

Heteroxenous coccidia increase the predation risk of parasitized rodents

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(Received 22 January 1998; revised 11 May 1998; accepted 11 May 1998)

SUMMARY

We have investigated the influence of heteroxenous coccidia (Apicomplexa: Eimeriorina) on the predation risk of intermediate hosts. Voles infected with *Frenkelia* spp. were found more frequently in buzzards' (*Buteo buteo*) prey than among snap-trapped rodents. To eliminate the possibility of traps selecting for uninfected rodents, a laboratory experiment was performed. Mice experimentally infected with *Sarcocystis dispersa* seemed to be more likely caught by the final host, the long-eared owl (*Asio otus*); this result was confirmed by a mathematical model. Field data confirmed the adaptive value of parasite-induced changes. The increase of predation is directed towards the specific final host only or is non-specific. In the populations studied the probability of predation of parasitized individuals by the specific predator was increased.

Key words: *Frenkelia*, *Sarcocystis dispersa*, coccidia, predation, manipulation hypothesis, birds of prey.

INTRODUCTION

Changes in the behaviour of parasitized animals have been found in many host-parasite systems; some behavioural changes lead to increased risk of predation (Moore & Gotelli, 1990). For heteroxenous parasites with predator-prey life-cycles, increased predation can facilitate transmission. The manipulation hypothesis predicts that the behaviour of an infected intermediate host is manipulated by the parasite to increase its chance of being eaten by the specific final host (for review see Moore & Gotelli (1990)). Among other parasites, heteroxenous coccidia (Apicomplexa: Eimeriorina) are reported to manipulate the behaviour of their intermediate hosts. *Toxoplasma gondii* induces a wide range of behavioural changes in infected mice (Arnott *et al.* 1990 and references therein) and rats (Webster, 1994; Webster, Brunton & MacDonald, 1994) which are beneficial to the parasite (Berdoy, Webster & MacDonald, 1995). *Sarcocystis rauschorum*-infected lemmings (*Dicrostonyx richardsoni*) changed their exploratory activity (Quinn, Brooks & Cawthorn, 1987). The increased infection rates of voles (*Microtus arvalis*) in the prey of kestrels (*Falco tinnunculus*) suggests that *Sarcocystis cernae* increases the predation risk of its intermediate host (Hoogenboom & Dijkstra, 1987).

Frenkelia are heteroxenous coccidia transmitted between buzzards (*Buteo* spp.) and small rodents. The 2 species described, *F. glareoli* and *F. microti*, differ in the intermediate host spectrum. Thus *F. glareoli* develops in *Clethrionomys* spp., whereas *F. microti* can develop in several genera of rodents (Dubey, Speer & Fayer, 1989). Cysts in the brain contain asexually multiplying merozoites, infective for the final host. After ingestion by the buzzard, merozoites invade the intestinal epithelium and undergo gamogony, sporogony and sporulation. Infective oocysts/sporocysts are shed with faeces. After ingestion by a suitable intermediate host, the parasites invade firstly the liver, multiply asexually and then migrate to the brain to develop cysts (Krampitz *et al.* 1976; Rommel, Krampitz & Giesel, 1977). The life-cycle of *Frenkelia* does not differ from related species of *Sarcocystis* with birds of prey as final hosts except that infective cysts in the intermediate host develop in the brain rather than in muscles.

During field studies, relatively high prevalence of *Frenkelia* was found in populations of common buzzard (*Buteo buteo*), more than a half of nestlings being infected (Svobodová, Votýpka & Voříšek, 1995). We were therefore interested to see if *Frenkelia* are also capable of increasing the predation risk of their intermediate hosts. However, field methods do not allow us to determine whether or not the apparent increased parasite prevalence in the prey is caused by selecting for uninfected control individuals or if the infection of the host could be a consequence of behavioural changes. Therefore,

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using a related heteroxenous coccidian *Sarcocystis dispersa* (owls *Tyto alba* or *Asio otus* – *Mus musculus* s. l.) we tried to confirm field data by laboratory experiments thus avoiding a bias which could be induced by different methods of rodent catching (predator/trap).

MATERIALS AND METHODS

Field observations

The study was carried out in the Biosphere Reserve Pálava (48° 48'–48° 51' N, 16° 39'–16° 44' E) (southern Moravia, Czech Republic) in a 22.2 km² oak-hornbeam forest. The data were collected in 1994, 1995 and 1997.

The buzzards' prey was studied during the breeding season (late April to early July). The nests with nestlings were inspected from hatching to fledging. All rodents found in the nests were determined, weighed and sexed; the head was cut for assessment of *Frenkelia* prevalence. Altogether, 247 inspections in 93 nests with young were carried out.

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Frenkelia spp. prevalence in available prey was studied in snap-trapped rodents. Snap-trap lines, each containing 50 traps at 3 m intervals, were set in all potential buzzards' hunting habitats (alfalfa fields, clear-cuts, ecotones) during the nesting period (late May, early June). In most cases, the trap lines were exposed during 3 consecutive nights. The lines were checked every morning; trap-nights totalled 5009.

Apodemus flavicollis and *A. sylvaticus* were evaluated as 1 category *Apodemus* spp. because of difficulties in determination of young individuals and therefore the risk of incorrect species determination.

The infection status of both preyed and snap-trapped rodents was examined microscopically (magnification $\times 32$ –50). Whole brain was removed from the cranium, squashed between 2 microscope slides and examined without staining. Animals weighing less than the smallest individual found infected were not included in the analysis (*C. glareolus* < 12 g, *M. arvalis* < 13 g, *Apodemus* spp. < 20 g), because dif-

ferent proportions of these animals in preyed and snap-trapped groups would bias the results.

Experimental procedures

Males of laboratory mice (B10.A \times BALB/c hybrids, 120 days old) from our breeding colony were used as the intermediate host of *Sarcocystis dispersa*. Each group (infected, uninfected) contained 21 individuals, which were kept individually before starting the experiment. The mice were infected orally using a stomach tube and controls were sham-inoculated 30 days prior to the experiment. The animals were therefore in the chronic stage of infection at the beginning of the experiment. The inoculum (1200 sporocysts/mouse) originated from a dead barn owl (*Tyto alba*) found in the study area in 1994. From previous experiments we knew that the sporocysts were infective for 100% of inoculated mice ($n = 297$). The long-eared owl (*Asio otus*) was placed in the experimental light controlled room (12L:12D, 4 \times 4.5 \times 3 m) for 6 days to habituate to hunting in the room. After that, mice were placed in the room 3 days prior to the experiment. Surplus shelters for mice differing in distance from feeding/drinking sites ($n = 4$) were available. The risk of predation during feeding/drinking was different for mice occupying individual shelters. The arrangement of feeding sites did not allow mice to store food in shelters. Mice (both infected and controls) were marked on lower incisors, allowing them to be identified in owl's pellets. Complete mice skulls were present in pellets, which were collected during daily room inspections. Numbers and infection status of mice caught by the owl were recorded. Two mice were killed accidentally during the room inspections: 1 control mouse on day 8 and 1 infected on day 13 of the experiment. It was impossible to determine status and time of catching (infected versus controls) of 5 mice caught in the last 4 days of the experiment because of rapid growth of marked incisors. Therefore, results from days 16–19 were pooled. Mice remaining in the experimental room were killed and their infection status determined by preparing squashed preparations from muscles.

The long-eared owl was a wild adult individual caught in nature at its wintering site, thus its breeding cycle was not affected by the experiment. The owl was released after the experiment.

RESULTS

Prevalence of *Frenkelia* spp. in preyed and snap-trapped rodents

The prevalence of *Frenkelia* infection was higher in rodents preyed upon by buzzards than in the trapped ones ($\chi^2_{(1)} = 8.30$, $P = 0.004$; all rodents, parasite species and years pooled). However, the pattern was

Table 1. Prevalence of *Frenkelia* spp. in the prey of buzzards and in snap-trapped rodents

Year	Catching mode	<i>Microtus arvalis</i>		<i>Clethrionomys glareolus</i>		<i>Apodemus</i> spp.		All species	
		Infected (%)	N	Infected (%)	N	Infected (%)	N	Infected (%)	N
1994	Prey	10 (8.9)	112	3	6	1	4	14 (11.5)	122
	Traps	3 (2.5)	118	1	9	0	108	4 (1.7)	235
1995	Prey	0	10	0	1	1	4	1 (6.7)	15
	Traps	3 (17.7)	17	0	2	2 (1.9)	106	5 (4.0)	125
1997	Prey	2 (9.1)	22	0	1	0	5	2 (7.1)	28
	Traps	0	19	12 (17.7)	68	0	46	12 (9.0)	133
1994–1997	Prey	12 (8.3)	144	3 (37.5)	8	2 (15.4)	13	17 (10.3)	165
1997	Traps	6 (3.9)	154	13 (16.5)	79	2 (0.8)	260	21 (4.3)	493

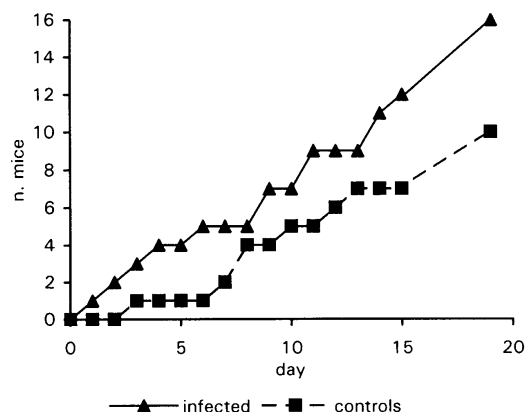


Fig. 1. Cumulative numbers of mice caught by the long-eared owl, the definitive host of *Sarcocystis dispersa*. At the beginning of the experiment, 21 *Sarcocystis*-infected and 21 sham-infected male mice were present in the experimental room. As the owl preyed upon the mice, their ratio changed.

different in particular years and species (see Table 1). Therefore, to test the influence of the method of rodent catching (traps versus buzzards) on the infection with *Frenkelia* we used logistic regression models. The first model was adjusted for year and rodent species, and interactions (the method of catching – year and method of catching – rodent species) were incorporated into the model. The deviance (*D*) of this model with 10 parameters was 241.2786. Another logistic regression model, without incorporating the method of rodent catching and its interactions (5 parameters, *D* = 258.7713) was compared with the first model by a likelihood ratio test. The models differed significantly in their ability to explain data ($\chi^2_{(5)} = 17.4927$, *P* = 0.004). Thus, we conclude that the method of catching (traps versus buzzard’s prey) is important in explaining the data.

Risk of predation in Sarcocystis dispersa-infected mice

Survival of mice under the predation pressure differed for *Sarcocystis dispersa*-infected and uninfected individuals (Fig. 1). After the end of the

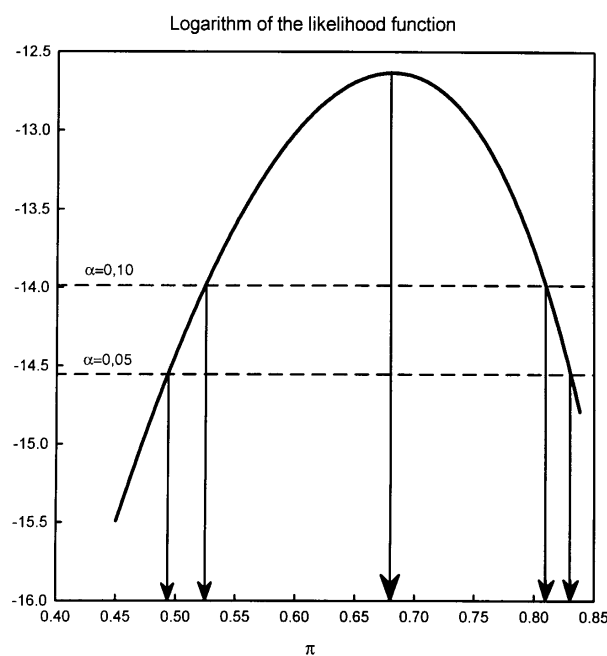


Fig. 2. Logarithm of the likelihood function of the parameter π (see text) with its maximum likelihood estimate *P* = 0.68. The arrows show 90 % confidence interval (0.52, 0.81) for π and its 95 % confidence interval (0.49, 0.83).

experiment, 4 infected and 10 uninfected mice remained in the experimental room. Therefore, 4 infected and 3 uninfected mice were preyed upon between days 16 and 19. Throughout the whole experiment, the owl caught 16 infected and 10 uninfected mice.

To describe the risk of being caught by the owl for 2 groups of mice, we developed a mathematical model. Let us assume that we have 2 mice, 1 infected and the other non-infected. Let us denote the probability that the owl will catch the infected mouse by π . Therefore, it will catch the non-infected mouse with the probability $1 - \pi$. It follows that the probability of being caught is proportional to πn^I for an infected mouse (n^I is the number of living infected mice in that time) and the same probability is proportional to $(1 - \pi)n^C$ for a non-infected mouse (n^C

is the number of living non-infected mice in that time). Therefore, we can compute the probability that in the given time-interval (1 or 4 days) d' infected mice will be caught where the total number d (infected or non-infected) mice were caught in this time-interval. Summing the logarithms of these probabilities over all time-intervals we can compute logarithm $l(\pi)$ of the likelihood function, which depends on the unknown parameter π . The maximization of $l(\pi)$ gives a point estimate $p = 0.68$. Because $2l(p) - 2l(\pi)$ has an asymptotic χ^2 distribution with 1 degree of freedom, we can determine the 95% confidence interval as (0.49, 0.83) and the 90% confidence interval as (0.52, 0.81). The last interval implies that the null hypothesis $\pi = 0.5$ tested against the alternative hypothesis $\pi > 0.5$ is rejected at the 5% level of significance. A null hypothesis that means that the survival of infected and uninfected mice is the same must be rejected. The infected mice were significantly more frequently caught by the owl than controls (Fig. 2).

DISCUSSION

The overall prevalence of *Frenkelia* spp. was 2.4 times higher in rodents preyed upon by buzzards than in snap-trapped controls. These results are similar to those obtained for *S. cernae*-infected voles (*M. arvalis*) caught by kestrels (*F. tinnunculus*) (Hoogenboom & Dijkstra, 1987).

Although the long-eared owl caught significantly more infected than uninfected mice, the direct mechanism of parasite influence remains unclear. Infected rodents could have different activity patterns, lower ability to detect the presence of predators or lower ability to escape predation (Hoogenboom & Dijkstra, 1987). The parasite could lower the social status of the infected individuals and limit their access to food and water. Because of the design of our experiment (surplus shelters, limited access to food and water, impossibility of food storing) the greatest risk of predation could be expected during feeding. The activity of infected mice is not changed in regard to the overall level nor the light/dark period distribution, and neither is the aggressivity (Votýpka, unpublished).

In our study, *S. dispersa*-induced changes in intermediate host's behaviour led to increased risk of predation by the final host. Our field studies showed that *Frenkelia* spp. increase the predation risk of the infected rodents by the specific final host. Our experiment with *S. dispersa*-infected mice confirms that the increased parasite prevalence in the diet of the birds of prey is due to the increased risk of predation. In conclusion, being parasitized by hetero-oxenous coccidia increases the predation risk of the

host and infected individuals are preyed upon with higher probability by final hosts. The behavioural changes of intermediate hosts are therefore adaptive for the parasite by increasing its transmission rate.

This study was partially supported by the Ministry of Education of the Czech Republic. We thank L. Bartoš for discussions on experimental design, P. Rödl and Administration of the Biosphere Reserve Pálava in Mikulov for logistic support.

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