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Natural infections with *Cryptosporidium* in the endangered spotted souslik (*Spermophilus suslicus*)

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Abstract

Cryptosporidium is an intestinal protozoan parasite prevalent in a wide range of mammals. Although it has been recorded in many hosts, its impact on endangered species is poorly understood. Here we present a preliminary study of four populations of the highly threatened spotted souslik (*Spermophilus suslicus*), living in the westernmost part of the species range. The populations inhabit fragmented habitats and suffer from loss of genetic variation. An IFA test revealed that 35.9% of sampled animals (41/114) was infected with *Cryptosporidium* and none with *Giardia*. The prevalence and infection intensity differed among the populations. In areas grazed by cattle it was about 3 folds higher, which suggests a possible transmission route. To the authors best knowledge the present study is the first report of *Cryptosporidium* infections in *S. suslicus*.

Keywords

Cryptosporidium, spotted souslik, *Spermophilus suslicus*, IFA, MeriFluor

Introduction

Cryptosporidium is an intestinal protozoan parasite prevalent in a wide range of mammals, including humans, domestic animals and pets. In individuals with either acquired or innate immunodeficiency *Cryptosporidium* infections cause persistent or re-emerging diarrhoea (Monis and Thompson 2003). Currently 14 *Cryptosporidium* species are considered valid and seven of them have been found to infect humans, namely *C. hominis*, *C. parvum*, *C. canis*, *C. felis*, *C. melegridis*, *C. muris* and *C. suis* (Xiao *et al*. 2004, Cacciò *et al*. 2005). Cryptosporidiosis is recognized as a zoonosis because only *C. hominis* is exclusively a human parasite (Monis and Thompson 2003). Most wildlife species investigated so far were infected with strains that currently seem to be of low public health importance (reviewed in Xiao *et al*. 2004). However, the strains that are not adapted to human host are often found in immunocompromised patients and the host specificity may change over time (Xiao *et al.* 2004). Hence, it seems useful to study natural infections with *Cryptosporidium* in wildlife to control for the overall pool of oocysts in the environment and to better understand the zoonotic potential of the parasite (Appelbee *et al*. 2005).

An important question often omitted in wildlife studies is an impact of the *Cryptosporium* on the host itself. Although *Cryptosporidium* spp. have been recorded in over 100 mammalian hosts (Appelbee *et al*. 2005) and their harmful effect on the health of young livestock and pets has been reported (Bajer 2008), the impact of protozoan infections on endangered host species remains poorly understood. Yet, monitoring health status of threatened taxa seems essential for developing accurate conservation activities. Theoretical models show that threatened populations should harbour fewer parasite species because the pathogens spread less efficiently when host population declines (Lyles and Dobson 1993). More detailed studies revealed however that a parasite may persist in declining population if it has a wide zoonotic reservoir or if the infection occurs via environment (Altizer *et al*. 2003, McCallum and Dobson 2002, DeCastro and Bolker 2005). *Cryptosporidium* is a good example of such a pathogen as it is transmitted not only by a direct contact, but also via contamined soil, water or food (Monis and Thompson 2003). Moreover, *Cryptosporidium* is harmful mostly to individuals in poor condition or with decreased immunocompetence, and it is likely in a case of threatened species which usually suffer from low genetic variance and negative effects of inbreeding (Radwan *et al*. 2010).

In the present paper we examine four populations inhabiting the westernmost part of the species range. Our aim was to assess the occurrence of intestinal protozoans (*Giardia* and

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Cryptosporidium) in the spotted souslik, and to evaluate possible transmission routes involving the host. The spotted souslik is classified as „Vulnerable" in the IUCN Red List and as „Endangered" in the Polish Red Data Book. It is a medium-size ground squirrel that inhabits open, grassy habitats, such as steppes, meadows and pastures in Eastern Poland, Ukraine, Moldova, and the European parts of Russia. The souslik lives in colonies in a system of burrows. Typically, a colony comprises up to several hundred animals and occupies a relatively small area (20–60 ha). In the last few decades, in the western part, of its range the spotted souslik has undergone a severe decline: of 143 colonies observed in Poland in the 1950s (Surdacki 1963) there are only seven left nowadays (Gondek 2004). Recent studies have shown that surviving populations suffer from low genetic diversity, what may make them particularly susceptible to pathogens (Biedrzycka and Konopiński 2007, Biedrzycka and Radwan 2008).

Materials and methods

Animals and samples

Samples were collected during June/July 2007 in a field study concerning the evolutionary ecology of the souslik (results to be published elsewhere; see Biedrzycka *et al*. 2011). The species is strictly protected by local law which prevented multiple sampling of one population over a season. Sousliks hibernate from early autumn till March, they mate in April and the offspring are born in May. Since we were permitted to capture animals only once, we chose the period when the animals were still active, but juveniles had already left the mothers (Próchnicki 2008).

We sampled four populations: two in Western Ukraine (Boriatyn, 24°11´E, 50°26´N and Murowane, 24°06´E, 50°26´N) and two in Eastern Poland (Chochłów, 24°01´E, 50°29´N and Popówka, 23°30´E, 50°45´N). Nature reserves have been established in the territories of both Polish colonies. In Ukraine, the spotted souslik is not protected. Population Boriatyn is situated on an extensively grazed pasture and population Murowane inhabits a meadow.

Sousliks were captured in self-made wire-mesh tubes placed at the entrance to burrows (Biedrzycka and Konopiński 2007). Trapped animals were weighed and sexed based on the anogenital distance (Brower 2006). In males the distance between anus and penis is much larger than the distance between anus and vagina in females. We were not able to determine unambiguously the sex of some juveniles (9 individuals) so they were excluded from analyses concerning differences between sexes. Captured sousliks were temporary marked by cutting their tail tips to avoid sampling the same individual twice, and to obtain blood and tissue for further analyses (not described here). Sousliks were kept in traps until defecation which usually occurred immediately after capturing. The pellets were collected and stored in 10% formaldehyde.

Field procedures were carried out according to the Ethical Committee decision no.: 48/OP/2002 and the Polish Ministry of Environment permits no.: DOPog-4201-04-73/03/jr and DOPog-4201-04-22/04/jr.

Detection of Cryptosporidium *and* Giardia

From each individual we collected approx. 1–1.5 ml of fresh faeces. Prior to analysis, preserved samples were diluted in 10 ml of phosphate buffered saline (PBS) pH 7.2 and left overnight in –4°C. The upper layer of the sediment was transferred to a test tube (1.5 ml), centrifuged and diluted in 10% formaldehyde. Aliquots (20 µl) were examined using direct immunofluorescence test (MeriFluor®Cryptosporidium/Giardia, Meridian Diagnostics, Cincinnati, Ohio, USA). The samples were stained according to the manufacturer's instructions. The cysts and oocysts were identified on the basis of shape (*Cryptosporidium*: round to slightly oval, 2–6 µm in diameter, *Giardia*: oval, 8–12 µm long) and fluorescent, bright green colour. We used positive and negative controls provided by the manufacturer.

Statistical analysis

The occurrence of oocysts in faeces was expressed as a binary variable (presence/absence) and analysed using generalized linear models with binomial error distribution and logit link function. The number of oocyst per ml of faeces describing infection intensity was log (x + 1) transformed and incorporated in generalized linear models with Poisson error and log link function. The models were fitted using glm functions in the R statistical package (R Development Core Team 2010).

The explanatory variables in both models included sampling site, host sex and body mass. First we fitted a saturated model including all the main effects and second-order interactions. The model was simplified by deleting the interactions that did not increased significantly the amount of variation explained. The significance of terms was deletion tested basing on the difference in model deviance between models containing the given explanatory variable and without it (Crawley 2007).

Results

Overall, we captured 114 animals from 4 sites. The number of sampled animals was roughly 5–10% of the estimated population size (Table I). In site Popówka the estimates of population size, performed by various authors, differed considerably (Biedrzycka and Konopiński 2007, Próchnicki 2008). There is, however, no reason to suppose that the trapping success in this site differed from other sites, as we used the same number of traps for a similar period of time. Hence, it is most likely that the number of animals captured in this colony was also close to 5% of the whole population size.

Table I. Age and sex of captured sousliks compared to estimated colony size in four sampling sites

Site	Estimated population size	Captured	By sex			By age	
			females	males	undetermined	adults	juveniles
Boriatyn (UA)	500[2]*	21	9	6	6	11	10
Chochłów (PL)	680[1]	52	30	19	3	28	24
Murowane (UA)	500[2]*	19	8	11	0	11	8
Popówka (PL)	50[2]–2570[1]	22	11	11	0	21	1
Overall	–	114	58	47	9	71	43

[1]Próchnicki 2008, [2]Biedrzycka and Konopiński 2007, *approximated value.

Table II. Prevalence and abundance of *Cryptosporidium* infections by sampling site

Site	% Infected	Abundance	
		geometric mean no. of oocysts/1 ml of sediment	95% CL
Boriatyn (UA)	52.3	86.90	13.00–549.81
Chochłów (PL)	23.0	3.52	0.39–14.31
Murowane (UA)	21.1	1.92	0–14.31
Popówka (PL)	63.6	21.91	0.79–292.77

CL – confidence limits.

None of the animals were infected with *Giardia* spp. and further analyses presented here concern *Cryptosporidium* infections exclusively (Fig. 1). The overall prevalence was 35.9% and infected animals were found in each of the sampled colonies (Table II). The average oocyst size was 4.35 µm (range 3.18–5.38 µm, SD = 0.43 µm) × 4.96 µm (range 3.97–

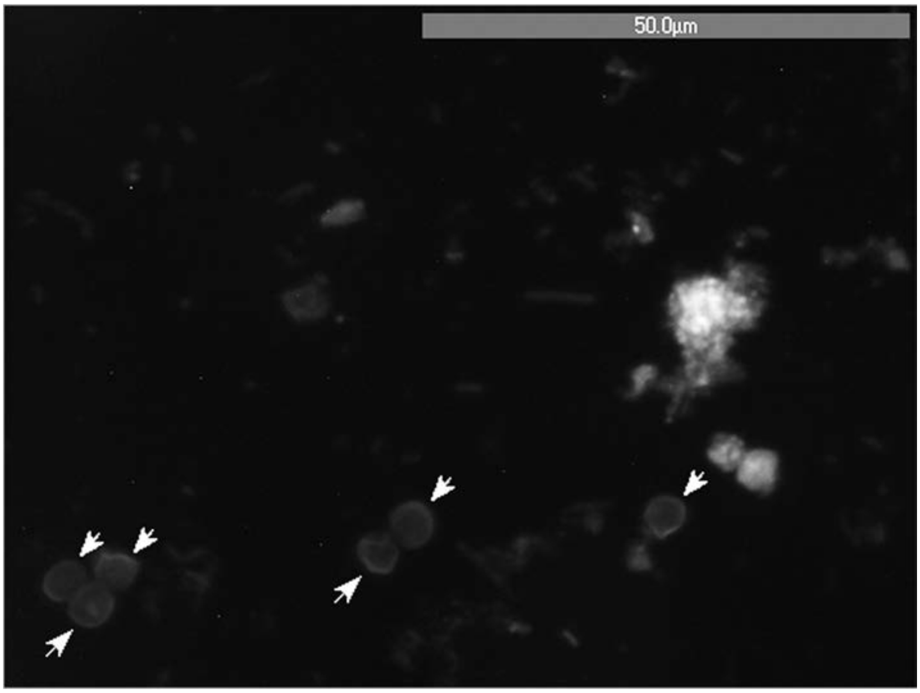

Fig. 1. Photograph of the *Cryptosporidium* oocyst detected via IFA MeriFluor test

Table III. Results for generalized linear models for (a) parasite occurrence and (b) infection intensity in the spotted souslik from 4 populations. The tables present p-values for all deletion-tested explanatory variables. Effects constituting the minimal model are given in bold

(a) Occurrence of infection

Effect	df	χ^2	p
mass × sex × population	3	0.114	0.990
mass × population	3	0.285	0.963
mass × sex	1	0.483	0.487
sex × population	**3**	**9.486**	**0.024***
population	**3**	**14.450**	**0.003****
body mass	1	0.002	0.965
sex	**1**	**0.039**	**0.844**

(b) Infection intensity

Effect	df	F	p
mass × sex × population	3	0.200	0.896
mass × population	3	0.107	0.954
mass × sex	1	0.230	0.632
sex × population	**3**	**2.655**	**0.053**
population	**3**	**4.452**	**0.006****
body mass	1	0.283	0.596
sex	**1**	**0.353**	**0.554**

df – degrees of freedom, χ^2 and F – test statistics, p –probability.

6.08, SD = 0.37), basing on 85 measurements of 85 random oocysts.

The reduced model of parasite occurrence comprised population, sex and sex × population interaction (Table IIIa). The occurrence of *Cryptosporidium* differed significantly between populations (χ^2 = 14.498, df = 3, p = 0.003): the number of infected animals in site Popówka (63.6%) and Boriatyn (52.3%) was about two times higher than in sites Chochłów and Murowane (23 and 21.1% respectively, Table II). We found also significant interaction between host sex and population (χ^2 = 9.486, df = 3, p = 0.024). In site Chochłów females were more often infected than males (33.3% vs 10.5%; Fig. 2a) but in other sites the pattern was opposite: males were infected more often, and in site Murowane males were the only sex excreting *Cryptosporidium* oocysts.

The reduced model of infection intensity consisted of the same explanatory variables as a model of parasite occurrence, i.e. population, host sex and interaction between sex and population (Table IIIb). The mean number of oocysts shredded by sousliks differed between populations (F = 4.4521, df = 3, p = 0.0057) and was highest in site Boriatyn and lowest in Murowane (Table II). The interaction between host sex and sampling site was marginally insignificant (F = 2.655, df = 3, p = 0.0533) and we observed a similar pattern as in the model for prevalence (Fig. 2b).

We did not observed any signs of diarrhea or visual evidence of weak condition in the infected animals which suggests that the infections were asymptomatic and rather mild. The body mass which may be considered as a predictor of host condition did not affect the risk of infection (χ^2 = 0.0019, df = 1, p = 0.965) nor on the number of oocysts per gram of faeces (F = 0.5957, df = 1, p = 0.2833).

Discussion

Studies focusing on the occurrence of *Cryptosporidium* in protected species are relatively rare compared to the numerous reports on this parasite in non-endangered wildlife. To our best knowledge, the present paper is the first report of *Cryptosporidium* in the highly threatened spotted souslik *S. suslicus*. The percentage of infected animals reported in the present study was similar to that recorded in wild rodents (54% in bank vole, 62% in common vole; Bajer 2008) but relatively high (21–63%) compared to other species of conservation concern. In Europe *Cryptoporidium* was found in 29% of faecal samples from the European bison, 19% from the beaver and 35% from the grey wolf (Paziewska *et al*. 2007). In tropical zone it was recorded in several endangered mammal species such as mountain gorillas (11% infected; Nizeyi *et al*. 2002), olive baboons (32% infected; Hope *et al*. 2004), and bilbies (39.2% infected; Warren *et al*. 2003).

The risk of infection seems to be significantly influenced by environmental factors. In gorillas most infections occurred in individuals living in proximity to humans (Nizeyi *et al*. 2002), and the prevalence in wild beavers was about three

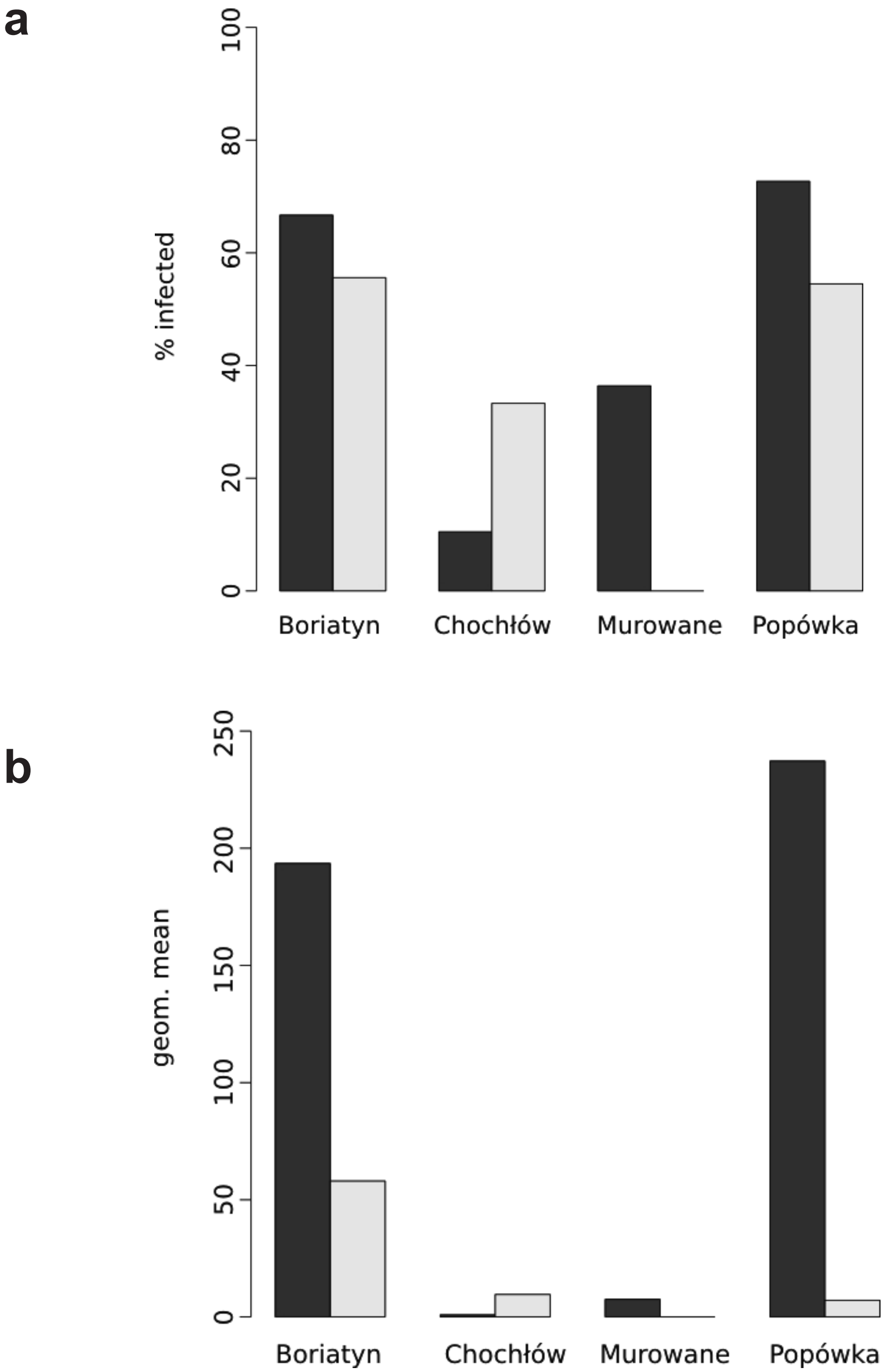

Fig. 2. The differences in (a) prevalence and (b) infection intensity among sexes. Dark-coloured bars represent males, light-coloured bars represent females

times higher than in captive ones (Paziewska *et al*. 2007). Also, the prevalence of parasites tends to be higher in animals living in fragmented habitats. Two primates, the Tana River red colobus and the mangabey, inhabiting endemically fragmented, forests in Eastern Kenya were considerably more often infected by gastrointestinal parasites than their conspecifics from other habitats (Mbora and McPeek 2009). Similarly, spotted sousliks from the central part of the species range were free from *Cryptosporidium* (Kloch, unpublished), but the prevalence in fragmented populations from the verge of the range was high, as reported in the current paper.

In the present paper we found significant differences between populations in the number of animals infected. As discussed below, we hypothesize that the observed pattern may arise from a presence of other host species that may act a main source of infection for sousliks. The high number of infected souslik does not, however, implicate that *Cryptosporidium* pose a serious threat to the host health as we found neither apparent symptoms of cryptosporidiosis in the infected animals nor an effect of infection on condition characterized by host body mass. However, the decline of the westernmost populations of the spotted souslik occurred relatively recently (Biedrzycka and Konopiński 2007, Biedrzycka and Radwan 2008) and one may hypothesize that the negative effects of inbreeding and/or loss of genetic variation have not yet been expressed in the populations examined in the current paper. Nonetheless, it is crucial to monitor potentially disastrous pathogens because it may help to undertake appropriate conservation activities if the condition of the population worsens.

We found significant differences in prevalence between sexes. Interestingly, in three of four sampling sites males were infected more often than females, which is opposite to the pattern usually observed in rodents (Bajer 2008). In the spotted souslik males wake from the hibernation about two weeks earlier than females and during the mating season they fight severely for females (Próchnicki 2008). This may cause them to be more vulnerable to infections as the androgens are known to act as immunosuppressants (Alexander and Stimson 1988).

Important aspect of the *Cryptosporidium* infection in spotted sousliks is their role as a zoonotic reservoir of the pathogen. Zoonotic potential of wildlife is currently widely discussed (Appelbee *et al*. 2005, Cacciò *et al*. 2005) and despite several reports on infections from wild hosts in rural areas, the significance of this transmission route remains unclear (Xiao *et al*. 2004). Wildlife most likely act as a secondary source of infection, although the prevalence of *Cryptosporidium* in rodents is often very high (e.g. Bednarska *et al*. 2007) and possible transmission from wild rodents to cattle has been recorded in English farms (Sturdee *et al*. 2003). Sousliks examined in this study inhabit pastures or meadows mown for hay. Importantly, we found that the prevalence in sites grazed extensively by cattle (Boriatyn, Popówka) was over two times higher than in sites where grass was used only for hay or grazed sporadically (Chochłów, Murowane). The shape and size of oocysts found in the current study suggest that sousliks are infected with *C. parvum* rather than *C. muris*. Although rodents are most often infected by the "mice" genotype of *C. parvum*, the "cattle" genotype was found in isolates from mice inhabiting farms in Australia, which suggests that transmission from livestock to wild rodents may occur (Morgan *et al*. 1999). Hence, transmission between sousliks and cattle in the investigated area seems possible. However, to evaluate the zoonotic potential of the spotted souslik further studies concerning molecular characterisation of the *Cryptosporidium* parasite are necessary.

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