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Article

Coprological Survey of Helminths in Reindeer (*Rangifer tarandus*) in 50 Selected Zoos and Menageries in Russia

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Abstract: Zoo conditions are unique for reindeer, since even domestic reindeer are not kept in captive facilities like cattle. In the zoo, reindeer are usually surrounded by many different animals they would never encounter naturally. Thus they might pick up some new helminths. Numerous petting zoos raise an issue of safety of tactile communications for human visitors. Our study is the first large-scale one. Qualitative and quantitative fecal analysis was carried out for 233 reindeer distributed over 50 Russian zoos according to National Standard of the Russian Federation (GOST R 54627-2011) *Ruminant animals—Methods of Laboratory Helminthological Diagnostics*. Where possible, DNA analyses of helminths were performed targeting internal transcribed spacer region. In result, *F. hepatica*, *Paramphistomum* sp., *Moniezia* sp. (including *M. expansa*), gastrointestinal strongylids (including *Nematodirus* spp.), *Dictyocaulus* sp., *E. rangiferi*, *Trichuris* sp., and *Capillaria* sp. were found in 106 (45%) zoo reindeer. All these helminths were previously reported for reindeer and pose no direct danger for humans. Intensity of invasions was mostly low. Fecal examination might be considered as an indirect method for mange diagnostics, as *Chorioptes* and *Demodex* mites were found in reindeer fecal samples. The latter may represent a novel species of mite specific for reindeer.

Keywords: reindeer; *Rangifer tarandus*; helminth; trematode; cestode; nematode; mite; zoo

1. Introduction

Helminths of reindeer (*Rangifer tarandus*) have already been studied quite thoroughly, and the knowledge on them was summarized in thematic books [1–3] and chapters [4–8]. Ongoing studies expand our views on many aspects of reindeer helminths [9–13], and sometimes even add new species to the checklist of parasitic worms of *Rangifer* [14,15].

Reindeer exist in domestic and wild forms totaling in more than 4 mln animals worldwide [16]. Their popularity as zoo cervids in the 21st century is unprecedentedly growing. According to Species360 ZIMS database, there are more than 500 *Rangifer* spp. distributed in more than 150 zoos around the world [17]. Some research on reindeer helminths in captive facilities were published [3,16,18–20], but they are still too rare and fragmentary.

Meanwhile zoo conditions are unique for reindeer, because even domestic reindeer mostly are not kept in farms and captive facilities of the kind like cattle. In the zoo, reindeer are usually surrounded by many different animals they would never encounter naturally. It might pose a threat of picking up some new helminths. Numerous petting zoos raise another issue: the safety of such tactile communications for human visitors.

Therefore, the aim of our study was to conduct a large-scale survey of reindeer helminths in Russian zoos. Fecal examination being non-invasive method (an essential trait for zoo animals) offers wide diagnostic capabilities, scoping gastrointestinal, lung, brain, and muscle parasitic worms. This study was done to find out, what kind of helminths inhabit zoo reindeer across Russia, how widespread they are, how typical they are, and how dangerous they are both for reindeer and humans.

2. Materials and Methods

2.1. Helminths recovery

Our study collected fecal samples from 233 reindeer kept in 50 selected zoos and menageries located in Russia (Table 1). Those include: all the state zoos in which collections reindeer are present (17); other state organizations having live reindeer for exhibition (Children's ecological station, Natural-ethnographic complex, and Arctic Tourism Center); private organizations-members of the *Union of Zoos and Aquariums of Russia* (5 private zoos and an urban farm); and 25 private menageries. The latter constitute of “clubs”, “parks: (“recreation-”, “ethnic-”, “fauna-”, and “safari park”), “reserve” (self-proclaimed), “mini zoo”, “ranch”, “farm”, “farmstead”, “pets' corner” and no name menageries. Many of these organizations participated in our research anonymously; therefore, for uniformity, we do not provide the actual names of all the 50 of them. However, their location combined with the type of ownership makes the data quite transparent. For brevity, further on all of these organizations would be referred to as zoos.

Table 1. Collection data for fecal samples from reindeer (*Rangifer tarandus*) in the Russian zoos (Federal subjects arranged from the West to the East).

Zoo ID	Ownership Type (S/P) ¹	Location (Federal Subject of Russia)	Coordinates (Decimal Degrees)	Number of Fecal Samples ³	Date Collected
1	P	Murmansk Oblast	68.98716 33.07261	2	November 2023
2	P	Murmansk Oblast	68.85722 33.19556	12	November 2023
3	P	Murmansk Oblast	67.56317 33.36571	2	November 2023
4	P	Murmansk Oblast	67.65276 33.66051	11	November 2023
5	P	Murmansk Oblast	69.16835 35.13280	3	November 2023
6	P	Republic of Karelia	65.76340 31.07419	1	March 2024
7	P	Republic of Karelia	66.43669 32.85459	7	June 2022
8	P	Republic of Karelia	62.33297 34.00604	3	March 2024

9	P	Republic of Karelia	61.87838 34.07819	9	March 2024
10	P	Leningrad Oblast	60.59067 30.00422	1	July 2020
11	P ²	Leningrad Oblast	60.59161 30.11204	8	May 2019
12	P	Leningrad Oblast	60.14195 30.32823	11	August 2018
13	P	Leningrad Oblast	59.94762 30.68122	3	May 2023
14	P	Saint Petersburg	59.84108 30.06470	2	June 2019
15	P	Saint Petersburg	59.98017 30.24438	3	March 2024
16	P	Saint Petersburg	59.97054 30.25672	4	January 2020
17	S ²	Saint Petersburg	59.95210 30.30891	9	March 2024
18	P	Saint Petersburg	59.67605 30.42401	2	February 2018
19	P	Tver Oblast	56.75051 36.36719	1	June 2020
20	S ²	Moscow Oblast	55.94012 36.21055	7	January 2024
21	P	Moscow Oblast	56.13343 36.50051	2	March 2024
22	S ²	Moscow	55.76347 37.57537	3	December 2023
23	P ²	Moscow	55.83306 37.62197	1	October 2023
24	S ²	Yaroslavl Oblast	57.67709 39.90005	6	November 2023
25	P ²	Nizhny Novgorod Oblast	56.33468 43.85420	6	November 2023
26	P	Nizhny Novgorod Oblast	56.92384 45.40185	4	August 2022
27	S ²	Republic of Mordovia	54.17500 45.18599	3	November 2023
28	S ²	Vologda Oblast	60.74784 46.17739	5	November 2023
29	P ²	Ulyanovsk Oblast	54.35485 48.52409	3	February 2024
30	P	Samara Oblast	53.34493 50.22240	21	August 2021
31	S ²	Udmurt Republic	56.86555 53.17413	2	November 2023
32	S	Nenets Autonomous Okrug	67.63436 53.24135	1	February 2024
33	S ²	Perm Krai	58.01672 56.23728	4	November 2023
34	P	Sverdlovsk Oblast	57.17585 60.65764	11	February 2024

35	S ²	Chelyabinsk Oblast	55.16894 61.36764	5	November 2023
36	P	Tyumen Oblast	56.99540 65.73485	7	February 2024
37	S	Yamalo-Nenets Autonomous Okrug	66.59257 66.85846	3	January 2024
38	S ²	Yamalo-Nenets Autonomous Okrug	66.07485 76.65427	2	November 2023
39	S ²	Omsk Oblast	56.08978 74.64219	5	November 2023
40	S ²	Novosibirsk Oblast	55.05612 82.88010	7	December 2023
41	P ²	Altai Krai	53.35593 83.68230	2	November 2023
42	S ²	Tomsk Oblast	56.60427 84.86807	1	March 2024
43	P	Krasnoyarsk Krai	69.42091 88.26126	1	February 2024
44	S ²	Krasnoyarsk Krai	55.96669 92.73100	5	November 2023
45	S ²	Republic of Sakha (Yakutia)	61.67818 129.35184	5	April 2024
46	P	Republic of Sakha (Yakutia)	62.03243 129.72416	5	August 2020
47	S ²	Khabarovsk Krai	48.62218 135.06819	6	December 2019
48	S ²	Sakhalin Oblast	46.96788 142.75403	2	December 2019
49	S ²	Kamchatka Krai	53.18850 158.38604	1	April 2019
50	P	Kamchatka Krai	55.92095 158.69500	3	March 2024

¹ S is for State ownership, P is for Private ownership. ² Member of the *Union of Zoos and Aquariums of Russia*. ³ Number of fecal samples equals the number of reindeer in the zoo.

Qualitative and quantitative fecal analyses, light microscopic study, and measurements of obtained eggs and larvae of helminths were performed as described by Loginova et al. [21].

2.2. DNA analysis

For trematodes, the QIAamp DNA Accessory Set, Micro kit (Quiagen, Netherlands) was used to extract DNA from embryonated eggs as described by Loginova et al. [22]. For nematodes, DNA was extracted either via digestion of first larval stages (L1) in presence of Proteinase K [23] as described by Loginova et al. [24] or by using the QIAamp DNA Accessory Set, Micro kit (Quiagen, Netherlands). We targeted the region of the internal transcribed spacer (ITS rDNA). For trematodes, we used BD1, BD2 primers [25] and corresponding protocols [26]. For nematodes we used either NC1, NC2 primers and protocols as described by Gasser et al. [27] or 18S, 26S primers [28] and protocols as described by Loginova et al. [24]. Visual check of PCR, cleaning of the PCR products, sequencing, and analysis of obtained chromatograms were performed as described by Loginova et al. [21].

3. Results

Flukes, tapeworms and round worms were found at the stage of egg or larva in feces of 106 reindeer (45%) kept in 31 zoos (62%). Identification of helminths was based on their morphology, morphometric data, and (where possible) DNA analysis (Table 2).

Table 2. Species of helminths recovered from the feces of zoo reindeer and identified genetically.

Zoo ID	Species	GenBank ¹	Vouchers ²
13	<i>Fasciola hepatica</i> ³	PP328913	IPEE_Parasites 14320
4	<i>Elaphostrongylus rangiferi</i> ⁴	PP843608	IPEE_Parasites 14319
5	<i>Elaphostrongylus rangiferi</i> ⁴	PP843600	IPEE_Parasites 14339
6	<i>Elaphostrongylus rangiferi</i> ⁴	PP843598	IPEE_Parasites 14343
7	<i>Elaphostrongylus rangiferi</i> ⁴	PP843592	IPEE_Parasites 14344
8	<i>Elaphostrongylus rangiferi</i> ⁴	PP845195	IPEE_Parasites 14345
9	<i>Elaphostrongylus rangiferi</i> ⁴	PP845196	IPEE_Parasites 14346
12	<i>Elaphostrongylus rangiferi</i> ⁵	MW848820	IPEE_Parasites 14282
13	<i>Elaphostrongylus rangiferi</i> ⁴	PP843584	IPEE_Parasites 14347
25	<i>Elaphostrongylus rangiferi</i> ⁴	PP845193	IPEE_Parasites 14348
29	<i>Elaphostrongylus rangiferi</i> ⁴	PP845194	IPEE_Parasites 14349
30	<i>Elaphostrongylus rangiferi</i> ⁴	PP845192	IPEE_Parasites 14350

¹ GenBank accession numbers for sequences from 65 embryonated eggs of *F. hepatica* and sets of 30 first larval stages (L1) of *E. rangiferi*. ² Voucher specimens with definitive identifications and accession numbers archived in the Museum of Helminthological Collections of the Parasitology Center at the A. N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences (Moscow, Russia). ³ Sequence previously in GenBank as reported by Loginova et al. [22]. ⁴ Sequence information reported here for the first time. ⁵ Sequence previously in GenBank as reported by Loginova et al. [24].

Appearance of eggs and larvae of helminths obtained from zoo reindeer feces is shown in Figure 1.

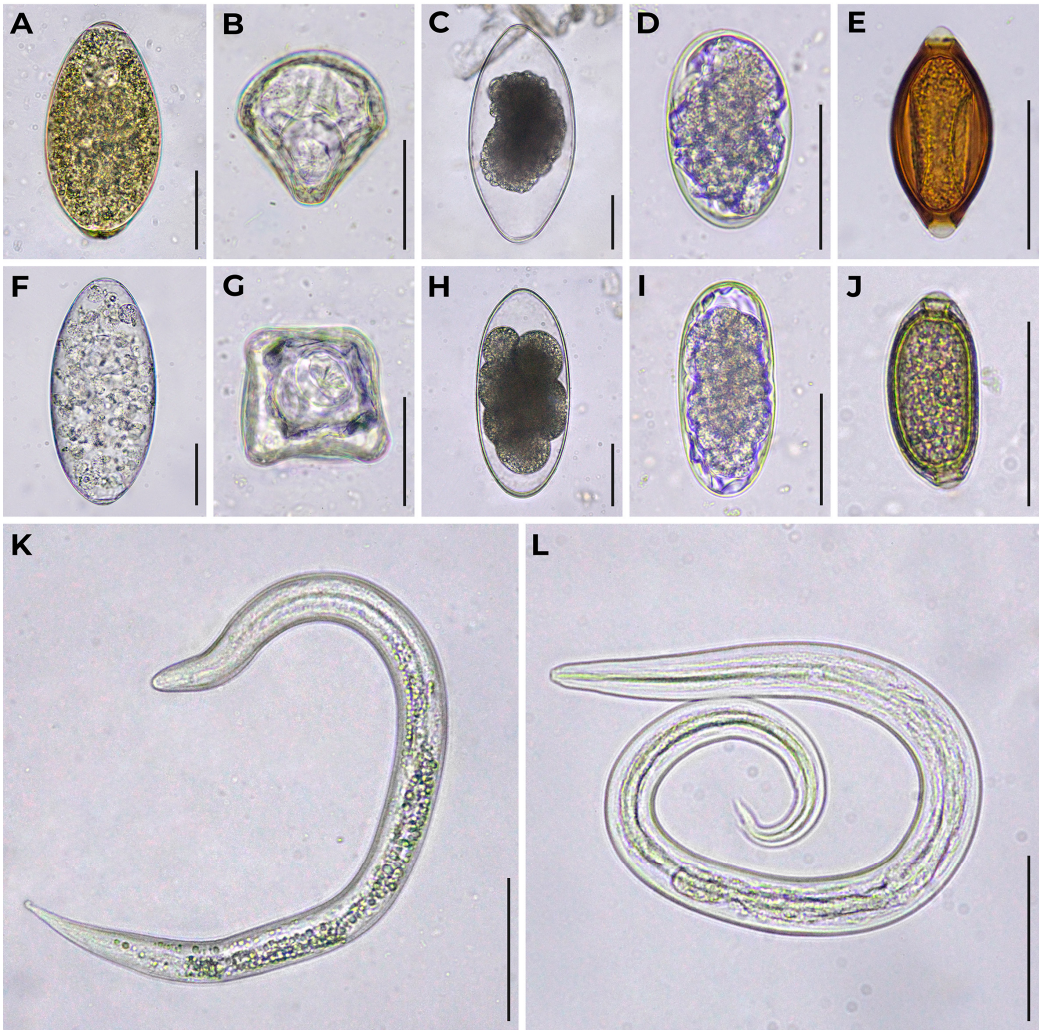


Figure 1. Diagnostic stages of helminths obtained from feces of zoo reindeer. (A) *Fasciola hepatica* egg; (B) *Moniezia expansa* egg; (C) *Nematodirus* sp. egg at late stage of embryonic development; (D) Rounded Strongyle-type egg; (E) *Trichuris* sp. egg; (F) *Paramphistomum* sp. egg; (G) *Moniezia* sp. egg; (H) *Nematodirus* sp. egg at the early stage of embryonic development; (I) Elongated Strongyle-type egg; (J) *Capillaria* sp. egg; (K) *Dictyocaulus* sp. L1; (L) *Elaphostrongylus rangiferi* L1. Bright field microscopy, 400× magnification. Scale bar equals 50 μm.

Prevalence rates and distribution of helminths obtained from zoo reindeer feces are summarized in Table 3. For brevity, only helminth-positive zoos are included.

Table 3. Helminths found in the feces of zoo reindeer.

Zoo ID	Trematodes		Cestodes			Nematodes			
	<i>Fasciola hepatica</i>	<i>Paramphistomum</i> sp.	<i>Moniezia</i> spp.	Strongyle-type	<i>Nematodirus</i> spp.	<i>Dictyocaulus</i> sp.	<i>Elaphostrongylus rangiferi</i>	<i>Trichuris</i> sp.	<i>Capillaria</i> sp.
2	–	–	–	–	–	–	–	–	4 (33%)
4	–	2 (18%)	–	–	–	–	4 (36%)	–	2 (18%)
5	–	2 (67%)	–	–	–	–	3 (100%)	–	–
6	–	–	–	–	–	–	1 (100%)	–	–
7	–	–	–	3 (43%)	–	–	4 (57%)	–	1 (14%)

8	–	–	–	–	–	–	1 (33%)	–	1 (33%)
9	–	3 (33%)	1 (11%)	–	–	–	6 (67%)	–	1 (11%)
10	–	–	–	–	–	–	–	–	1 (100%)
11	–	4 (50%)	–	–	–	–	–	–	–
12	–	–	–	4 (37%)	2 (18%)	–	7 (63%)	–	5 (45%)
13	1 (33%)	2 (67%)	–	–	–	–	1 (33%)	–	–
14	–	–	1 (50%)	2 (100%)	–	–	–	1 (50%)	–
16	–	–	–	–	–	–	–	–	2 (50%)
18	–	–	–	–	–	–	–	–	1 (50%)
21	2 (100%)	–	–	–	–	–	–	–	–
22	–	–	–	3 (100%)	–	–	–	1 (33%)	–
24	–	–	1 (17%)	1 (17%)	–	–	–	–	–
25	–	–	–	–	–	–	1 (17%)	–	1 (17%)
26	–	–	1 (25%)	–	–	–	–	–	2 (50%)
28	–	–	–	–	–	–	–	–	1 (20%)
29	–	–	–	–	–	–	2 (67%)	–	–
30	–	8 (38%)	–	1 (5%)	–	1 (5%)	6 (29%)	–	3 (14%)
34	–	–	1 (9%)	2 (18%)	–	–	–	–	2 (18%)
36	–	–	–	–	2 (29%)	–	4 (57%)	–	–
37	–	1 (33%)	–	–	–	–	3 (100%)	–	–
38	–	2 (100%)	–	2 (100%)	–	–	–	–	–
39	–	4 (80%)	–	–	–	–	–	–	–
44	–	–	–	–	1 (20%)	–	–	–	–
46	–	–	–	–	–	–	5 (100%)	–	–
47	–	–	–	–	–	–	–	–	1 (17%)
48	–	–	–	–	–	–	–	–	2 (100%)

Prevalence rates for total 233 reindeer examined are shown in Figure 2.

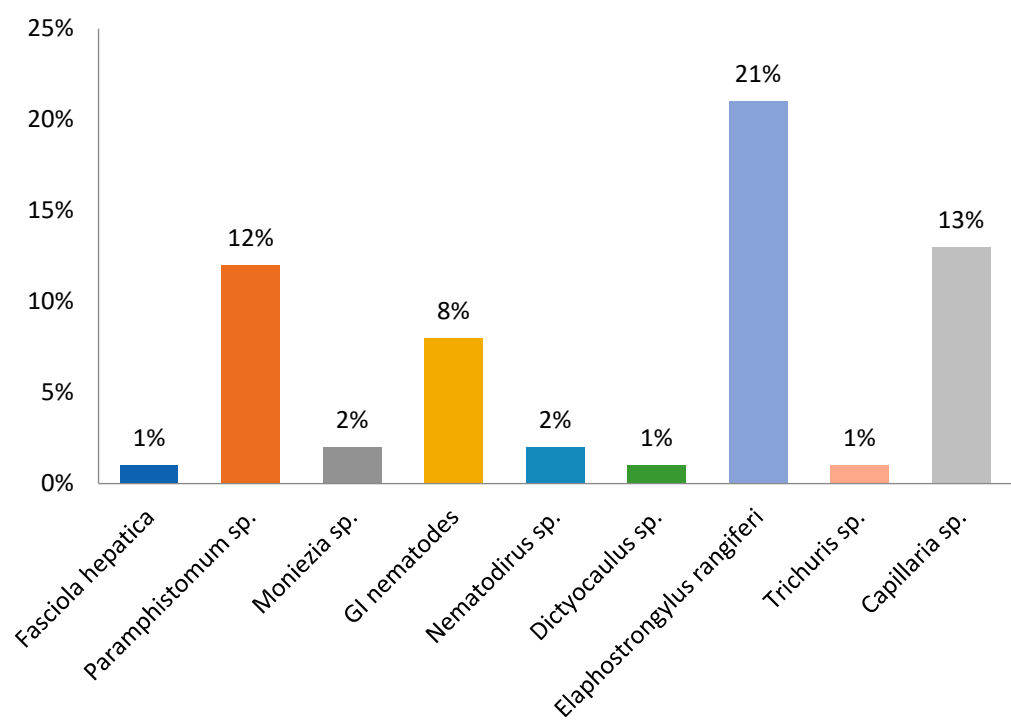


Figure 2. Prevalence rates (%) of helminths found in feces of zoo reindeer.

Intensity of invasion was mostly low in terms of National Standard of the Russian Federation (GOST R 54627-2011) *Ruminant animals—Methods of Laboratory Helminthological Diagnostics* [29]. Nematode eggs / gram of feces ranged from 1 to 56 in all reindeer samples. Nematode larvae / gram varied from 1 to 98. Cestode eggs / gram ranged from 1 to 17. Trematode eggs / gram varied from 1 to 8. Exceptions were: fecal sample of reindeer from the zoo #9 that showed 537 *E.rangiferi* larvae / gram; fecal sample of reindeer from the zoo #30 that showed 56 *Paramphistomum* sp. eggs / gram. Those numbers are estimated as medium intensity (GOST R 54627-2011).

4. Discussion

Our study revealed trematodes (*Fasciola hepatica* and *Paramphistomum* sp.), cestodes (*Moniezia expansa* and *Moniezia* sp.), and nematodes (so called small strongylids, *Nematodirus* spp., *Dictyocaulus* sp., *Elaphostrongylus rangiferi*, *Trichuris* sp., and *Capillaria* sp.) in zoo reindeer via fecal examination. All of these helminths were previously reported for reindeer [3,8].

In many samples, only one kind of helminths was found. However, we have also discovered combinations of two or three kinds of helminths (Table 4).

Table 4. Combinations of helminths found in the feces of zoo reindeer.

Helminth 1	Helminth 2	Helminth 3
<i>Fasciola hepatica</i>	<i>Paramphistomum</i> sp.	–
<i>Paramphistomum</i> sp.	small strongylids	–
<i>Paramphistomum</i> sp.	<i>Dictyocaulus</i> sp.	–
<i>Paramphistomum</i> sp.	<i>Elaphostrongylus rangiferi</i>	–
<i>Paramphistomum</i> sp.	<i>Elaphostrongylus rangiferi</i>	<i>Capillaria</i> sp.
<i>Moniezia expansa</i>	<i>Moniezia</i> sp.	–
<i>Moniezia</i> sp.	small strongylids	–
<i>Moniezia</i> sp.	<i>Elaphostrongylus rangiferi</i>	–
<i>Moniezia</i> sp.	<i>Trichuris</i> sp.	–
<i>Moniezia</i> sp.	<i>Capillaria</i> sp.	–
small strongylids	<i>Elaphostrongylus rangiferi</i>	–
small strongylids	<i>Trichuris</i> sp.	–
small strongylids	<i>Capillaria</i> sp.	–
<i>Nematodirus</i> sp. type 1	<i>Nematodirus</i> sp. type 2	–
<i>Elaphostrongylus rangiferi</i>	<i>Nematodirus</i> sp.	–
<i>Elaphostrongylus rangiferi</i>	<i>Capillaria</i> sp.	–

Common liver fluke *F. hepatica* inhabits bile ducts of its host. Previously it was reported for reindeer only in Eurasia, and one of the cases also involved zoo reindeer [3]. It is a rare finding for reindeer, and the latter is considered as an accidental host. Infestation of reindeer with *F. hepatica* usually is associated with other ruminants in contact and captive facilities [3]. In two private zoos where *F. hepatica* was found in reindeer, there were sheep, goats and sika deer (*Cervus nippon*) also infected.

Rumen fluke *Paramphistomum* sp. is widespread in Russian reindeer. When numerous, they cause atrophy of the rumen papillae and may lead to death [3,8]. Reindeer from the private zoo #30 with medium level of invasion with rumen flukes died within 6 month afterwards.

Tapeworms found were present with *M. expansa* (easily recognizable by its triangular eggs) and *Moniezia* sp. These helminths inhabit small intestine and may be quite pathogenic, especially for calves [3,8]. However, in our study it was found only in adults. Reindeer is considered an accidental host for *M. expansa*, and its infestation is also associated with sympatric livestock and captive facilities [3].

Supposedly, small strongylids found in zoo reindeer are of two different genera (Figure 1D,I) which may belong to fam. Chabertiidae, Cooperiidae, Haemonchidae or Trichostrongylidae.

Coproculture is needed to obtain larvae and clarify the diagnosis. In any case, small strongylids inhabit gastrointestinal tract, and their impact depends on many circumstances [8,10].

Eggs of *Nematodirus* obtained from the reindeer fecal sample from the zoo #40 certainly belong to two different species. Apart from their differences in size and shape, their development rates were also different. Figure 1H shows *Nematodirus* sp. with 8 blastomeres, whereas Figure 1C shows *Nematodirus* sp. with gastrulated embryo. These two types of eggs were found in the same fecal sample. In total, 4 eggs (two per type) were obtained from that sample. We made an attempt to get third larval stages (L3s) for DNA analysis, but did not succeed. However, light micrographs (taken daily during three weeks after the finding of these eggs) show their embryonic development, and are available as Supplementary Materials. Helminths of *Nematodirus* sp. inhabit small intestine. In reindeer, they are often accompanied by *Nematodirella longissimespiculata* [3], but in this study the latter was not found.

Lung worm *Dictyocaulus* sp. is the most concerning finding, because its presence may be life threatening. In the only zoo (#30) where it was found, there was also a sika deer infected with *Dictyocaulus* sp. It is hard to tell, which animal was the source of invasion, or was it a coincidental infestation.

Brain worm *E. rangiferi* was the most widespread helminth in our research. Its pathogenicity is tricky, because it may as well cause lethal epizootics or have no manifestation of its presence [3,8,30]. Due to localization of adult worms in the nervous system or between the muscles, there is no effective treatment. On the other hand, authors observed few reindeer that were severely infected in 2018 and eventually outlived their brain worms. At least, no *E. rangiferi* larvae have been found in their fecal samples since 2023. Probably, it would be impossible in the wild, but in human care reindeer can live much longer and, apparently, get rid of some of their parasites naturally [Loginova, unpublished].

Whipworms *Trichuris* sp. inhabit large intestine and can cause hemorrhagic diarrhoea [8]. They are more expected in captive animals. There are reindeer specific species and those common to ruminants that can also infect reindeer [3]. Egg morphology is not sufficient for the identification up to the species level.

Round worm *Capillaria* sp. inhabits intestine and is often associated with young animals [3,8]. Still the impact of this parasite is not known. Infection with *Capillaria* sp. was previously reported for zoo reindeer as well [3].

Thus, via fecal examination we managed to find liver and rumen flukes, GINs, lung worms, brain worms, whipworms, and capillarids. However, this method is not suitable for those helminths that inhabit ligaments (like *Onchocerca*), abdominal cavity (like *Setaria*), muscles, lungs, liver and brain (like larval stages of Taeniidae). Therefore, despite the large scale of our study, the helminth status of zoo reindeer is still incomplete.

Another bias of this work concerns different seasons of fecal sampling (which, probably, affected reproductive activity of helminths) and differences in age and sex of studied animals [3,8]. These circumstances should be kept in mind while comparing obtained results between zoos.

As we said earlier, popularity of reindeer as zoo animals in 21 century is outstanding. Only one zoo (#17) has 100 years of its history of keeping reindeer in captivity. It is not surprising that fecal samples from their reindeer were helminth negative not only in this study, but years prior to it. Many other zoos got their first reindeer only in 2023. Majority of the private zoos purchase domestic reindeer from agricultural husbandries. The latter are all private and run by families of indigenous peoples of the North. Zoos are able to buy only those reindeer that indigenous people are willing to sell. Reindeer herders want to keep the best individuals in the herd, and reindeer for sale may be less resistant to helminths.

Domestic reindeer in contrast to wild reindeer can be pure white (leucism, not albinism) throughout the year [31,32]. White reindeer attract most people [33], and numerous private zoos tend to have exactly the white reindeer. However, reindeer herders noticed that white reindeer are not good survivors [33,34]. It might add another disadvantage to such zoo reindeer in terms of helminth resistance, and requires special research.

Apart from husbandry “heritage”, how can zoo reindeer become infected with helminths? In particular, through the birds. Helminths eggs were reported to survive through sparrow digestive system and remain invasive [35]. Thus, birds can be vectors for helminths within the zoo and out of its boundaries.

It is noteworthy that even in helminth positive zoos, about 30% (0 to 83%) reindeer fecal samples were helminth free, probably, due to the different immune status of reindeer.

Besides helminths, we found mites in reindeer fecal samples (Figure A1). They were either ingested during autogrooming and passed digestive tract or contaminated fecal samples afterwards. Having found a mite egg in the sample from the zoo #30, we contacted that zoo and shared our considerations. It turned out that the zoo indeed had problems with mange caused by mites (Figure A1E). One adult mite found in a sample from the zoo #40 was identified as *Chorioptes* sp. (Figure A1D). *Chorioptes* mites were reported for reindeer in Canada, Norway, and Finland [8]. Another adult mite found in reindeer fecal sample (zoo #12) met morphological criteria for *Demodex* sp. To authors' knowledge, *Demodex* has never been reported for reindeer [3,8,36]. This finding also requires additional research which may result in the description of the new species.

Tactile contact between humans and animals in petting zoos may pose a risk of helminth infestation. For instance, red fox (*Vulpes vulpes*) and Arctic fox (*V. lagopus*) are recognized definitive hosts for *Echinococcus* spp. The latter produces numerous eggs which are shed with feces. Those eggs are very light and flying, and easily contaminate animal's fur. Stroking such sick fox or even breathing closely to it may result in ingestion of *Echinococcus* eggs which are instantly invasive [37].

Among helminths found in zoo reindeer in our research, there are few genera, reported for humans. Those are *Fasciola*, GINs (*Trichostrongylus*, *Oesophagostomum*), *Trichuris*, and *Capillaria* [37–40]. However, infected reindeer pose no direct threat for human visitors or zoo employees for the following reasons. Trematode *F. hepatica* is indeed the species, that can infect both cervids and humans, but its life cycle requires intermediate host (fresh water mollusk) [3]. Eggs shed by reindeer are not the infective stage for any vertebrate host. Moreover, eggs are excreted without miracidium, and it takes time for it to develop (that is possible only in water). Species of *Trichostrongylus*, *Oesophagostomum*, *Trichuris*, and *Capillaria* that can infect humans are not the same species that infect reindeer [3,8,37,40]. Besides, all these nematodes shed eggs that are not embryonated by the moment of their excretion with feces. Larvae development also requires few days or weeks. Thus, if reindeer dung is regularly removed, even reindeer infected with helminths are safe for humans.

5. Conclusions

In our study *F. hepatica*, *Paramphistomum* sp., *Moniezia* spp. (including *M. expansa*), gastrointestinal strongylids (including *Nematodirus* spp.), *Dictyocaulus* sp., *E. rangiferi*, *Trichuris* sp., and *Capillaria* sp. were found in 106 Russian zoo reindeer out of 233 ones via fecal examination. All these helminths were previously reported for reindeer and pose no direct danger for humans. Intensity of invasions was mostly low. Fecal examination might be considered as an indirect method for mange diagnostics, as *Chorioptes* and *Demodex* mites were found in reindeer fecal samples. The latter may represent a novel species of mite specific for reindeer.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Figures S1–S25: Light microscopic images of the two types of *Nematodirus* eggs showing the development of embryos from 13 December 2023 to 04 January 2024 respectively. Eggs were obtained from the same fecal sample from a reindeer from the zoo #40. Eggs were placed in a watch glass filled with tap water. Pictures were taken via photo camera of the smart phone Xperia2 DS Black (H4113) (Sony, China) and the optical microscope Micmed-6 (LOMO-MA, Russia).

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Appendix A

Mites found in the feces of zoo reindeer and manifestation of mange caused by mites in reindeer is shown in Figure A1.

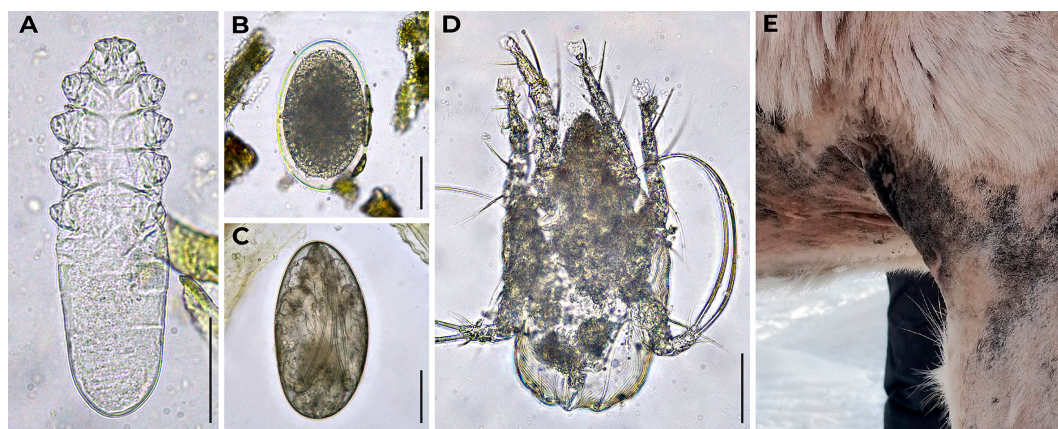


Figure A1. Mites of reindeer. (A) *Demodex* sp. obtained from feces of reindeer from the zoo #12; (B) Egg of mite at early developmental stage obtained from the feces of reindeer from the zoo #30; (C) Egg of mite at late developmental stage obtained from the feces of reindeer from the zoo #48; (D) *Chorioptes* sp. obtained from the feces of reindeer from the zoo #40; (E) Manifestation of mange caused by mites in reindeer from the zoo #30 (hairless front leg and chest are shown); photo courtesy: Kristina Zabarina. Light micrographs were made via bright field microscopy, 400× magnification. Scale bar equals 50 µm.

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