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Heteroxenous Coccidia (Apicomplexa: Sarcocystidae) in the Populations of Their Final and Intermediate Hosts: European Buzzard and Small Mammals

Milena SVOBODOVÁ[1], Petr VOŘÍŠEK[2], Jan VOTÝPKA[1] and Karel WEIDINGER[3]

[1]Department of Parasitology, Faculty of Science, Charles University, Prague; [2]Czech Society for Ornithology, Prague; [3]Laboratory of Ornithology, Palacký University, Olomouc, Czech Republic

Summary. Factors influencing prevalences of heteroxenous coccidia in the populations of small mammals and buzzards (*Buteo buteo*) were studied in the Czech Republic. Seventy one percent of buzzard broods were positive for *Frenkelia*-like sporocysts. Prevalence increased with nestling age and number, and reached 100 % at nest desertion. The prevalences of brain sarcosporidia (*Frenkelia glareoli* and *F. microti*) in rodents were higher in ecotones than in open habitats, in spring than in autumn, in heavier individuals, and on 2nd and 3rd day of trapping. These factors were significant although the overall prevalence was different in different host species (*Clethrionomys glareolus, Microtus arvalis, Apodemus flavicollis, A. sylvaticus*). The prevalences of muscle sarcosporidia in rodents and *Sorex araneus* were also positively influenced by habitat and host weight, while only for *M. arvalis* was the prevalence higher in spring. Host sex, locality and year did not show any effects on the prevalences. Besides two *Frenkelia* species, five *Sarcocystis* species were found (*S. putorii, S. cernae, S.* cf *sebeki*, and two undescribed species from *C. glareolus* and *Sorex araneus*). Natural infections of *C. glareolus* with *F. microti* and of *A. flavicollis* with *F. glareoli* are reported for the first time. Our study demonstrates that prevalences of brain and muscle sarcosporidians in small mammals are influenced by similar factors (intermediate host habitat and age) in different host-parasite combinations.

Key words: *Apodemus, Buteo, Clethrionomys, Frenkelia*, life cycles, *Microtus, Sarcocystis, Sorex,* transmission, wildlife parasites.

INTRODUCTION

Heteroxenous coccidia (Apicomplexa: Sarcocystidae) are dixenous parasites circulating among their vertebrate intermediate hosts and carnivorous final hosts. Many species have been described from raptors and their prey. These parasites were shown to influence their mammalian intermediate hosts by increasing the probability of predation by the definitive host (Hoogenboom and Dijkstra 1987, Voříšek *et al.* 1998). However, in natural host populations prevalences are generally not known, especially concurrently in both final and intermediate hosts of the parasite.

Frenkelia spp. are dixenous coccidian parasites of buzzards (*Buteo buteo, B. jamaicensis, B. borealis*) and their rodent prey. In the buzzard final host, parasite development is limited to the intestine, and infective sporocysts are excreted in faeces. In rodents, infective tissue cysts develop in the brain (Rommel and Krampitz 1975, Krampitz and Rommel 1977, Tadros and Laarman 1982, Upton and McKown 1992).

Two *Frenkelia* species are recognized, which differ in the morphology of brain cysts, and intermediate host

Address for correspondence: Milena Svobodová, Department of Parasitology, Faculty of Science, Charles University, Viničná 7, 128 44 Prague 2, Czech Republic; Fax: +420 2 24919704; E-mail: milena@natur.cuni.cz

spectrum. The rounded cysts of *F. glareoli* occur in bank voles (*Clethrionomys glareolus*), grey-sided voles (*C. rufocanus*), ruddy voles (*C. rutilus*) and southern water voles (*Arvicola sapidus*) (Erhardová 1955, Zasukhin *et al.* 1958, Doby *et al.* 1965). The lobulated cysts of *F. microti* can develop in a wide range of rodents. Natural infections have been found in field voles (*Microtus agrestis*), meadow voles (*M. pennsylvanicus*), common voles (*M. arvalis*), muskrats (*Ondatra zibethicus*), lemmings (*Lemmus lemmus*), and porcupines (*Erethizon dorsatum*) (Findlay and Middleton 1934, Frenkel 1953, Jírovec *et al.* 1961, Enemar 1962, Karstad 1963, Tadros and Laarman 1982, Kennedy and Frelier 1986). The experimental host spectrum is even wider, and includes the wood mouse (*Apodemus sylvaticus*), yellow-necked mouse (*A. flavicollis*), striped field mouse (*A. agrarius*), golden and common hamster (*Mesocricetus auratus*, *Cricetus cricetus*), rat (*Rattus norvegicus*), house mouse (*Mus musculus*), multimammate rat (*Mastomys natalensis*), chinchilla (*Chinchilla laniger*), and rabbit (*Oryctolagus cuniculus*) (Krampitz and Rommel 1977, Rommel and Krampitz 1978).

The validity of the genus *Frenkelia* was recently questioned by molecular studies, which place it in the genus *Sarcocystis*. To avoid this paraphyly, synonymization of *Frenkelia* with *Sarcocystis* was proposed (Votýpka *et al.* 1998, Modrý *et al.* 2004). In this study, we use *Frenkelia* as a subgeneric name, to distinguish it from muscle sarcosporidians, the latter with final hosts different from buzzards. Besides brain-invading sarcosporidia, rodents can serve as hosts for several species of muscle-cyst forming species, both with bird and mammalian final hosts (Odening 1998).

The aim of this study was to compare the prevalences of *Frenkelia glareoli* and *F. microti* in wild rodents. The influence of intrinsic (host age, sex) and extrinsic (season, year, habitat) factors on parasite prevalence was studied in several localities in the Czech Republic. To determine if the same factors are important for the occurence of brain and muscle sarcosporidia, prevalences of *Sarcocystis* in muscles were also studied. *Frenkelia* prevalence in the common buzzard, the final host, was studied simultaneously.

MATERIALS AND METHODS

Study area. The study was carried out in three localities in the Czech Republic: Locality 1 (50° 23' N, 016° 02' E) is located in the surroundings of the town Česká Skalice (Eastern Bohemia), characteristic by farmland with arable land, meadows and smaller woodland patches and riparian forests (woodland proportion between 10 to 20%) as the main habitat types (Diviš 1990). Habitats in locality 2 (50° 00' N, 016° 13' E), near the town Choceň (Eastern Bohemia), are similar to those in locality 1 with farmland covering nearly 60% and woodland 20% of the area (Voříšek 1995). Mixed forests are typical for woodlands in both localities. Locality 3 (48° 48' N, 016° 38' E) is located in Biosphere Reserve Pálava near the town Mikulov (South-eastern Moravia). The study was carried out in a 22 km^2 oak-hornbeam forest complex (Voříšek 2000).

Population density of buzzards has differed between the study sites: the density was similar in Česká Skalice and Choceň (20-40 breeding pairs per 100 km^2, Diviš 1990, Voříšek 1995) while the density in Mikulov has reached one of the highest values ever known (30-50 breeding pairs per 22 km^2, Voříšek 2000).

Prevalence of *Frenkelia*-like sporocysts in buzzards. The data for final hosts (buzzards) were collected in 1989-1993 in Česká Skalice, 1989-1992 in Choceň, and 1993-1995 in Mikulov. Faecal samples were collected during buzzard nest inspections (late April to early July). Mixed samples from individual nests were stored in 2% $K_2Cr_2O_7$ at 4°C. Before microscopic examination, samples were centrifuged for 10 min at 200 g, the sediment was mixed with 33% $ZnSO_4$, recentrifuged, and flotated for 20 min.

In Choceň and Česká Skalice, one sample per nest was collected. In Mikulov, nests were inspected repeatedly, and faecal samples were collected at each inspection. Age of buzzard's nestlings was estimated using wing length (Voříšek and Lacina 1998), the age of the oldest nestling in a nest was used for further analysis.

Prevalence of sarcosporidia in small mammals. The data for intermediate hosts (rodents and insectivores) were collected in 1990-1994 in Česká Skalice, 1990-1992 in Choceň, and 1993-1995 in Mikulov.

Small mammals were snap-trapped. Snap-trap lines, each containing 50 traps at 3 m intervals, were set in potential buzzards' hunting habitats (open habitats: meadows, alfalfa fields, clear-cuts; ecotones: riparian forests and wood edges) in spring (late March, early April) and autumn (late September, early October). Six trap lines were exposed during three consecutive nights at each trapping session. The lines were checked every morning. Mammals were identified, weighed using Pesola spring scale, and sexed. Correct species identification was difficult in young individuals of wood and yellow-necked mice, thus these individuals were categorized as *Apodemus* sp.

The infection status of snap-trapped small mammals was determined microscopically. Whole brains were checked for the presence of *Frenkelia* cysts in fresh squashed preparations. For the detection of *Sarcocystis*, femoral muscles were homogenized using a tissue grinder, centrifuged (10 min at 200 g), and the sediment was used for smear preparation. Smears were air-dried, fixed with methanol, stained with Giemsa, and checked for 10 min under immersion objective. Cystozoites from positive samples were measured for species determination.

Data analysis. Raw prevalence (in %) was calculated as a proportion of positive samples (broods or individuals). Effects of multiple predictors on infection status (coded 1 = positive, 0 = negative) were analysed by fitting generalized linear mixed models (GLMM) with logit link and binomial error distribution (SAS-based macro Glimmix, Littel *et al.* 1996). Significance of fixed effects was assessed

by the Type III F-test with denominator df estimated using the Satterthwaite method. Restricted maximum-likelihood estimates of model parameters are presented.

Effects of brood age and brood size on the prevalence of *Frenkelia*-like sporocysts in common buzzard nestlings were evaluated by fitting separate models to two partly overlapping data sets: (A) data from all three study localities using only one sample per brood (the last sample in Mikulov data; n = 229 broods/samples); (B) data from only the locality Mikulov using multiple samples per brood (n = 88 broods/271 samples). Random effects included in the models were: locality and year (model A); year, brood and brood × age, the last two nested within year (model B). This means, that both intercept and slope for age effect were allowed to vary among broods in model B.

Prevalence of *Frenkelia* and *Sarcocystis* in small mammals was analysed using two hierarchical subsets of the total data, for which a complete set of predictor variables was available: (i) host species, habitat type, season, trapping day, body weight; (ii) all the above variables with addition of host sex. Only those species with >1 positive individuals for the corresponding response variable were included in the models; all unidentified individuals of field mice were excluded. Body weight was centered within species to obtain a relative value, presumably related to age. Random effects of locality, year and trapping sample (nested within locality × year) were included. Apart from the main effects, all two-way interactions of species with the other effects were also examined.

The smaller data set, including host sex as a predictor variable, was heavily unbalanced (low number of individuals and/or zero prevalence for some combinations of predictors). Hence, the exact logistic regression (LogXact 5; Cytel Software Corporation, 2002) was used to fit simplified models. These included host species, sex, habitat type and season as the categorical predictors, and locality-year combination as the stratification variable. Two-way interactions of host sex with the other effects were examined.

RESULTS

Prevalence of *Frenkelia*-like sporocysts in buzzard nestlings

Overall prevalence of *Frenkelia*-like sporocysts in common buzzard nestlings was 71% (n = 229 broods) and varied from 43 to 89% among locality-year samples (Table 1). Nevertheless, the GLMM model A did not reveal significant random component of variation among localities (approximate z-test, P = 0.32) or years (P = 0.17), and a similar result was obtained with locality and year entered as fixed effects (locality: $F_{2,217} = 1.66$, P = 0.192; year: $F_{6,217} = 1.52$, P = 0.172). Hence, the spatio-temporal variation in prevalence could be accounted for by differences in mean age and size of the sampled broods (Table 1). The two GLMM models, based on different type of data, provided similar results for the direction of fixed effects - prevalence increased with both age and size of broods (number of nestlings), but the increase with age was steeper in larger broods (significant interaction term; Table 2, Fig. 1). The parameter estimates from the model B (multiple samples per brood) should be considered more realistic, because they were derived from data representing wider range of brood ages (Table 1). In accordance with model A, the random component of variation among years was not significant (approximate z-test, P = 0.19) also in model B, but there was significant (P < 0.001) random variation among individual broods in mean prevalence (intercept) and effect of age (slope).

Prevalence of *F. glareoli* and *F. microti* in rodents

The number of examined individuals and the raw (not adjusted for multiple effects) prevalence is shown in Table 3.

The GLMM model fitted to the larger data set (n = 1316; Table 4, Fig. 2) showed that the prevalence of *Frenkelia* was significantly higher in mammals caught in ecotone than in open habitat, in spring than in autumn, on the 2nd and the 3rd trapping day than on the 1st one (1st *vs.* 2nd: $t_{1302} = -3.6$, P < 0.001; 1st *vs.* 3rd: $t_{1303} = -2.5$, P = 0.013; 2nd *vs.* 3rd: $t_{1296} = 0.6$, P = 0.520), in relatively heavier individuals and in those positive for *Sarcocystis*.

Although the four mammalian host species differed in overall prevalence (P < 0.001 for all pair-wise comparisons, except for yellow-necked mouse *vs.*wood mouse: $t_{1306} = -1.7$, P = 0.097), effects of the above factors were consistent across all host species (nonsignificant interactions with species effect; all P > 0.1). The model suggests significant random component of variation among trapping samples (approximate z-test, P = 0.010), but not among localities or years (both P > 0.9).

The exact logistic regression applied to subset of the larger data set, for which host sex was available as an additional predictor (n = 578), corroborated the direction and significance of the effects of habitat (P = 0.013) and season (P < 0.001), but did not reveal an effect of host sex (slope for males ± SE, logit scale: 0.303 ± 0.361, P = 0.505) or interactions between sex and the other effects.

Prevalence of *Sarcocystis* spp. in rodents and insectivores

Number of examined individuals and the raw prevalence is shown in Table 3.

The GLMM model fitted to the larger data set (n = 888; Table 5, Fig. 3) showed that prevalence of *Sarcocystis* was significantly higher in mammals caught

Table 1. Raw prevalence (% of samples) of *Frenkelia*-like sporocysts in common buzzard nestlings at three localities in different years. Broods in Mikulov were sampled repeatedly throughout the nestling period, hence the multiple samples per brood. The mean age and number of nestlings in the sampled broods is shown.

Locality	Year	Broods (samples)	Prevalence (%)	Brood age (days)		Brood size	
				Mean ± SD	Range	Mean ± SD	Max [c]
Skalice	1989	15	60.0	22.8 ± 4.8	14 - 30	2.4 ± 1.0	4
Skalice	1990	49	65.3	22.0 ± 5.7	7 - 30	2.1 ± 0.9	4
Skalice	1991	16	87.5	23.8 ± 3.4	16 - 30	2.1 ± 0.8	4
Skalice	1992	15	80.0	24.3 ± 3.8	16 - 30	2.1 ± 0.9	4
Skalice	1993	10	60.0	20.5 ± 4.2	14 - 28	2.3 ± 1.3	4
Choceň	1989	8	75.0	17.6 ± 4.0	12 - 25	2.0 ± 0.5	3
Choceň	1990	7	42.9	18.9 ± 4.8	10 - 25	2.4 ± 0.8	3
Choceň	1991	6	66.7	18.4 ± 5.1	8 - 21	1.5 ± 0.5	2
Choceň	1992	15	73.3	15.9 ± 7.3	6 - 30	2.1 ± 0.9	4
Mikulov [a]	1993	34	55.9	25.0 ± 5.7	3 - 36	1.8 ± 0.7	4
Mikulov [a]	1994	35	88.6	29.3 ± 6.4	2 - 36	1.9 ± 0.8	4
Mikulov [a]	1995	19	78.9	24.9 ± 7.4	3 - 33	1.4 ± 0.5	2
Mikulov [b]	1993	120	28.3	16.2 ± 4.5	1 - 36	1.9 ± 0.3	4
Mikulov [b]	1994	100	54.0	18.4 ± 6.4	2 - 36	2.2 ± 0.5	4
Mikulov [b]	1995	51	62.7	18.7 ± 5.0	2 - 33	1.7 ± 0.4	3

[a] One (the last) sample per brood. [b] Multiple samples per brood. [c] Minimum = 1, in all cases.

Table 2. Fixed effect part of the GLMM models (binomial error, logit link) relating prevalence of *Frenkelia*-like sporocysts in common buzzard nestlings (positive = 1, negative = 0) to brood age (days) and brood size (number of nestlings). Model A was fitted to data from all three localities, using one sample per brood (n = 229); locality and year were included as random effects. Model B was fitted only to data from Mikulov, using multiple samples per brood (n = 271 samples and 88 broods); year, individual brood and brood × age were included as random effects.

Fixed effect	Estimate ± SE	Type III test		
		DDF [a]	F	P
Model A				
Intercept	0.370 ± 2.037			
Age	0.007 ± 0.087	218	< 0.1	0.939
Brood size	-2.000 ± 0.999	221	4.0	0.047
Age × brood size	0.105 ± 0.046	221	5.3	0.022
Model B				
Intercept	-5.473 ± 0.981			
Age	0.212 ± 0.039	242	29.4	0.001
Brood size	-0.413 ± 0.350	221	1.4	0.240
Age × brood size	0.070 ± 0.019	240	13.6	0.001

[a] Denominator df estimated by the Satterthwaite method; numerator df = 1 in all cases.

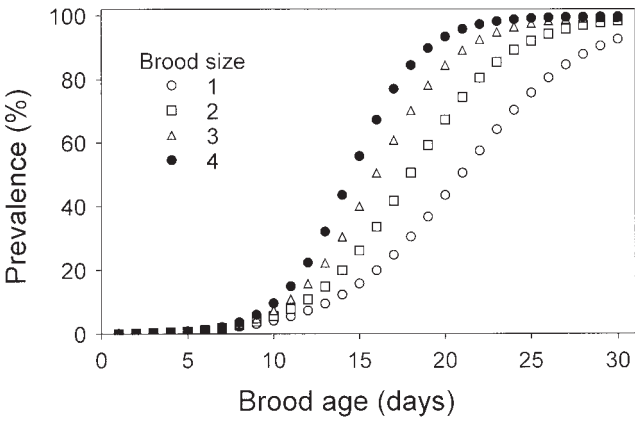

Fig. 1. Prevalence of *Frenkelia* sp. in common buzzard nestlings in Mikulov, predicted by the GLMM model (Table 2: model B) for broods of different age and size (number of nestlings).

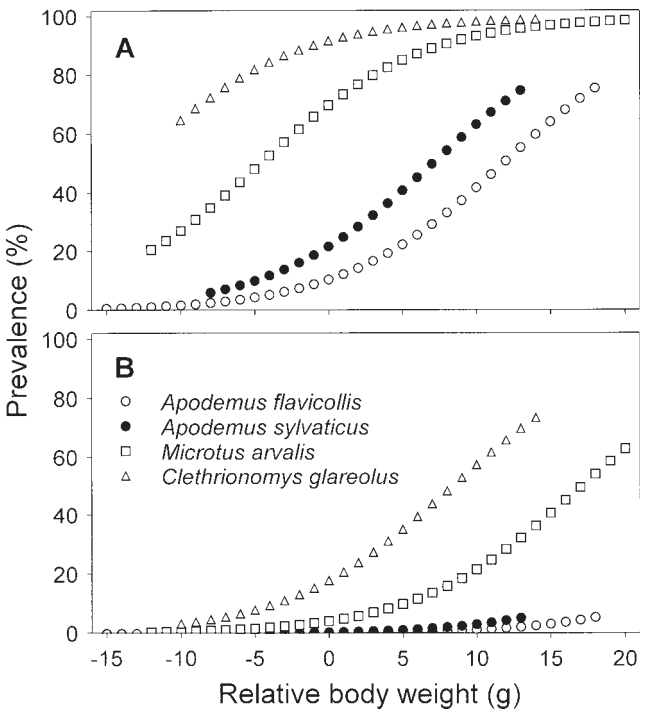

Fig. 2. Prevalence of *Frenkelia microti* (*Apodemus flavicollis*, *A. sylvaticus*, *Microtus arvalis*) or *F. glareoli* (*Clethrionomys glareolus*), predicted by the GLMM model (Table 4) for either the edge habitat in spring (**A**), or the open habitat in autumn **(B)**. Values of the other predictors were held constant (the 2nd trapping day, positive for *Sarcocystis*). Body weight was centered within species.

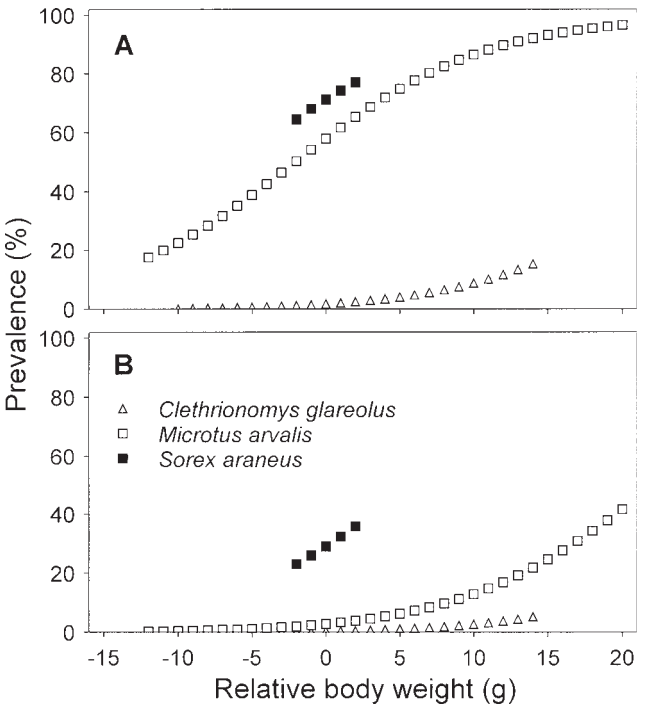

Fig. 3. Prevalence of *Sarcocystis* spp. predicted by the GLMM model (Table 5) for either the edge habitat in spring (**A**), or the open habitat in autumn (**B**). Values of the other predictors were held constant (the 2nd trapping day, positive for *Frenkelia*). Body weight was centered within species.

in ecotone than in open habitat, in relatively heavier individuals and in those positive for *Frenkelia*; which effects were consistent across all host species (nonsignificant interactions with species effect; all $P > 0.1$). The effect of season differed among host species (significant interaction, Table 5): prevalence was significantly higher in spring than in autumn only in common vole (2.59 ± 0.60, $t_{29} = 4.3$, $P < 0.001$) but not in common shrew (0.63 ± 0.68, $t_{17} = 0.9$, $P = 0.374$) and bank vole (-0.03 ± 0.73, $t_{38} = -0.05$, $P > 0.9$). The overall prevalence differed among the three mammal host species ($P < 0.001$ for all pair-wise comparisons); no significant effect of trapping day was found. The model did not reveal significant random component of variation among localities (approximate z-test, $P = 0.32$), years ($P = 0.24$) or trapping samples ($P = 0.17$).

The exact logistic regression applied to a subset of the larger data set, for which host sex was available as an additional predictor ($n = 365$), failed to reveal significant effects of habitat ($P = 0.9$), season ($P = 0.09$), host sex (slope for males: 0.274 ± 0.714, $P > 0.9$) or interactions between sex and the other effects.

Parasite and host species

The species identification revealed that the main host of *F. glareoli* is the bank vole, and that of *F. microti* is

Table 3. Raw prevalence [% of individuals (n)] of *Frenkelia microti* (apofla, aposyl, aposp, micarv), *F. glareoli* (clegla) and Sarcocystis spp. in different mammalian host species. Prevalence is shown for the total sample and separately for the three localities, three trapping days, two habitat types, two parts of year and host sex.

Sample	*Frenkelia*					*Sarcocystis*		
	apofla	aposyl	aposp	micarv	clegla	sorara	micarv	clegla
Skalice	4.7 (107)	0.9 (115)	1.2 (82)	5.9 (256)	42.0 (293)[aa]	20.0 (50)	3.5 (257)	1.4 (293)
Choceň	0.0 (15)	5.3 (94)	--	5.9 (170)	35.4 (65)[a]	8.3 (36)	2.9 (70)	1.7 (58)
Mikulov	0.8 (128)	2.6 (77)	0.0 (36)	3.1 (194)	32.0 (128)	33.3 (9)	2.1 (194)	1.6 (128)
1st day	0.9 (109)	2.0 (149)	0.0 (58)	5.4 (223)	35.7 (224)[aa]	14.9 (47)	2.7 (186)	2.3 (221)
2nd day	3.6 (84)	3.3 (90)	2.9 (35)	5.8 (225)	40.5 (158)[a]	20.0 (25)	2.6 (192)	0.6 (155)
3rd day	3.5 (57)	4.3 (47)	0.0 (25)	3.5 (172)	41.4 (104)	17.4 (23)	3.5 (143)	1.0 (103)
Ecotone	3.1 (194)	3.7 (218)	1.9 (53)	12.9 (170)	41.8 (373)[aaa]	21.9 (73)	2.3 (173)	1.9 (365)
Open	0.0 (56)	0.0 (68)	0.0 (27)	2.5 (363)	14.3 (63)	0.0 (22)	3.8 (261)	0.0 (64)
Spring	7.3 (55)	8.7 (46)	6.7 (15)	13.0 (46)	63.0 (100)[a]	21.7 (23)	15.2 (46)	3.0 (99)
Autumn	1.0 (195)	1.7 (240)	0.0 (103)	4.4 (574)	32.1 (386)[aa]	15.3 (72)	1.7 (475)	1.1 (380)
Male	0.0 (66)	0.0 (43)	0.0 (12)	4.5 (112)	29.7 (74)	50.0 (4)	1.8 (114)	2.7 (74)
Female	2.6 (77)	0.0 (40)	3.8 (26)	3.1 (127)	32.8 (64)	16.7 (6)	1.6 (129)	1.5 (65)
Total	2.4 (250)	2.8 (286)	0.8 (118)	5.0 (620)	38.5 (486)[aaa]	16.8 (95)	2.9 (521)	1.5 (479)

[a] Cases of *F. microti* ([a] one case, [aa] two cases, [aaa] three cases). Host species: apofla = *Apodemus flavicollis*, aposyl = *A. sylvaticus*, aposp = *Apodemus* sp., micarv = *Microtus arvalis*, clegla = *Clethrionomys glareolus*, sorara = *Sorex araneus*.

the common vole. However, three individuals of bank vole were infected with *F. microti*, and in one case, *F. glareoli* cysts were found in the brain of yellow-necked mouse.

Several species of *Sarcocystis* occured in small mammals, which differed in the length and morphology of their cystozoites. In common voles, *S. cernae* and *S. putorii* was found. Species from bank vole and common shrew are probably undescribed species. One yellow-necked mouse had sarcocysts in muscles, which probably belonged to *S. sebeki*.

DISCUSSION

This study describes for the first time prevalences of heteroxenous coccidians in populations of both their final and intermediate hosts.

The prevalence of *Frenkelia*-like sporocysts in buzzard faecal samples increased with the age of nestling, and reached 100% at the time of nest desertion. During the first week of life, chicks did not shed sporocysts due to the prepatent period of 7-9 days in both *Frenkelia* species (Rommel and Krampitz 1975, Krampitz and Rommel 1977). First positive samples are therefore found at the 2nd week of nestling life. The increase is not linear, because the daily amount of food consumed by chicks increases with age.

The prevalence increases with the size of brood, and the increase is steeper in bigger broods. The absolute amount of consumed prey is higher in bigger broods at a certain age, and the probability of finding sporocysts in a mixed faecal sample is higher for a bigger brood at a certain time point.

Although differences between study localities (habitats, buzzard's breeding density) are relatively large, no significant effect of locality or year on prevalence was found. The results show that the age of nestlings and the number of nestlings in the nest explain variation in prevalence in buzzards.

Species identification of *Sarcocystis* based on sporocyst morphology is not possible. The numbers of sporo-

Table 4. Fixed effect part of the GLMM model (binomial error, logit link) relating prevalence (positive = 1, negative = 0) of *Frenkelia* to effect of host species (*Apodemus flavicollis*, *A. sylvaticus*, *Clethrionomys glareolus*, *Microtus arvalis*), habitat type (ecotone *vs.* open), season (spring *vs.* autumn), relative body weight (centered within species), trapping day (three days) and infection by *Sarcocystis* (positive *vs.* negative). Model predictions shown in Fig. 2.

Fixed effect (modelled level)	Estimate ± SE	Type III test			
		NDF	DDF[a]	F	P
Intercept	-3.097 ± 0.653				
Species (apofla)	-2.980 ± 0.476	3	1266	59.9	0.001[b]
Species (aposyl)	-2.113 ± 0.420				
Species (clegla)	1.586 ± 0.234				
Habitat (ecotone)	2.251 ± 0.489	1	33	21.2	0.001
Season (spring)	1.553 ± 0.436	1	22	12.7	0.002
Body weight	0.182 ± 0.021	1	1262	73.5	0.001
Day (1st)	-0.530 ± 0.213	2	1300	6.9	0.001[c]
Day (2nd)	0.137 ± 0.213				
Sarcocystis (+)	1.509 ± 0.516	1	1302	8.6	0.004

[a] Denominator df estimated by the Satterthwaite method. Total n = 1316. [b] Test of species effect. [c] Test of trapping day effect.

Table 5. Fixed effect part of the GLMM model (binomial error, logit link) relating prevalence (positive = 1, negative = 0) of *Sarcocystis* to effect of host species (*Clethrionomys glareolus*, *Microtus arvalis*, *Sorex araneus*), habitat type (ecotone *vs.* open), season (spring *vs.* autumn), relative body weight (centered within species), trapping day (three days) and infection by *Frenkeli*a (positive *vs.* negative). Model predictions shown in Fig. 3.

Fixed effect (modelled level)	Estimate ± SE	Type III test			
		NDF	DDF [a]	F	P
Intercept	-0.316 ± 0.893				
Species (clegla)	-4.112 ± 0.616	2	356	26.8	0.001[b]
Species (micarv)	-2.552 ± 0.460				
Habitat (ecotone)	1.174 ± 0.517	1	17	5.2	0.037
Season (spring)	0.627 ± 0.686	1	8	5.6	0.045
Body weight	0.155 ± 0.037	1	598	18.0	0.001
Day (1st)	-0.420 ± 0.362	2	854	1.1	0.321[c]
Day (2nd)	-0.565 ± 0.390				
Frenkelia (+)	1.864 ± 0.467	1	678	15.9	0.001
Species × season (clegla)	-0.660 ± 0.889	2	152	5.2	0.006[d]
Species × season (micarv)	1.966 ± 0.851				

[a] Denominator df estimated by the Satterthwaite method. Total n = 888.[b] Test of species effect. [c] Test of trapping day effect. [d] Test of species × season interaction.

cysts in the samples were usually low, probably due to the small amounts which are shed at the beginning of the patent period. Sporocysts found in the faeces of buzzard might belong to other *Sarcocystis* species infecting raptors. However, to our knowledge, only *S. citellibuteonis* is known to infect buzzards (Pak *et al.* 1989), and its intermediate host, the yellow suslik (*Spermophilus fulvus*), is absent in the study area. Muscle sarcocysts were found in the examined rodents, but in considerably lower prevalences than brain cysts; moreover, only a part of those were species with bird final host [e. g., *S. cernae* of kestrel (*Falco tinnunculus*) in common vole]. Of the *Sarcocystis* species with avian final host, only *S. dispersa* from owls was described from two host genera (Černá *et al.* 1978). *Sarcocystis* species with small mammals as intermediate hosts are

generally more specific for their final host than those with large mammals as hosts (Cawthorn and Speer 1990). Therefore, we suppose that sporocysts in buzzards belonged to the subgenus *Frenkelia*.

The prevalence of *F. glareoli* in wild populations reported in other studies ranges from 2% to 30-50%, and may be as high as 85% (Jírovec *et al.* 1961, Skofitsch 1980, Enemar 1962, Kepka and Krampitz 1969). However, comparison among studies is difficult as the data were obtained in different habitats and seasons. In our study, prevalence of *F. glareoli* in bank vole was 32% in autumn, 63% in spring; 42% in ecotones and 14% in open habitats (Table 3).

Mammals are more frequently infected in ecotones than in open habitats, and the prevalence is higher for both *Frenkelia* and *Sarcocystis* spp. Buzzards and other potential final hosts use wood edges and riparian forests as perches and for roosting, and faeces may concentrate at these sites, resulting in higher probability of intermediate host contact with infective sporocysts. Small mammals differ in their habitat preferences. The common vole lives in open habitats, namely fields and meadows, while the bank vole is a sylvatic species, typical for deciduous and mixed forests with undergrowth. The wood mouse is opportunistic but mainly lives in open habitats near wood edges or in bushes, yellow-necked mouse prefers deciduous or mixed forests (Anděra and Horáček 1982). As a result, mammalian species are exposed to different risks of infection, and differ in their potential to serve as intermediate hosts. Other studies have also found habitat differences in prevalence. In Germany, infected bank voles were found mostly near "forests along the rivers" (Kepka and Skofitsch 1979), in Udmurt (Russia) in "broad-leaved" and "dark coniferous", in comparison with "small-leaved" tree forests; in pine forests parasites were absent (Kalyakin *et al.* 1973). Intermediate host habitat is probably one of the most important factors in sarcosporidia transmission.

Prevalence of both *Frenkelia* and *Sarcocystis* is higher in heavier individuals. Body weight correlates with the age of small rodents and shrews (Šebek 1959, Pucek 1970, Zejda 1971), therefore, heavier individuals are, on average, older. Since the host remains infected till the end of his life, this result is not surprising. Double infections with *Frenkelia* and *Sarcocystis* are found significantly more frequently than would be expected by chance. This suggests that at least some factors influencing the probability of infection are similar; indeed, in both parasites, the habitat and host body weight significantly influence the prevalences in the same direction.

Frenkelia prevalences were higher in spring than in autumn in all host species, while in the case of *Sarcocystis*, only the common vole was more often infected in spring. Higher prevalences in spring than in autumn are probably attributable to the higher age of overwintering animals. Higher prevalences of *Frenkelia* in spring have been reported also in other areas, e. g. in Russia and Germany (Kalyakin *et al.* 1973, Skofitsch 1980).

Prevalence of *Frenkelia* in snap-trapped rodents is lower the first day of trapping than in the consecutive days. This suggests that host behaviour may be influenced by the parasites. Changes in host behaviour that enhance transmission have been reported in several host-parasite combinations including heteroxenous coccidia. Rodents naturally infected with sarcosporidians were found more frequently in the prey of the final hosts than in snap traps (Hoogenboom and Dijkstra 1987, Voříšek *et al.* 1998), and predation experiment using *S. dispersa* in mice and long-eared owl (*Asio otus*) as a predator confirmed that the results were not biased by the snap-traps preferentially trapping uninfected rodents (Voříšek *et al.* 1998). Differences in the host social status or neophilia could explain our result. However, trapping day has no effect on muscle *Sarcocystis* prevalences in small mammals. This suggests that brain and muscle dwelling sarcosporidians differ in their effects on host behaviour.

In rodents, higher prevalence of *F. glareoli* than that of *F. microti* seems surprising. Buzzards in Central Europe prey mostly on common voles, while bank voles are only occasionaly found in the prey (Haberl 1995, Voříšek *et al.* 1997). During winter, bank vole represents only 1% of prey (Ševčík 1981). In Poland, common vole represented 36% of prey during breeding, while bank vole only 14% (Goszczyński and Piłatowski 1986). In Česká Skalice, the proportion of bank vole does not exceed 10% in the buzzard prey but is probably lower (Diviš, pers. comm.). Moreover, *F. microti* is able to infect several rodent genera including the bank vole.

Although *F. microti* has a wide host spectrum, its prevalence is however higher in common voles than in bank voles, even in individuals from the ecotones, where we could expect the same risk of infection. However, differences in host food could cause different exposure to sporocysts. Common voles feed on green plant parts, while bank voles have a diverse food which includes seeds and fruits, green plant parts, fungi, and insects

(Holišová 1959, Obrtel and Holišová 1974). Insects may be very important in the transmission, as they may serve as transporting or paratenic hosts (Smith and Frenkel 1978, Markus 1980). The bank vole diet therefore does not explain the lower prevalence of *F. microti*. Rather, the infectivity of *F. microti* sporocysts is different for those rodents. In the original studies on host spectrum, sporocysts of *F. microti* were not infective to bank voles (Krampitz and Rommel 1977, Rommel and Krampitz 1978). In fact, in this paper we report for the first time natural infection of bank vole with *F. microti*. Our results suggest that in Central Europe, common voles are the main host for *F. microti*, while infections in bank vole are rather occasional. The same is probably true for yellow-necked mouse infected with *F. glareoli*, which was reported only once from a genus different than *Clethrionomys* (Doby *et al.* 1965), and in our study we report it for the first time from the genus *Apodemus*.

Relatively few studies have been done on protozoan parasites of small mammals in Central Europe, and most of them only described the parasite species spectrum (e. g., Šebek 1975a, b). Few studies report factors influencing parasite prevalences, and the results differ depending on parasite species studied. Apicomplexan infections (*Babesia*, *Hepatozoon*) were more prevalent in adult rodents, while trypanosomes in younger ones (Wiger 1979, Healing 1981). On the other hand, in a study of bank vole haemoparasites in Poland, temporal and seasonal variation was detected in prevalences, while age and sex were not important (Bajer *et al.* 2001). In our case, host sex did not influence prevalences of sarcosporidians, which is consistent with most other studies on bacterial, protozoan, and helminth parasites of rodents (Turner 1986, Healing 1981, Bajer *et al.* 2001, Behnke *et al.* 2001). Increased *S. muris* infection intensity was demonstrated for male house mouse (*Mus domesticus*) and male hybrids with *M. musculus* (Derothe *et al.* 2001), but the animals were kept under laboratory conditions. In nature, more males were found infected with *Babesia microti* than females (Krampitz and Baumler 1978).

Our study demonstrates that prevalences of brain and muscle sarcosporidians in small mammals are influenced by similar factors in different host-parasite combinations; these intrinsic factors include intermediate host habitat and age. Locality and year did not show any effect on prevalences, as well as host sex. Although the prevalence of *F. glareoli* is significantly higher than prevalence of *F. microti* in their respective intermediate hosts, both parasites successfully cycle in their final and intermediate host populations. The efficiency of transmission is proven by the prevalence of sporocysts in buzzard, the final host, which reaches 100% already at the time of nest desertion.

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