HELMINTHOLOGY - ORIGINAL PAPER

Gastrointestinal parasites of arctic foxes (*Vulpes lagopus*) and sibling voles (*Microtus levis*) in Spitsbergen, Svalbard

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Abstract

The arctic fox (*Vulpes lagopus*), an apex predator with an omnipresent distribution in the Arctic, is a potential source of intestinal parasites that may endanger people and pet animals such as dogs, thus posing a health risk. Non-invasive methods, such as coprology, are often the only option when studying wildlife parasitic fauna. However, the detection and identification of parasites are significantly enhanced when used in combination with methods of molecular biology. Using both approaches, we identified unicellular and multicellular parasites in faeces of arctic foxes and carcasses of sibling voles (*Microtus levis*) in Svalbard, where molecular methods are used for the first time. Six new species were detected in the arctic fox in Svalbard, *Eucoleus aerophilus*, *Uncinaria stenocephala*, *Toxocara canis*, *Trichuris vulpis*, *Eimeria* spp., and *Enterocytozoon bieneusi*, the latter never found in the arctic fox species before. In addition, only one parasite was found in the sibling vole in Svalbard, the *Cryptosporidium alticolis*, which has never been detected in Svalbard before.

Keywords Parasites · Svalbard · Arctic fox · Sibling vole · Coprology

Introduction

Arctic foxes have a circumpolar distribution and are apex predators in Arctic ecosystems (Audet et al. 2002). In the

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High Arctic Svalbard archipelago, they are scavengers and top predators of both marine and terrestrial ecosystems (Ehrich et al. 2015; Eide et al. 2012). The arctic fox is an important vector of zoonotic pathogens, and knowledge of their health status is of great importance for the ecosystem (e.g. Andreassen et al. 2017; Skírnisson et al. 1993; Sørensen et al. 2005). Gastrointestinal parasites may cause effects on vital demographic parameters like decreased survival and fecundity rate (Anderson and May 1978). It can also contribute to documented increased mortality rates in arctic foxes, especially in juveniles (Meijer et al. 2011). In combination with other stressors such as harvesting, pollutant levels, and climate change, parasites may contribute to unknown cumulative effects on Arctic fox populations.

Arctic foxes are apex predators and scavengers with an opportunistic and generalistic feeding behaviour (Eide et al. 2012; Ehrich et al. 2015). They live in two main tundra types, inland and coastal, that differ in the type of available food sources (Braestrup 1941). Rodents represent the main food source for the inland fox populations, e.g. in North America, Eurasia, and in east Greenland, while the coastal ecotype lives in areas where rodents like lemmings are absent, with a diet mainly from the marine food web like seabirds, eggs, fish, crustaceans on the seashore, or carrions of seals and reindeer (Angerbjörn et al. 2004; Hersteinsson and Macdonald 1996;

Prestrud et al. 1992). As top predators, living in both inland and coastal ecosystems, one might expect a broad species diversity of intestinal parasites in arctic foxes.

In Svalbard, arctic foxes belong to the coastal ecotype and they have to survive even in the absence of rodents, apart from a small introduced population of sibling voles (*Microtus levis*). The sibling voles were introduced with the cattle feed between 1920 and 1960, but with a very restricted area of distribution (Henttonen et al. 2001). Other year-round resident species in Svalbard that are important in the diet of the arctic fox are carcasses of Svalbard reindeer (*Rangifer tarandus platyrhynchus*) and the Svalbard rock ptarmigan (*Lagopus muta hyperborea*). In summer, the archipelago is visited by numerous numbers of migrating seabirds and terrestrial birds (e.g. geese), and there is a large variety of food items from the marine ecosystem (Eide et al. 2012). With the lack of small rodents like lemmings, Svalbard has relatively low biodiversity, making the number of parasite species in arctic foxes low, compared with other parts of the Arctic (e.g. Jónsdóttir 2005). However, arctic foxes may still carry a considerable number of parasites (e.g. Henttonen et al. 2001).

To date, several intestinal parasites have been recorded in Arctic foxes in Svalbard (Fuglei et al. 2008; Henttonen et al. 2001; Stien et al. 2010). The documented parasite fauna includes cestodes *Echinococcus multilocularis*, *Diphyllobothrium* sp., *Taenia ovis krabbei*, *T. crassiceps*, and *T. polyacantha*. The latter two cestodes together with *E. multilocularis* use rodents as intermediate hosts and depend on sibling voles that inhabit a very restricted area in Svalbard, while *T. ovis krabbei* uses reindeer and *Diphyllobothrium* sp. uses fish or invertebrates as intermediate hosts. Moreover, two additional parasites were found: an unidentified ascarid nematode and an unidentified acanthocephalan. Metacestodes of *E. multilocularis* were also detected in sibling voles in 1999 and 2001–2006, and in 2003, two voles were positive for *T. crassiceps* (Henttonen et al. 2001; Stien et al. 2010).

In the course of this study, faeces were collected around arctic fox dens during the annual den surveys conducted by the Norwegian Polar Institute as well as when found on the ground while doing field work. Sampling was followed by microscopy together with extended use of molecular methods for the detection of parasites. Part of the samples originated from the sibling vole distribution area in Longyearbyen, located 10 km east of Grumantbyen, the core area of the sibling voles (Henttonen et al. 2001). For that reason, we also captured sibling voles and included data based on their dissections.

Materials and methods

Faeces of Arctic foxes were collected during the summer seasons in 2012 ($n = 10$), 2013 ($n = 12$), and 2015 ($n = 40$) from three different sites in Longyearbyen, Sassendalen, and Billefjorden in the central part of Spitsbergen, the largest island of the high Arctic Svalbard archipelago (Fig. 1). Faeces samples were collected from the ground; thus, it cannot be excluded that more samples were collected repeatedly from a single host individual. Faeces were stored in sealed plastic bags, and subsequently forwarded for laboratory analyses up to 3 weeks post collection to laboratories in Centre for Polar Ecology, University of South Bohemia in České Budějovice, Czech Republic). All samples were transported and stored at 4–8 °C prior to being used for laboratory analyses.

Sibling voles ($n = 63$) were trapped (spring-loaded bar mousetrap) during summer in 2017 (research registered in The Research in Svalbard Database, permission RiS-10852). Traps were baited with almond and placed at two locations in Longyearbyen the largest of the four human settlements in Svalbard. One site was located around a horse stable (78° 14′ 32″ N 15° 31′ 42.5″ E) and one in Nybyen by an abandoned dog yard (78° 11′ 44″ N 15° 33′ 39.4″ E). Traps were checked every 2–3 h with voles dissected immediately after capture. Vole carcasses from the 2014 and 2015 seasons were acquired from local people from whole Longyearbyen (caught during the winter and spring and kept frozen until summer) and were used for the purpose of this study. Vole organs were examined macroscopically for the presence of metacestodes, and with special attention to liver for the presence of *E. multilocularis*. The intestinal contents were stored in screw top plastic tubes in 5% potassium dichromate solution at 4–8 °C and subsequently forwarded for laboratory analyses (laboratories in Centre for Polar Ecology, University of South Bohemia in České Budějovice, Czech Republic).

Faecal samples of foxes and voles' intestinal content from necropsy were initially screened for helminth and protozoal stages using the light microscopy (× 200 and × 400 magnifications, Olympus BX51, camera Olympus Camedia C-5060, Quick PHOTO MICRO 2.3 software) following two methods of concentration (i) Sheather's sugar flotation method (Sheather 1923; Smith et al. 2007) and (ii) AMS III sedimentation (Hunter et al. 1960).

Faecal samples of foxes and vole intestinal contents were homogenised with 0.5-mm glass beads (Biospec Products, Inc., Bartlesville, OK, USA) in a FastPrep-24 Instrument (MP Biomedicals, Santa Ana, CA, USA) at a speed of 5 m/s for 1 min followed by DNA extraction using the Exgene™ Stool DNA mini kit (GeneAll, South Korea) following the manufacturer's protocol. Nested PCR protocols were used to amplify the ITS (Internal Transcribed Spacer) region of the rRNA gene of *E. bieneusi* as previously described by Buckholt et al. (2002), the ITS region of the rRNA gene of *Encephalitozoon* spp. using the primer sets of INT580F and INT580R according to Didier et al. (1995) and MSP3 and MSP4A primer sets for the secondary PCR reaction according to Katzwinkel-Wladarsch et al. (1996), the partial sequence of
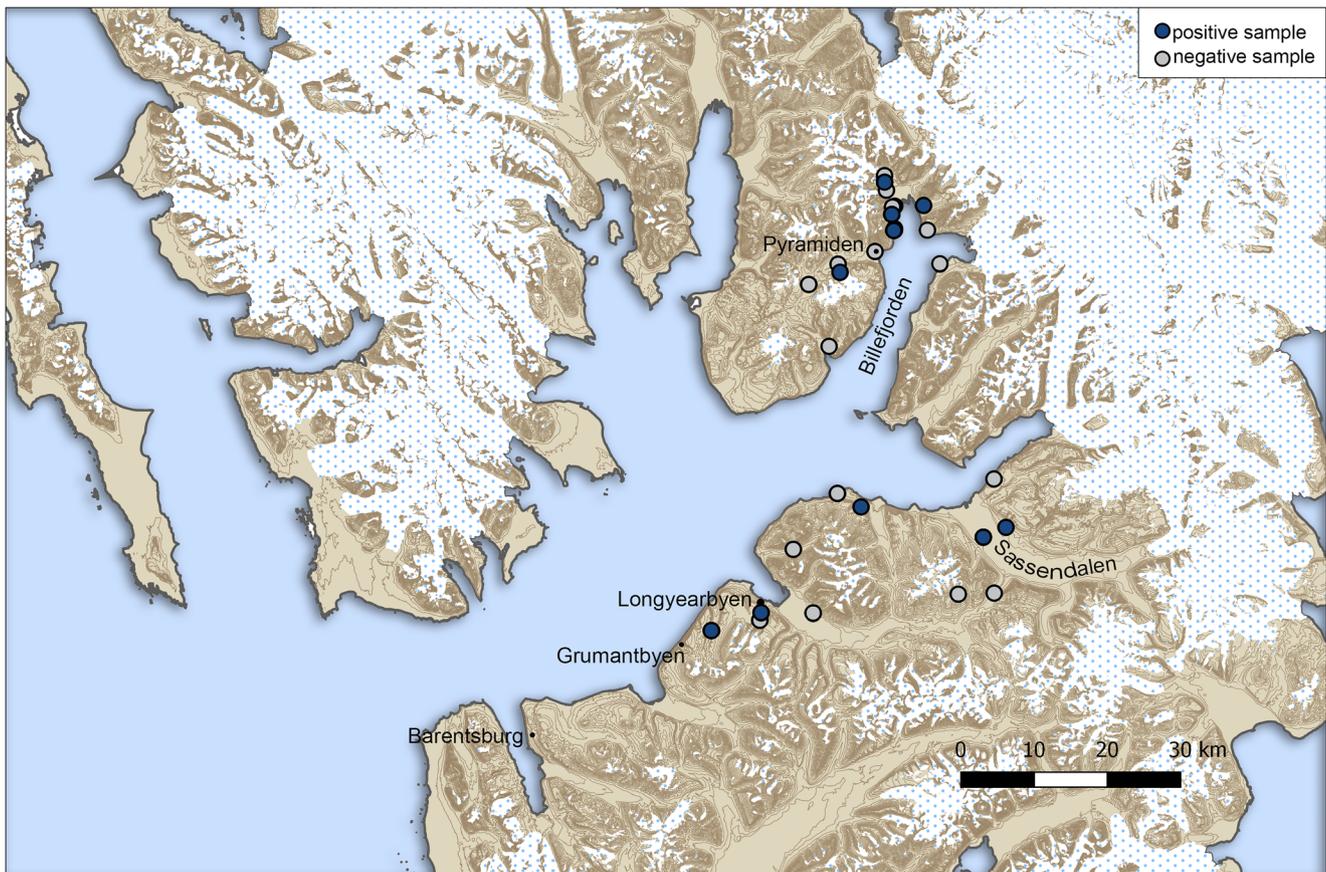
Fig. 1 Map showing the main sites (Longyearbyen, Sassendalen, and the Billefjorden area) of collected arctic fox faeces samples in Spitsbergen, Svalbard. Dark buttons, positive samples; light buttons, negative samples. Map created in QGIS Development Team (2018) QGIS Geographic information system. Open source geospatial foundation project. Available at: http://qgis.osgeo.org; Basemap data from Norwegian Polar Institute. Available at: https://geodata.npolar.no/

TPI (Triosephosphate Isomerase) gene of *Giardia* spp. described by Sulaiman et al. (2003), the partial sequence of the *Cryptosporidium* small ribosomal subunit rRNA (18S rRNA) gene described by Jiang et al. (2005), the ITS region of nematodes of the family Ascaridae using the primer set AsITS1 described by Carlsgart et al. (2009), the partial sequence of COI (Cytochrome c oxidase I) and 18S rRNA genes of the family Eimeriidae (Kvičerová et al. 2008; Schwarz et al. 2009), and following individual genes were amplified by PCR using the two primer pairs for tapeworms: near the D1–D3 region of lsrDNA with LSU5 and 1500R (Littlewood et al. 2000; Olson et al. 2003) and mitochondrial gene NAD1 (Trachsel et al. 2007). The primary PCR reaction containing 12.5 μl 1× Plain PP master mix (Top-Bio, Praha, Czech Republic; contain Taq polymerase), 400 nM each primer, 1.0 μl of template DNA and molecular grade water up to a volume 25 μl. The reaction conditions for secondary PCR were similar to those described above for the primary PCR, with the exception that 2 ul of the primary PCR product was used as the template. A negative (molecular grade water) and positive controls (DNA of *C. varanii*, *E. cuniculi* genotype III, *E. bieneusi* genotype D, *G. intestinalis* assemblage E, *E. intestinalis*, *E. multilocularis*, and *A. suum*) were included in each appropriate PCR reaction set. Each cycle consisted of denaturation of 94 °C for 45 s, annealing temperature that was specific for each of the targeted genus (according to mentioned reference publications), and extension of 72 °C for 60 s. Initial denaturation at 94 °C for 3 min and the final extension at 72 °C for 7 min/10 min were included. The number of cycles (35 for *Cryptosporidium*, *Giardia*, Eimeriidae COI, and microsporidia, 34 for Ascaridae, and 30 for Eimeriidae 18S rRNA) was set based on the above-mentioned reference publications. PCR products were visualised on 1% agarose gel containing 0.1 g/ml ethidium bromide, positive samples of expected sizes were isolated from the gel by the Expin™ Combo GP (GeneAll, South Korea) and sequenced with PCR primers for Ascarids and secondary PCR primers for the remaining three parasite taxa on an ABI 3130 sequence analyzer (SEQme, Dobříš, Czech Republic). Each sample was sequenced in both directions. Sequences were aligned and assembled using the Geneious 9.1.5 software (http://www.geneious.com, Kearse et al. 2012), and compared with sequences in the GenBank database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequences obtained in this study were

deposited in the GenBank database under the accession numbers MK315211-MK315221.

Ethical approval

The protocol was approved by the Committee on the Ethics of Animal Experiments of the University of South Bohemia, and also by the Ministry of the Environment of the Czech Republic (Permit Numbers MZP/2017/630/854). All procedures performed in studies involving animals were in accordance with the ethical standards of the Norwegian Animal Welfare Act. The research was also registered in The Research in Svalbard Database, RiS-10852. All handling/usage with biological samples were allowed by the University of South Bohemia in České Budějovice in accordance with the law of the Czech Republic (Act No. 166/1999), regulation of European Parliament (Act. No. 1069/2009), and Commission Regulation (EU) (No. 142/2011).

Results

Out of the 62 samples collected from arctic fox faeces, 17 were microscopically positive for endoparasites. Specifically, four samples were positive for eggs of *Toxascaris leonina* 13.3 % (Fig. 2a), one for *Trichuris vulpis* 1.7 % (Fig. 2c), two for *Eucoleus* sp. 3.3 % (Fig. 2b), and one sample for *Uncinaria stenocephala* 1.7 % (Fig. 2d; Table 1). In addition, five samples were positive for several different morphotypes of *Eimeria* spp. oocysts 8.3 % (Fig. 3; Table 1).

Only two samples had more than one species of parasite: *T. leonina* with *T. canis* (season 2015), and *T. leonina* together with *Eimeria* spp. (season 2013). Nevertheless, three positive samples (each containing only single parasitic taxon, *Eucoleus* sp., *U. stenocephala*, and *Eimeria* spp.) collected in 2013 originated from the same locality in Billefjorden. These samples could originate from a single individual. Summary of positive samples from the seasons 2012, 2013, and 2015 are provided in Fig. 1.

Three sequences of eimerians found in the faeces of arctic foxes were obtained (8.3 %; Table 1). The partial cytochrome c oxidase I (COI) sequence of eimerians present in the sample 63A clustered inside the eimerians from gallinaceous birds. The partial COI sequence of eimerians found in sample 153 clustered individually, being most closely related to the eimerians infecting rodents and pangolin. The partial sequence of 18S rDNA of eimerians occurring in sample 125 also clustered individually, on the basal position of the eimeriids infecting warm-blooded vertebrates (Online Resource1-2).

No sample was microscopically positive for spores of microsporidia. However, *Enterocytozoon bieneusi*–specific DNA was detected by molecular tools in one arctic fox (1.7 %; Table 1).

Additionally, specific DNA of *T. leonina* and *Toxocara canis* was detected in four (13.3 %) and one (1.7 %) microscopically negative samples, respectively (Table 1). All samples from foxes and voles were molecularly negative for any tapeworm (Table 1).

None of the 63 sibling voles was microscopically positive for any endoparasites (Table 1). A sample from one sibling vole contained specific DNA of *Cryptosporidium* (1.7 %) and following phylogenetic analysis of the 18S rRNA gene showed the presence of *Cryptosporidium alticolis* (100 % identity with the bootstrap value 100, Table 1). However, none of the samples from foxes was positive neither for *Cryptosporidium* spp. nor for *Giardia* spp. and also none of the sibling voles contained DNA of *Giardia* spp., eimerians or microsporidia.

Discussion

We were able to successfully detect exogenous life stages and specific DNA of endoparasites in collected samples via the microscopy and molecular tools, respectively. Parasitological examination of faecal material always poses a risk of underestimation of the parasite species richness. Limitation of coprological examination resides in the fact that it is able to detect only those parasites that shed eggs/cysts/oocysts in sufficient quantity over the detection limit of the method used during the sampling period (Martínez-Carrasco et al. 2007). Therefore, we also employed molecular methods to complement the traditional method, and to be able to detect parasites that were not detected during the microscopical examination due to low infection intensity or stage sizes that are difficult to be detected (e.g. microsporidia spores). Specifically, arctic fox samples that were microscopically negative for *T. leonina* and *E. bieneusi* were molecularly positive, showing the usefulness and the improvement by using different parasitological approaches.

In contrast to Stien et al. (2010) that focused on materials from arctic fox GI tracts that reported high abundance of tapeworms, we did not detect any tapeworm stages in the collected arctic fox faecal material. On the other hand, we recorded six new parasites that have not been previously reported in Svalbard, specifically, a respiratory tract worm *Eucoleus* sp. (Fig. 2b), hookworm *U. stenocephala* (Fig. 2d), whipworms *Trichuris vulpis* (Fig. 2c), *T. canis*, *Eimeria* spp., and *E. bieneusi*. All of them are common parasites of foxes, and although they have been detected in other Arctic areas (Aguirre et al. 2000; Meijer et al. 2011; Skírnisson et al. 1993), this is the first record in Svalbard.

Toxascaris leonina was the most prevalent parasite for arctic foxes in this study, detected at all sites and it was more
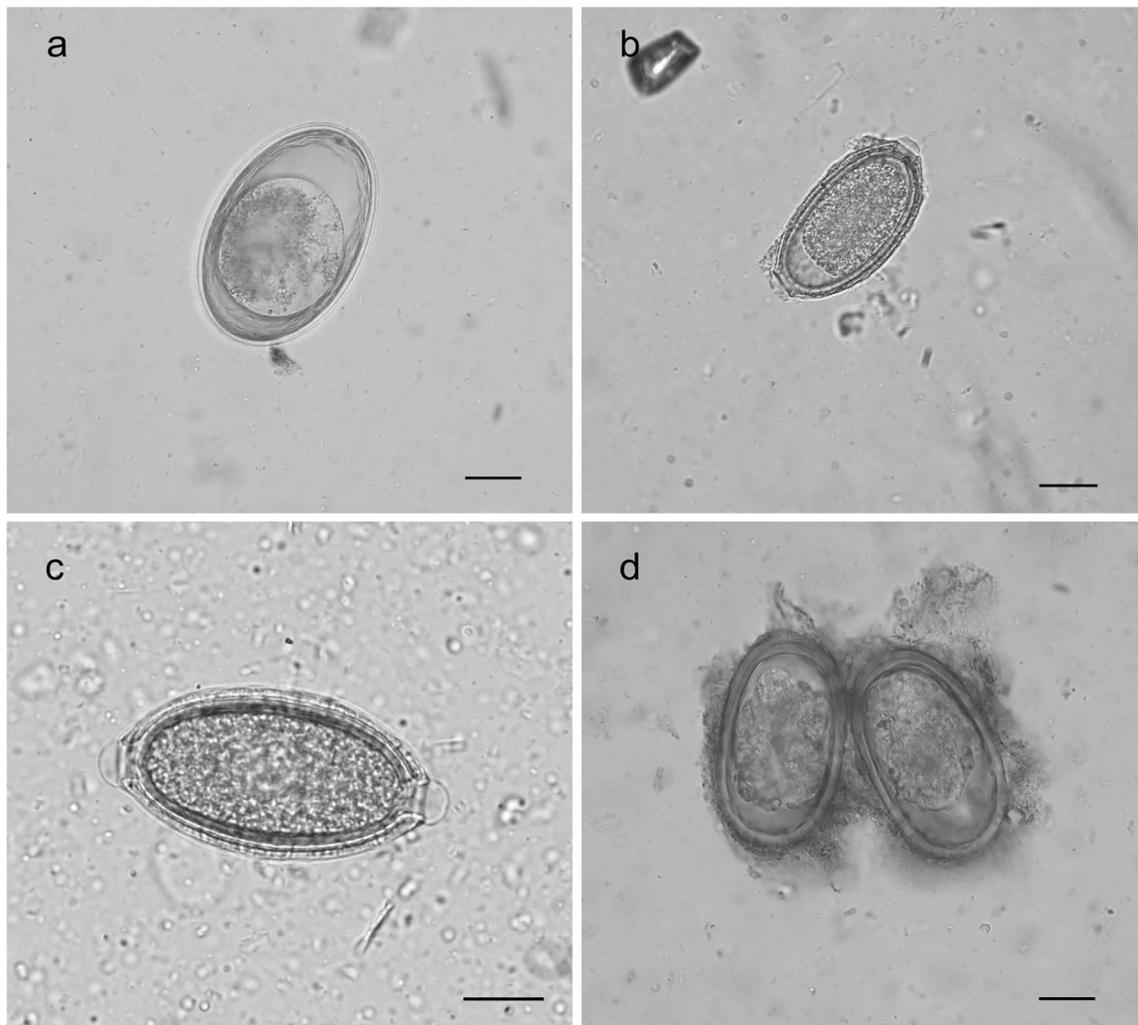
Fig. 2 Nematode eggs observed in the arctic fox faecal samples. **a** *Toxascaris leonina*. **b** *Eucoleus* sp.. **c** *Trichuris* sp.. **d** *Uncinaria stenocephala*. The scale bar 20 μm

abundant than *T. canis* (Meijer et al. 2011; Elmore et al. 2013). In general, ascarid nematodes (mostly *T. leonina* and *T. canis*) are frequent intestinal parasites of arctic foxes. The prevalence found in this study (13.3%) is lower compared with the study (33 %) of Stien et al. (2010), both from Svalbard. The difference may be caused by a lower number of examined samples in our study or year to year differences. *Toxascaris leonina* may use rodents as paratenic hosts, and Stien et al. (2010) found a decreased prevalence of *T. leonina* with distance to the sibling vole core area of Grumantbyen (see the map in Fig. 1). Because of our low samples size, we were not able to analyse for such an effect. The presence of *T. canis* is for the first time documented in arctic foxes in Svalbard and is striking. Predominant species in high latitudes is *T. leonina*, an ascarid roundworm that is able to survive at low temperatures and can generally adapt to a greater variety of climate conditions than *Toxocara* spp. (Okoshi and Usui 1968). *Toxocara canis* has previously been detected in arctic foxes only in Iceland (one single case), and in Sweden (common parasite,

several cases) (Meijer et al. 2011; Skírnisson et al. 1993). Stien et al. (2010) detected *T. leonina* but there was also large numbers of unidentified nematode individuals present.

The differentiation of *Eucoleus* spp. from *Calodium hepaticum* eggs was performed according to Fugassa et al. (2008). *Eucoleus* sp., a respiratory nematode common in foxes and other carnivores, is documented for the first time in Svalbard. Its pathogenic role in foxes is not well recognised, but it usually causes only minor clinical signs (Lalošević et al. 2013). However, it has also been considered as a cause of massive mortality in farmed silver foxes (*Vulpes vulpes*; Skryabin et al. 1957). The life cycle is direct; animals become infected by ingesting the embryonated eggs from the environment (Taylor et al. 2007). It is difficult to distinguish via microscopy whether these are eggs of the genus *E. aerophilus* or *E. boehmi* because of the high morphological similarity of their eggs (Traversa et al. 2010). The first mentioned has been reported in arctic foxes from Iceland and Sweden (Aguirre et al. 2000; Meijer et al. 2011; Skírnisson et al. 1993) and

Table 1 Parasite species in arctic foxes (*Vulpes lagopus*) and sibling voles (*Microtus levis*) in central Spitzbergen, Svalbard. Samples are characterised by microscopy and PCR analysis with prevalence (%) of parasites found in 62 faecal samples and in 63 trapped sibling voles. Positive samples are completed with locality and year of sampling (2 = 2012; 3 = 2013; 5 = 2015). Parasites written in bold are new species to Svalbard

Arctic foxes	Microscopy (*n* = 62)	Molecular analysis (*n* = 62)	Prevalence (%)	Positive sample	
				Year	Locality
Toxascaris leonina	4	5 *one	12.9	2; 3; 5	All locations[†]
Eucoleus sp.	2	0	3.2	2; 3	Billefjorden
Trichuris sp.	1	-	1.6	5	Billefjorden
Toxocara canis	0	1	1.6	5	Sassendalen
Uncinaria stenocephala	1	-	1.6	3	Billefjorden
Eimeria spp.	5	3 *all	8.1	2; 3; 5	Billefjorden
Enterocytozoon bieneusi	0	1	1.6	2	Billefjorden
Encephalitozoon sp.	0	0	0	-	-
Giardia sp.	0	0	0	-	-
Cryptosporidium sp.	0	0	0	-	-
Cestodes	0	0	0	-	-
Sibling voles	Microscopy (*n* = 63)	Molecular analysis (*n* = 63)	Prevalence (%)	Positive sample	
				Year	Locality
Cryptosporidium alticolis	0	1	1.6	5	Longyearbyen

0 = negative sample; - = not tested; * = microscopically positive; [†] = Billefjorden, Sassendalen, Longyearbyen

the latter from Greenland (Andreassen et al. 2017). The hookworm *U. stenocephala* has been previously reported in arctic foxes in Iceland, Greenland, and Sweden (Meijer et al. 2011; Rausch et al. 1983; Skírnisson et al. 1993). Unlike another hookworm *Ancylostoma caninum*, this parasite has a higher tolerance to lower temperatures (Balasingam 1964). The life cycle of *U. stenocephala* usually follows a faecal-oral route of transmission, ingestion of paratenic host (rodents), or less frequent the larvae migrate through the skin (Chu et al. 2013; Zajac and Conboy 2012). Eggs of *Trichuris vulpis*, a common parasite of red foxes (*Vulpes vulpes*; Borecka et al. 2013; Miterpáková et al. 2009), have only been reported in arctic foxes in Sweden (Meijer et al. 2011). The life cycle of this parasite is also direct, and animals become infected through a faecal-oral transmission (Venco et al. 2011).

Stien et al. (2010) recorded several species of tapeworms (*Taenia ovis krabbei*, *T. polyacantha*, *T. crassiceps*, *Diphyllobothrium* sp., and *Echinococcus multilocularis*), and acanthocephala, whereas all of our samples were negative for these parasites despite the same sites of origin of some samples for both studies.

Fuglei et al. (2008) found a strong spatial pattern in the occurrence of the tapeworm *E. multilocularis* in arctic fox faeces on Svalbard. They found a high *E. multilocularis* prevalence within the core area of the sibling vole, namely the Grumantbyen. However, similar to our study, in the area from Bjørndalen (2–6 km from the vole core area Grumantbyen) and in more distant areas on Nordenskiöld Land, no faeces

were positive for *E. multilocularis*, except one faeces found at a very far distance (110–130 km). Also, the prevalence of the *E. multilocularis* has been analysed in sibling voles in Svalbard. Henttonen et al. (2001) and Stien et al. (2010) trapped 224 (26 % positive) and 506 (19 % positive) voles in the vole core area in Grumantbyen, respectively, between the years 1999 and 2006. Their findings were different compared with this study, as all 63 captured voles in our study were negative. The reason for this is most probably because our sibling voles came from Longyearbyen about 20 km from Grumantbyen, and Grumantbyen is the only locality where *E. multilocularis* is found in voles in Svalbard. The reason for this may be that in the vicinity of Longyearbyen, compared with Grumantbyen, both hosts are fewer, the intermediate host (the sibling vole) and arctic foxes, the definitive hosts of this tapeworm, and therefore less infected voles (Fuglei et al. 2008).

Based on the molecular analyses (Online Resource1-2), *Eimeria* sp. occurred in one faecal sample (63A) from the arctic fox. Our sample clustered inside with *Eimeria* spp. infecting gallinaceous birds. Because eimerians tend to be usually highly host specific (Vrba and Pakandl 2015), it points to a probable origin from birds, and passages through the gastrointestinal tract of the fox. In the case of sample 63A, several different morphotypes of *Eimeria* spp. oocysts were present (Fig. 3c, d) and it is not possible to distinguish which one the sequence belongs. The second eimerian sample (sample 153) clustered individually, close to the eimerians infecting rodents
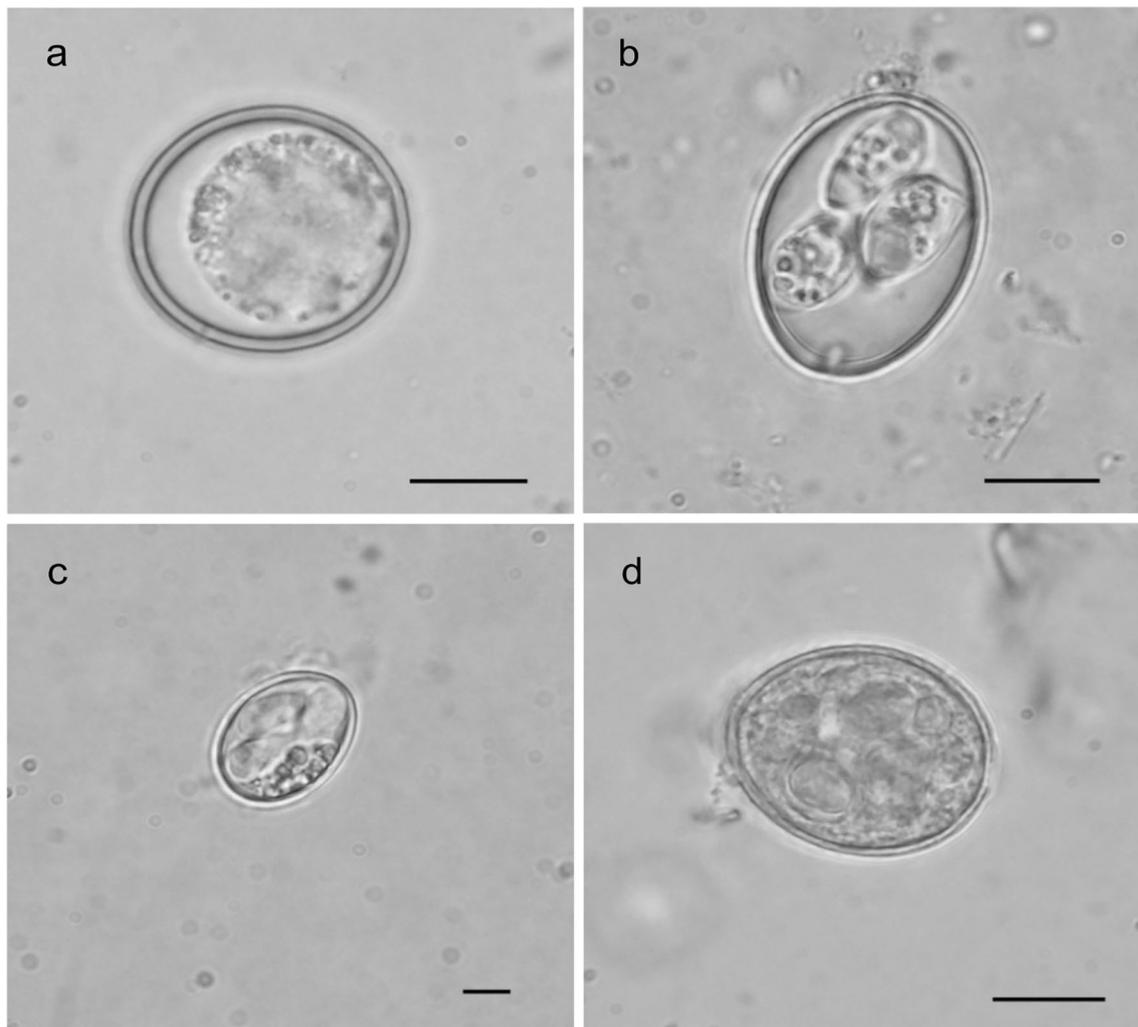
Fig. 3 Different morphotypes of *Eimeria* spp. oocysts observed in the arctic fox faecal samples. **a**, **b** From sample number 153. **c**, **d** From sample number 63A from Billefjorden. In the case of **a** and **d**, these are nonsporulated oocysts. The scale bar 10 μm

(field mice, squirrels, dormouse) and pangolin; thus, we suppose that these eimerians may infect the foxes, or may represent rodent eimerians just passed through the gastrointestinal tract of the fox. Eimerians present in the third sample (sample 125) clustered individually, on the basal position of the eimeriids infecting warm-blooded vertebrates; thus, we presume that these are real parasites of arctic foxes.

Encephalitozoon spp. and *Enterocytozoon bieneusi* are the most widely spread microsporidia in mammals (Hinney et al. 2016). Only *E. bieneusi* was molecularly positive in one arctic fox sample. However, this is the first report for arctic foxes. The sequence share 99 % sequence identity (bootstrap support 88) with genotype WL15 (GenBank AY237223) that has been found both in humans and animals. Arctic foxes may be infected by contaminated water with spores or from their prey (birds, rodents; Thellier and Breton 2008). Alternatively, the detection of a single case of *E. bieneusi* in fox could also be explained by simple passage of spores through the digestive tract without any ongoing infection.

Even though series of previous reports characterised *E. cuniculi* as a predominant parasite in farmed blue foxes in the Scandinavian countries (Arnesen and Nordstoga 1977; Mohn et al. 1974; Nordstoga and Westbye 1976), wild populations of arctic foxes in Iceland (Hersteinsson et al. 1993) and red foxes in England, Ireland, and the Czech Republic (Wilson 1979; Murphy et al. 2007; Hůrková and Modrý 2006),we have not detected this parasite in the samples in this study.

None of our samples tested positive for *Cryptosporidium* spp. The prevalence of *Cryptosporidium* seems to be low in the Arctic in general, and in some areas, the parasite has not been detected at all. For example, in Greenland, there is no record of this protist neither from animals nor from humans (Jenkins et al. 2013).

However, we molecularly detected the presence of *Cryptosporidium* in one sibling vole from Longyearbyen, which is the first detection in Svalbard. The sequence was 100 % identical to *C. alticolis* isolated from a common vole (*M. arvalis*) from the Czech Republic (Horčičková et al. 2018;

Genbank KY644657). Low prevalence correlates with the environmental effects of the *Cryptosporidium* life cycle: oocysts of *Cryptosporidium* are highly sensitive to extreme temperatures, and in temperatures under zero they remain viable only for a short period of time (Fayer et al. 1998).

Even though Svalbard is situated at high latitudes with a high degree of isolation, the parasite species richness in arctic foxes is comparable with populations from other areas of their distribution such as Iceland, Greenland, and Canada, at least with regard to herein examined groups of parasites (Aguirre et al. 2000; Andreassen et al. 2017; Eaton and Secord 1979; Elmore et al. 2013; Kapel and Nansen 1996; Meijer et al. 2011; Rausch et al. 1983; Skírnisson et al. 1993). One of the reasons for this resemblance is probably that the Svalbard fox population is connected to other Arctic continents over the sea ice (Geffen et al. 2007; Norwegian Ice Service – MET Norway: http://polarview.met.no/; Carmichael et al. 2007; Norén et al. 2011) and the foxes can migrate over long distances and shed and share their parasites. However, only from locations that are connected with sea ice.

Eucoleus sp., *U. stenocephala*, *T. canis*, *Trichuris vulpis*, and Eimeria have never been detected in Svalbard; however, they are parasites of arctic foxes in other arctic locations (Meijer et al. 2011; Rausch et al. 1983; Skírnisson et al. 1993). We declined the possibility of transmission via dogs present in the island as they are under strict veterinary control (SYSSELMANNEN: http:// www.sysselmannen.no/en/ Shortcuts/Pets/). The occurrence of these parasites in Svalbard may be explained by transmission over the sea ice inside the GI tract of an arctic fox. Foxes can migrate over long distances, and Svalbard serves as a "meeting" point from both the east and west (Norén et al. 2010).

As this study shows, the coprological studies are keys for assessing the parasitic fauna and potential health risk of wild animals in the Arctic. The present documentation of as many as six new parasites for Svalbard shows the importance of the host-parasite studies in the Arctic ecosystem. It is also crucial for mapping the parasite richness of arctic foxes throughout their distribution range, enabling us to evaluate the impact of climate change that will affect the Arctic most profoundly.

Compliance with ethical standards The protocol was approved by the Committee on the Ethics of Animal Experiments of the University of South Bohemia, and also by the Ministry of the Environment of the Czech Republic (Permit Numbers MZP/2017/630/854). All procedures performed in studies involving animals were in accordance with the ethical standards of the Norwegian Animal Welfare Act. The research was also registered in The Research in Svalbard Database, RiS-10852. All handling/usage with biological samples were allowed by the University of South Bohemia in České Budějovice in accordance with the law of the Czech Republic (Act No. 166/1999), regulation of European Parliament (Act. No. 1069/2009), and Commission Regulation (EU) (No. 142/2011).

Conflict of interest The authors declare that they have no conflict of interest.

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