly among the voles. Seasonal variation was also ap-

parent but did not follow a consistent pattern, peaking in all 3 seasons (5 of 9 peaks in summer and 2 in the spring) depending on host and year combination (Fig. 3A). The age effect arose from the generally milder infections among the oldest animals (Fig. 3B), notably among *C. glareolus*, but also in 1998 among *M. arvalis* and in 1997 among *A. flavicollis*.

Variation in the abundance of *Giardia* spp. is illustrated in Fig. 4, where it can be seen that the principal sources of variation were host species and year of study. These 2 main effects had the major influence on our data, and there were only relatively small contributions from the 2 significant interactions (see legend to Fig. 4). *A. flavicollis* carried much milder infections than the 2 voles throughout the study. As with *C. parvum*, 1998 was a year in
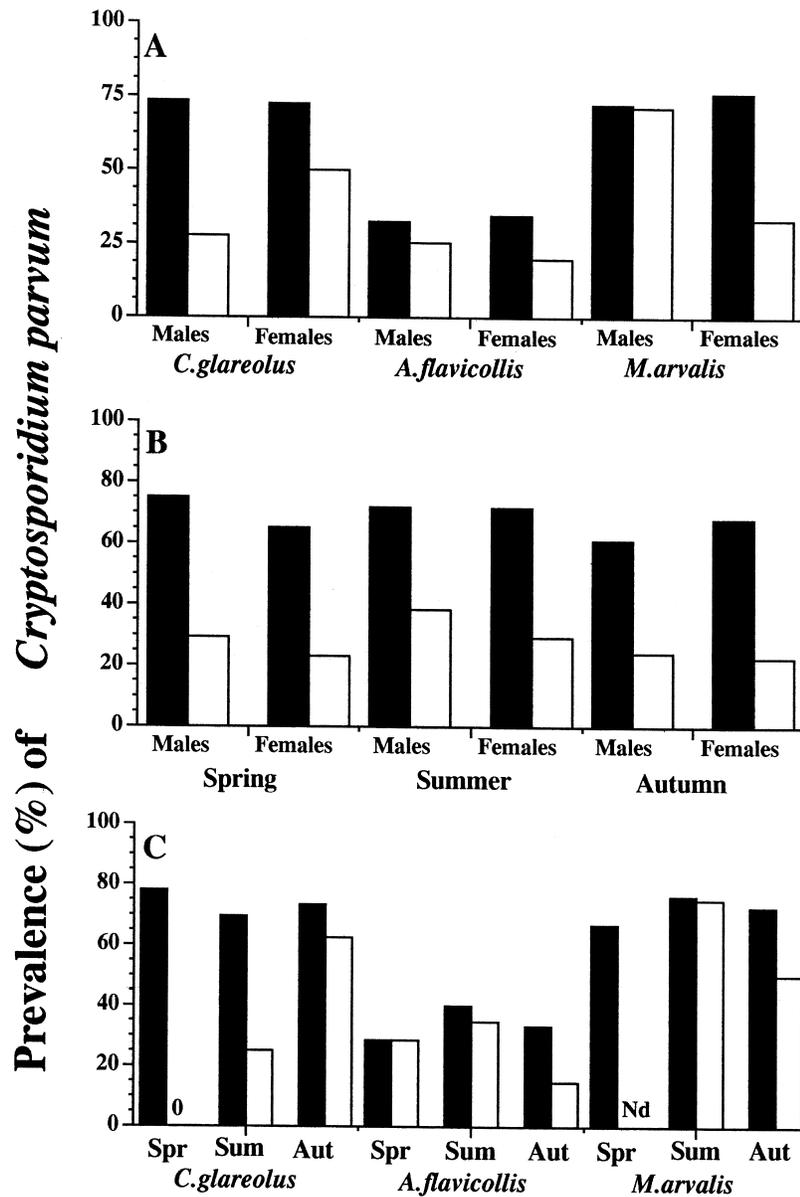
Fig. 5. Co-occurrence of infections with *Giardia* spp. and *Cryptosporidium parvum*. The figures show the percentage of animals infected with *C. parvum* among subsets of animals either carrying (■) or not infected (□) with *Giardia* spp. (A) Co-occurrence in relation to host species and sex (species × sex × *C. parvum* × *Giardia* spp., $\chi^2_2 = 11 \cdot 5$, $P = 0 \cdot 003$). (B) Co-occurrence in relation to season and host sex (season × sex × *C. parvum* × *Giardia* spp., $\chi^2_2 = 9 \cdot 8$, $P = 0 \cdot 008$). (C) Co-occurrence in relation to host species and season (season × species × *C. parvum* × *Giardia* spp., $\chi^2_4 = 17 \cdot 1$, $P = 0 \cdot 002$). Eight additional interactions comprising the minimum sufficient model included various combinations of extrinsic and intrinsic factors and in 5 cases one of the species (not shown). Test of the goodness of fit of the minimum sufficient model gave $\chi^2_{400} = 161 \cdot 95$, $P = 1 \cdot 0$). See text for full explanation. (Spr = spring, Sum = summer, Aut = autumn).

which infections were more intense than in other years, and even among *A. flavicollis* infections were heavier than in the preceding year, but there was essentially no difference between *C. glareolus* and *M. arvalis*. No overall consistent pattern of seasonal changes was evident, with peaks occurring in all seasons (4 of 9 peaks in spring and 3 in the autumn) depending on the combination of host species and year. Interestingly, there was no apparent difference in abundance among the 3 age classes in *C. glareolus* across all 3 years. The age contribution to the 4-way

statistical interaction arose from the declining abundance of infection with increasing age in *A. flavicollis* in 1998, and the increasing abundance with age among *M. arvalis* in 1997.

Associations between parasites based on categorical data

We tested first for interactions between parasites at the level of co-occurrence/exclusion by maximum likelihood analysis of prevalence data, including all
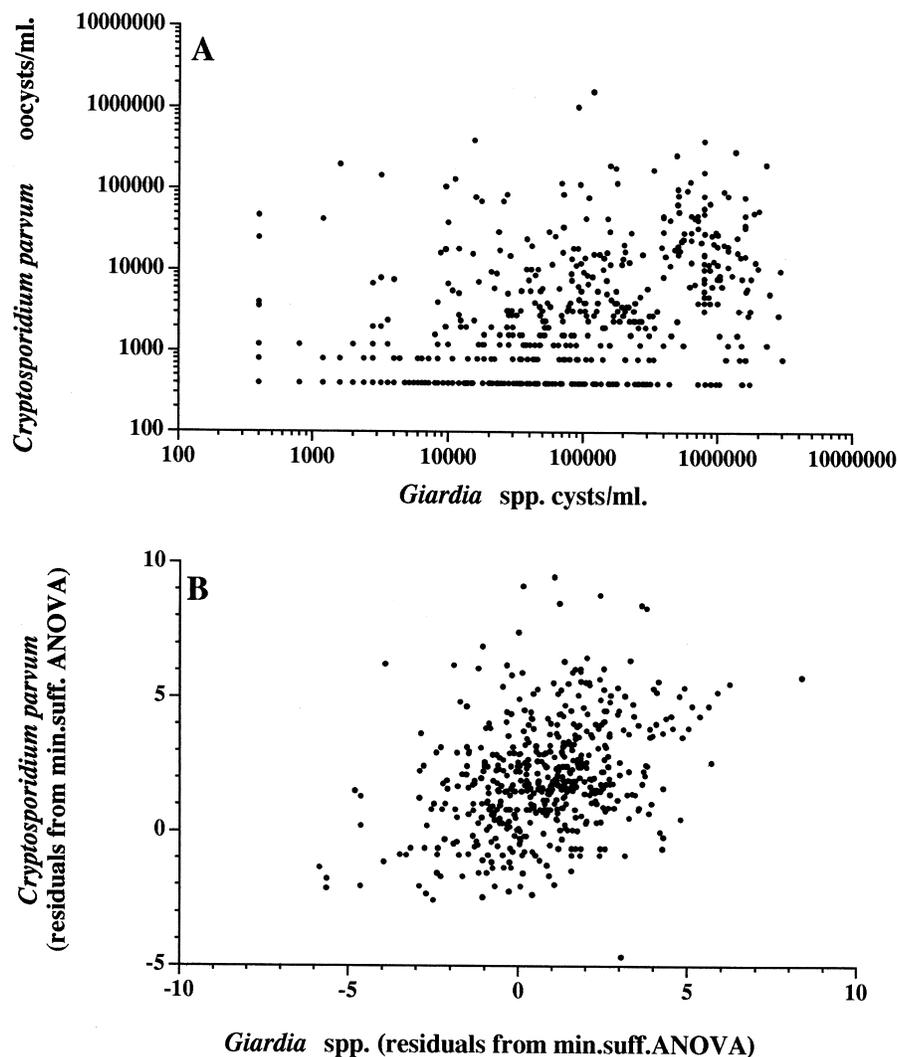
Fig. 6. Positive association between the abundance of infection of *Giardia* spp. and *Cryptosporidium parvum*. (A) Raw abundance data. (B) Residuals from minimum sufficient ANOVAs to control for the confounding effects of host species, year, season, host sex and age. See text for statistical analysis.

Table 5. Spearman Rank order correlation coefficients for the quantitative association between *Cryptosporidium parvum* and *Giardia* spp. by host species

	Raw data			Resids. from min suff. model*		
Species	r_s†	n‡	P§	r_s†	n‡	P§
Clethrionomys glareolus	0·506	305	< 0·0001	0·259	305	< 0·0001
Apodemus flavicollis	0·164	33	N.S.	−0·011	33	N.S.
Microtus arvalis	0·408	191	< 0·0001	0·348	191	< 0·0001

* Residuals from minimum sufficient 5-way ANOVAs for all host species combined derived from the analyses given in legends to Figs 3 and 4.
† Spearman Rank order correlation coefficient.
‡ Sample size, based on animals which carried both species.
§ Probability for acceptance of the null hypothesis.

intrinsic and extrinsic factors recorded in the study. Three interactions incorporated both species of parasites but each interaction was confounded by the influence of 2 additional factors. It is quite clear that across most of the combinations illustrated in Fig. 5 (excepting only the spring *A. flavicollis* (Fig. 5C) and male *M. arvalis* Fig. (5A)) prevalence of *C. parvum* was higher among mice that also harboured *Giardia* spp. Positive co-occurrence of the 2 species was particularly marked in male *C. glareolus* and female

M. arvalis (Fig. 5A), when species and age classes were combined among both sexes right across all 3 seasons (Fig. 5B), and in *C. glareolus* in the spring and summer (Fig. 5C).

Interactions between species based on quantitative data

Quantitative associations were tested only in mice carrying both species of parasites. We first analysed the raw data for each species and this gave a highly significant positive correlation (Fig. 6A; $r_s = 0.465$, $n = 529$, $P < 0.001$). However, to control for the possibility that the relationship may have arisen through aggregations of particularly heavily infected animals among some of the subsets of data, we also tested the correlation between the residuals of minimum sufficient models derived from 5-way ANOVAs, as described earlier. This analysis gave $r_s = 0.365$, ($n = 529$, $P < 0.0001$ and Fig. 6B), supporting the idea of a positive correlation between the intensity of infection of *C. parvum* with *Giardia* spp.

Finally we broke down the analysis by host species (Table 5) and this showed that the correlations were highly significant in both vole species irrespective of whether the analyses were carried out on the raw data or on residuals, but not in *A. flavicollis*.

DISCUSSION

The results reported in this paper firmly establish that the common woodland and grassland wild rodents in N.E. Poland constitute significant and major reservoirs of infection with *Giardia* spp. and *C. parvum* throughout 3 seasons of the year. *Giardia* spp. were clearly very common parasites with prevalence between 48 and 96% among the 3 rodent species examined. The overall prevalence of *C. parvum* infections was generally lower, 28–73%, but nevertheless sufficient for these animals also to constitute an important natural reservoir.

Whilst high overall in both species, prevalence of infection varied significantly in relation to the factors that we included in our analyses. Perhaps the most important of these was the species of host. Both voles (*C. glareolus* and *M. arvalis*) showed high prevalence and intensity, but each parameter of infection for both species of parasites was considerably lower (abundance was typically 1–2 orders of magnitude lower) in *A. flavicollis*. The low prevalence of *C. parvum* infection in the yellow-necked mouse population (28%) is similar to values reported elsewhere for Muridae: it was comparable with our preliminary results (27% in Bajer, Bednarska & Sinski, 1997; Sinski *et al*. 1998) and with prevalence in wood mice *A. sylvaticus*, in the UK (21 and 22%, Chalmers *et al*. 1995, 1997*a*, respectively) and in Spain (35%, Torres *et al*. 2000). Thus mice seem to be least important as reservoir hosts for this protozoan. The

discrepancy in susceptibility/resistance to infection is likely to have an intrinsic (e.g. genetic) rather than an extrinsic basis because both mice and bank voles inhabit the same forest habitats, rely on similar food resources and live in close proximity under the same climatic conditions. The much higher prevalence (70.6%) and abundance of *C. parvum* in *C. glareolus* appears typical of this region but differs markedly from studies elsewhere (UK 8.9, 13 and 7.9% (Chalmers *et al*. 1995, 1997*a*; Bull *et al*. 1998) and Spain 20% (Torres *et al*. 2000)). Similarly prevalence in common voles (73%), whilst comparable with earlier studies in the region (68%, Bajer *et al*. 1997; Sinski *et al*. 1998), differed markedly from values reported in Finland for other Microtidae (0.8% *M. agrestis*, 2.4% *C. glareolus*, 0% *M. oeconomu*; Laakkonen, Soveri & Henttonen, 1994).

Throughout the world rodents commonly serve as reservoirs of infection for *Giardia* spp., although prevalence varies by region and species (Chilvers *et al*. 1998). Prevalence can be very high, as in our study and elsewhere (e.g. 100% of *M. richardsoni* and *M. longicaudus* in the USA, Pacha *et al*. 1987; 75% of muskrats in Germany, Karanis *et al*. 1996*b*). We detected the highest prevalence (96.3%) in *M. arvalis* from post-agricultural habitats comprising abandoned fields and previously extensively grazed meadows. Prevalence in bank voles was only marginally lower (94%) but these hosts were exclusively sampled from the woodland areas in our study sites. In addition to our earlier paper (Bajer, Bednarska & Sinski, 1998), only 1 other study has reported on the prevalence of *Giardia* spp. in small woodland rodents from Europe (*C. glareolus*, *A. flavicollis* and *A. sylvaticus*), but the data are pooled and an overall prevalence of > 50% is reported (Karanis *et al*. 1996*a*).

The second significant factor influencing prevalence and abundance of infection was temporal. Quite clearly, there were significant changes between the years, in the case of *C. parvum*, with maximum prevalence and abundance in 1998. This temporal variation in *C. parvum* in Poland contrasts with the relative stability of prevalence in *C. glareolus*, *A. sylvaticus* and *Rattus norvegicus* in the UK (Chalmers *et al*. 1997*a*; Quy *et al*. 1999). However, between-year differences in prevalence have been observed among *Mus musculus* (12.4% in 1992, 22.5% in 1993, and 50% in the spring 1994; Chalmers *et al*. 1997*a*) and *C. glareolus* (16.3% in 1994 and 1.5% 1995; Bull *et al*. 1998) but with prevalence markedly lower than our values. Although we detected a between-year difference in the prevalence of *Giardia* spp., the overall prevalence with these species was so high that the annual fluctuations were minor in comparison. Interestingly, maximum prevalence and abundance was detected in 1998, as with *C. parvum*.

The third source of variation in prevalence and abundance arose from seasonal effects. For *C.*

parvum, our data indicate that peaks are more likely to occur in the spring and summer, rather than the autumn, but there was no convincing strong pattern across the seasons during the 3 years of the study. These findings generally concur with earlier reports (Torres *et al*. 2000; Quy *et al*. 1999) which have reported contrasting seasonal changes in prevalence among different rodent species, inhabiting various ecosystems. A seasonally low prevalence in the autumn was observed among rats on livestock farms in the UK, but no obvious peak in other seasons, whilst on arable farms prevalence dropped markedly in the winter (Quy *et al*. 1999). Similarly low prevalence was observed in the winter among three species of rodents, followed by higher prevalence in the summer and an autumnal peak (Chalmers *et al*. 1997*a*), coinciding with calving periods on local farms. However, there were no active farms in the vicinity of our study sites and the seasonal changes therefore reflect fluctuations in transmission among the wild animal reservoir. The seasonal effect was not as marked for *Giardia* spp., although fluctuations were more evident in *A. flavicollis*, in which prevalence rose from summer to autumn. The only comparable data are from a *Giardia* spp. survey in a wide range of hosts from New Zealand (Chilvers *et al*. 1998), where no seasonal differences in prevalence were found among house mice and ship rats.

Interestingly, intrinsic factors played a lesser role in determining prevalence and abundance of infection. Firstly, the prevalence of *C. parvum* did not vary between the sexes in all 3 host species in our study, in line with some earlier reports (Chalmers *et al*. 1997*a*; Bull *et al*. 1998) but contrasting with Quy *et al*. (1999; prevalence higher among male rats) and Torres *et al*. (2000; prevalence higher among female *A. sylvaticus* and *C. glareolus*).

Secondly, there was a significant age effect and some indication that it arose principally through generally lower prevalence and abundance of *C. parvum* among the oldest animals in our study. This declining prevalence with increasing age might indicate some development of immunity to re-infection among the older animals, which would have had a greater opportunity to be exposed to infection. Nevertheless, even among these animals overall prevalence was high, so at best, this might relate only to a relatively small subset of the population. Young animals are expected to disperse from their parents' territory (intrapopulation migrants) and therefore are likely to be important in spreading the parasite to new locations. Much the same conclusion emerged from the studies of Quy *et al*. (1999), in which overall prevalence was lower than in our study (24%) but varied significantly in relation to age with 40% of juvenile rats carrying infection in contrast to just 12% of adults. Similarly, a higher prevalence was observed in juveniles by Torres *et al*. (2000) but only in *C. glareolus*, and not in *A. sylva-*

ticus. Other field studies have not detected an age effect in rodents (Bull *et al*. 1998; Chalmers *et al*. 1997*a*), and since the average life-expectancy of voles in the wild is just 1·5–3·0 months (Pucek, Ryszkowski & Zejda, 1969/1970) and *C. parvum* does not pose a life-threatening infection, it is possible that there is little selection pressure for the evolution of strong protective responses. For the longer-lived species such as yellow-necked mice (mean life length in wild = 2·9–3·6 months and under laboratory conditions 4–5 years; Bobek, 1969) resistance to *C. parvum* may bring fitness benefits and in our study both prevalence and abundance were low in the oldest class of these rodents.

Under laboratory conditions male mice experience more severe and longer lasting infections with *Giardia* spp. than females (Daniels & Belosevic, 1995) but in accordance with other field studies (Marino *et al*. 1992; Chilvers *et al*. 1998) neither prevalence nor abundance of *Giardia* spp. were affected by host sex or age in our study. Although we do not present data here, we also examined recaptured animals and among these, *C. parvum* infections were often persistent, some animals among both sexes continuing to excrete oocysts for more than 3–6 months.

The two intestinal protozoa showed significant co-occurrence and in animals carrying both species there was a strong positive correlation between the abundance of infection with each, especially in voles. The trophozoites of these two species occupy different microhabitats in the intestine: *C. parvum* trophozoites are intracellular and freely mobile *Giardia* spp. trophozoites are loosely attached to villi. The presence of one might facilitate infection with the second species or both species may be focused in susceptible animals with weakened resistance or those showing behavioural patterns predisposing to infection. During the survey on Skomer bank voles (Bull *et al*. 1998) the majority of *C. parvum* infections were detected in voles co-infected with *C. muris*. These species also occupy different habitats (intestine and stomach, respectively) and thus their co-occurrence is very possible.

Finally, this study constitutes the first comprehensive report and analysis of the prevalence and abundance of infection with 2 medically important intestinal protozoa, *C. parvum* and *Giardia* spp., in 3 rodent species from N.E. Europe. To our knowledge, apart from species identification in different hosts including humans, rodents and livestock (Bednarska, Bajer & Sinski, 1988; Sinski *et al*. 1998; Majewska *et al*. 1999*a*, *b*), no other studies from this region of Europe have probed as deeply the factors that influence both prevalence and abundance of these opportunistic protozoan parasites. Our data establish firmly that at least 2 species of voles inhabiting 2 quite different habitats maintain these pathogens in the natural environment throughout the years and

seasons. Thus they may act as zoonotic reservoir of *C. parvum* and *Giardia* spp. for transmission to other animals including humans, although whether rodent genotypes, and specifically those parasitizing voles in our study sites, are infective to humans and livestock, remains to be established.

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