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Gastrointestinal parasites and the first report of *Giardia* spp. in a wild population of European brown bears (*Ursus arctos*) in Croatia

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ABSTRACT

Ninety-four European brown bear (*Ursus arctos*) faecal samples, collected in three counties of Croatia, were examined for the presence of gastrointestinal parasites. Five genera were identified, including the nematodes *Baylisascaris transfuga* and *Syngamus* sp., and the protozoan enteropathogens *Cryptosporidium* sp., *Eimeria* sp., and *Giardia* sp. Ancylostomatid eggs were also recovered. Cestodes, trematodes and acanthocephalan eggs were absent from all samples. This is the first parasite survey of brown bears in Croatia in thirty years and the first report of *Giardia* in this species from the region.

Key words: brown bear, *Ursus arctos*, gastrointestinal parasites, *Giardia*, Croatia

Introduction

The brown bear (*Ursus arctos*) in Europe is present in 22 countries. Based on the existing distribution data, as well as a range of geographic, ecological, social and political factors, they can be clustered into ten populations: Scandinavian, Karelian, Baltic, Carpathian, Dinaric-Pindos, Eastern Balkan, Alpine, Central Apennine, Cantabrian, and Pyrenean. The estimated total number of brown bears in Europe is approximately 17,000

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individuals. Most of these populations are strictly protected and are currently increasing (KACZENSKY et al., 2013).

The third largest bear population in Europe is found in the largely ecologically and biologically conserved Dinaric-Pindos mountain range, which includes Croatia (HUBER et al., 2008). The brown bear in Croatia is managed as a game species with an annual hunting quota of 10 % of the population or approximately 100 bears. In the core bear area, the density can reach 20 bears per 100 km^2, which is amongst the highest globally (HUBER et al., 2008).

Hunting organisations in Croatia are permitted to use supplementary feeding with plant and meat products, including domestic animal carcasses, to attract bears. At these sites, bears, which are normally solitary animals, associate with their conspecifics, as well as with other species. These sites may therefore act as a platform for parasite transmission.

There are limited reports of ursid parasites from this geographic region. These include a recent Croatian wildlife study, specifically of *Giardia* (BECK et al., 2011), and wildlife parasite surveys performed at least thirty years ago, two in Croatia (HUBER and EHRLICH, 1981; HUBER and ŠTAHAN, 1983) and one in Slovenia (BRGLEZ and VALENTINČIČ, 1968). This pilot study was conducted to obtain preliminary baseline data on the gastrointestinal parasite fauna of brown bear in Croatia.

Materials and methods

Ninety-four fresh bear faecal samples were collected from 8 different sites in Croatia (Fig. 1) during the autumn of 2009. Bear faeces were recognized by their distinctive size, shape and smell, and only scats deposited within the past 24 hours were collected. The samples from Štrbački buk were from a mother and cub that had been killed by a train, and from Klek, they included one hunted bear. We collected adult *Baylisascaris transfuga* from two of these bears at necropsy. All samples were stored in 95 % ethanol and shipped to the Royal Veterinary College (RVC), London, UK.

Faeces (approximately 4 g) were analysed using standard sedimentation and Foreyt's (FOREYT, 1994) standard sugar flotation (specific gravity 1.3), for the detection of helminth eggs, protozoa-oocysts and -cysts. Additionally, faecal smears were stained with carbol-fuchsin (HEINE, 1982) for the detection of *Cryptosporidium* spp. oocysts. The slides were examined using an Olympus CX31 light microscope (Olympus Microscopy, Southend-on-Sea, UK) at x 400. DNA was extracted and PCR performed on seven of the *B. transfuga* infected faecal samples identified by flotation, as described by DE AMBROGI et al. (2011).

Fig. 1. Study area in Croatia showing the eight sampling sites: Ravna Gora (1), Gomirje (2), Vrbovsko (3), Velika Kapela (4), Risnjak (5) and Smrekova draga (6) in Primorje-Gorski kotar county, Klek (7) in Karlovac county and Štrbački buk (8) in Lika-Senj county

Results

The overall prevalence for gastrointestinal parasites in our study was 33 %. The prevalence of single nematodes was 11.7 % for *B. transfuga*, 10.6 % for the Ancylostomatidae, and 1.1 % for *Syngamus* sp. (Table 1). Most of the ancylostomatid-eggs found carried morulae stages instead of larvae stages. Twenty one per cent of the samples carried at least one of these nematodes. Using flotation, we found *B. transfuga* eggs in eleven faecal samples from four sites, three in the Primorje-Gorski kotar county and one in the Karlovac county. Ancylostomatid eggs were found in ten scats from three sites in Primorje-Gorski kotar, one in Karlovac, and one in Lika-Senj. *Syngamus* sp. was found in one faecal sample from Gomirje.

The prevalence for single gastrointestinal protozoa was 8.5 % for *Cryptosporidium* sp., 4.2 % for *Giardia* sp. and 1.1 % for *Eimeria* sp. (Table 1). *Giardia*-cysts had a rather oval form and an average size of 15 µm (Fig. 2A) whereas *Cryptosporidium*-oocysts had a rounded shape and an average size of 5 µm (Fig. 2B). Fourteen percent of the samples carried at least one of these protozoan genera. We found *Giardia* in four scats from four sites, and *Cryptosporidium* sp. in eight scats from three sites, all in Primorje-Gorski county. *Eimeria* sp. was found in one faecal sample from Ravna Gora.

Table 1. Gastrointestinal parasites found in brown bears (*Ursus arctos*) from Croatia detected by coprological examination (flotation/sedimentation techniques) and the faecal smear method (carbol-fuchsin staining) according to their geographic sites

		Nematodes			Protozoa		
Geographic sites	Sample number	Ancylostomid	*Baylisascaris transfuga*	*Syngamus* sp.	*Cryptosporidium* sp.	*Giardia* sp.	*Eimeria* sp.
Ravna Gora	40	0	0	0	10.0 %	2.5 %	2.5 %
Gomirje	22	9.1 %	13.6 %	4.5 %	13.6 %	4.5 %	0
Vrbovsko	11	0	0	0	9.1 %	0	0
Velika Kapela	7	14.3 %	71.4 %	0	0	0	0
Risnjak	3	0	0	0	0	33.3 %	0
Smrekova draga	4	100.0 %	25.0 %	0	0	25.0 %	0
Klek	5	40.0 %	40.0 %[1]	0	0	0	0
Štrbački buk	2	50.0 %	0[1]	0	0	0	0
Total	94	10.6 %	11.7 %[1]	1.1 %	8.5 %	4.2 %	1.1 %

[1] Two bears were positive for *B. transfuga* at necropsy, but were negative for this species on flotation. If the necropsy results are included the total prevalence for this species becomes 13.8 %. These bears were from Klek and Štrbački buk, so the prevalence becomes 60 % and 50 % for these sites respectively.

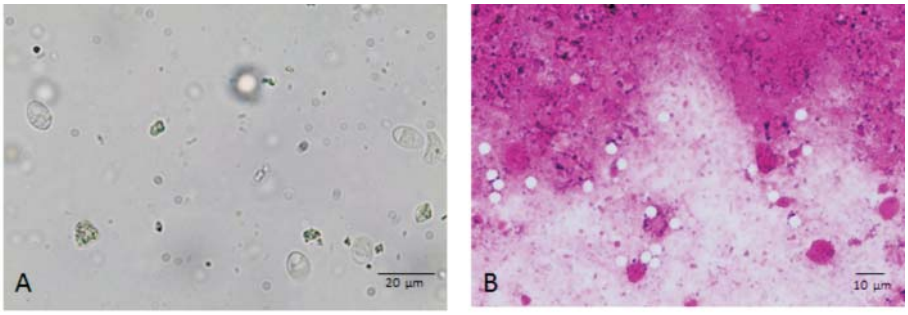

Fig. 2. Protozoan parasite stages found in faecal samples of brown bears (*Ursus arctos*). (A) *Giardia* spp. cysts, (B) *Cryptosporidium* spp. oocysts detected by conventional carbol fuchsin staining method

Discussion

Previous studies in this region reported *B. transfuga* and strongylid parasites from bear faecal and gastrointestinal samples in Croatia (HUBER and EHRLICH, 1981; HUBER and ŠTAHAN, 1983) and *B. transfuga*, *Trichuris* sp., and adult and larval *Taenia* sp. (probably *T. hydatigena*) from gastrointestinal post-mortem samples in Slovenia (BRGLEZ and VALENTINČIČ, 1968). All three of these studies stated that more complete examinations could have been performed. In our samples we did not find trematodes or cestodes, but we did find three genera of protozoan parasites, *Cryptosporidium*, *Giardia*, and *Eimeria*, which have not been previously reported from bears in this region. None of the studies in this region, including ours, reported acanthocephalans from bears.

Nematode eggs can often only be identified to genus by microscopy. We identified only *B. transfuga* to species level, which was done as part of another project to develop a species-specific PCR for these parasites (DE AMBROGI et al., 2011). We did not perform additional analyses, such as PCR and sequencing, for other parasite genera because this was a pilot study.

Surprisingly, flotation results for the two animals from which adult *B. transfuga* were obtained at necropsy, were negative for parasites. The adult *B. transfuga* obtained from both these animals included gravid females. We suggest that the false flotation *B. transfuga*-negative results may be a consequence of differential egg shedding rates, as has been demonstrated for *B. procyonis* in raccoons (REED et al., 2012). Our PCR assay detected *B. transfuga* DNA in faecal samples from both animals. This suggests that the flotation-derived *B. transfuga* prevalence is an underestimate. If we include the two necropsied bears, the prevalence becomes 13.8 %. *Baylisascaris transfuga* has been widely reported from black and grizzly bears in North America, including CRUM et al.

(1978), GAU et al. (1999) and FOSTER et al. (2004). In addition to the reports from Croatia (HUBER and EHRLICH, 1981; HUBER and ŠTAHAN, 1983; DE AMBROGI et al., 2011), and Slovenia (BRGLEZ and VALENTINČIČ, 1968), this species has also been reported from Slovakia (FINNEGAN, 2009). Of these European studies, Brglez and Valentinčič reported a *B. transfuga* prevalence of 20 % in spring and autumn, and Finnegan reported an autumn prevalence of 70.8 %, both of which were higher than the prevalence reported in this study.

Morphologically, we could not establish whether the Ancylostomid eggs we found were a species of *Ancylostoma* or of *Uncinaria*. PCR identification of strongyle eggs would therefore be optimal for future studies. This, in conjunction with necropsies of hunted bears, should establish the actual prevalence of strongylids infecting bears, as opposed to the presence of spurious parasites. *Ancylostoma caninum* and *A. tubaeforme* have been reported from black bears (CRUM et al., 1978; FOSTER et al., 2004), and *U. yukonensis* and *U. rauschi* have been reported from black and grizzly bears (CHOQUETTE et al., 1969; OLSEN, 1968). Also, in 1953 and 1962, scientists from the former USSR documented *U. stenocephala* in brown bears (ROGERS and ROGERS, 1976).

We found *Syngamus* sp. in only one faecal sample and we do not consider it to be pathogenic to bears, as it is unlikely that this genus lives in the bear gastrointestinal tract, and was probably part of an intestinal passage after ingestion of birds or earthworms.

Our prevalence for *Cryptosporidium* sp. was lower than that (55.6 %) of RAVASZOVA et al. (2012) for bears of the Slovak Republic. The latter results were obtained using a *Cryptosporidium* spp. coproantigen ELISA. When these authors retested the samples using faecal smears, they obtained a prevalence of 26.98 %. Faecal smears require the presence of fully developed oocysts of *Cryptosporidium* sp. and are therefore less sensitive than coproantigen ELISAs, but ELISAs may yield false-positive results (CIRAK and BAUER, 2004). It would be optimal to use commercial tests, such as a coproantigen ELISA, in addition to our methods for future studies. We do not know whether the bears in our study were suitable definitive hosts for *Cryptosporidium* or whether the oocysts merely passed through the intestine after ingestion of an infected dietary item. In support of the former, intestinal developmental stages of a new genotype of *C. parvum* have been described from black bear (DUNCAN et al., 1999; XIAO et al., 2000).

The very low prevalence of *Eimeria* in our samples could indicate that they occurred incidentally via infected prey. However, the genus *Eimeria* is generally monoxenic and broadly distributed in wild animal populations (BARRET and DAU, 1981), thus these oocysts could be enzootic in brown bears. The oocysts were all unsporulated, and therefore not adequate for morphological species identification. They had a thin, white-grey double oocyst wall and no micropyle. Future studies should involve additional methods such as PCR to further identify these parasites, and gastrointestinal histology

to determine whether bears in this region serve as definitive hosts for this genus. Only three *Eimeria* species have been described from bears, *E. albertensis* and *E. borealis* from black bears (HAIR and MAHRT, 1970) and *E. ursi* from brown bears (YAKIMOFF and MATSCHOULSKY, 1935). GAU et al. (1999) reported 14 % coccidia prevalence in grizzly bear scats, but did not further identify them.

The presence of *Giardia* sp. in four faecal samples was unexpected. To our knowledge, this is the first report of giardiosis in bears from Croatia. This is in contrast to BECK et al. (2011), who, using immunofluorescence microscopy, did not find *G. duodenalis* cysts in bear scats from Croatia. Their sample size may have been too low (n = 19) for *Giardia* detection. Also, the faecal samples were from hunted bears over three years old, and *Giardia* is most prevalent in young animals (JOHNSTON et al., 2010). It would be of interest therefore to apply immunofluorescence techniques to a greater number of samples and to ensure that they include young animals. *Giardia* has been reported from grizzly bear scats in Canada (ROACH et al., 1993). Although there is no literature available on *Giardia* spp. infections in European brown bear, there is evidence that cross-species transmission of *Giardia* may occur where ecological overlap between species is high (JOHNSTON et al., 2010), such as at supplementary feeding sites. However, despite several studies examining faecal samples in areas with enzootic *Giardia*, including DAVIES and HIBLER (1979) and ROACH et al. (1993), there are no reports from black bears. Indeed, Davies and Hibler failed to infect a black bear cub experimentally with *Giardia* cysts.

This pilot study was undertaken to provide preliminary baseline data on the parasite fauna of ursids in Croatia. The results have revealed that this species may be infected by a range of nematode and protozoan parasites, including *Giardia* sp., which to our knowledge, has not been reported from brown bear in this region. We call for additional, funded parasite surveys of bears in the region, utilizing commercial tests and genus-specific PCRs in addition to faecal flotation and sedimentation. The gastrointestinal tracts of hunted bears should also be examined for parasites to distinguish true infections from the presence of spurious parasites. Finally, *Giardia* sp., *Cryptosporidium* sp., and some species of the family Ancylostomatidae, may be zoonotic under certain conditions. However, currently these are not known to be zoonotic with bears as the source of infection. Nevertheless, because of the lack of recent parasite survey data for the European brown bear, the relationship between these species and humans may warrant further evaluation.

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SAŽETAK

Devedeset i četiri uzorka izmeta europskoga smeđeg medvjeda (*Ursus arctos*), sakupljena u tri županije u Hrvatskoj, pregledana su na prisutnost želučano-crijevnih nametnika. Utvrđeno je ukupno 5 rodova, uključujući nematode *Baylisascaris transfuga* i *Syngamus* sp., te enteropatogene protozoe *Cryptosporidium* sp., *Eimeria* sp. i *Giardia* sp. Utvrđena su i jaja ankilostomatida dok jaja cestoda, trematoda i akantocefala nisu pronađena. Ovo je prvo parazitološko istraživanje smeđeg medvjeda u Hrvatskoj u posljednjih 30 godina i prvi nalaz *Giardia* spp. u ove vrste s ovog područja.

Ključne riječi: smeđi medvjed, *Ursus arctos*, želučano-crijevni nametnici, *Giardia*, Hrvatska