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EPIZOOTIC MONITORING OF PROTOZOOZES IN THE FUR FARMS OF THE KALININGRAD REGION (2018-2020)

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Abstract

Parasitic diseases are widespread in fur-bearing animals, especially in minks. Coccidiidoses occupy a special place among invasive diseases, as they often occur without any symptoms and in some cases are not timely diagnosed. Despite the mild clinical manifestation of invasion, it causes serious damage to animal health and significant economic damage to fur-bearing animal farms. After analyzing the literature data, it became clear that the distribution of eimerioses of fur-bearing animals in the Kaliningrad region has not been studied well enough. In particular, there is no information about the prevalence rates, invasion intensity, and age dynamics of the mink eimeriosis in the fur farms of the region. The purpose of this work was to study the epizootic situation on mink (*Mustela vison*, Linnaeus, 1761, *Neovison vison* Schreber, 1777) coccidiidoses in fur-bearing animal farms in the Kaliningrad region. Investigations were performed in three fur-bearing animal farms of the Kaliningrad region. The species composition of protozoa in minks was determined by the morphological features of coccidia and by deep sequencing of the 18S rDNA V4 region. OTUs (operational taxonomic units) revealed by the bioinformatic analysis were used to establish the taxonomic affiliation of pathogens which confirmed the results of light microscopy. In analyzing the obtained results and choosing research methods, the age, sex of the animals, as well as housing and feeding conditions were taken into account. Young minks of 5-6 months and adult livestock, 1-2 year-old females and males, were surveyed. In total, 561 animals were examined in three farms, including farm 1 — 273 minks (198 young animals, 75 adults — 33 males and 42 females); farm 2 — 160 minks (68 young animals, 92 adults — 44 males and 48 females), and farm 3 — 128 minks (28 young animals, 100 adults — 44 males and 56 females). In all the farms surveyed, we found the protozoan of the *Eimeriidae* family. The greatest prevalence rates (56 %) and the widest variety of coccidia species occurred in the animals of farm 1 under the invasion of two eimeria, *Eimeria vison* and *E. furonis*, and two isospores, *Isospora laidlawi* and *I. eversmanni* (the *I. eversmanni* we have earlier identified in minks in the Kaliningrad region for the first time). In all fur farms in the Kaliningrad region, eimerioses more often occur as mono invasions. In mixed invasions, 68.55 % were two-parasite invasions, 23.67 % were three-parasite invasions. Young minks are more susceptible to eimeriidoses than adults. In the animals of the current year of birth, *E. vison* (18.36 %), *I. laidlawi* (16.32 %), and *E. vison* + *I. laidlawi* (11.90 %) prevailed.

Keywords: *Mustela vison*, *Neovison vison*, minks, *Eimeria vison*, *Eimeria furonis*, *Isospora laidlawi*, *Isospora eversmanni*, mono invasion, mixed invasion, invasion extensity, invasion intensity, fur farms, Kaliningrad region

Fur animals in fur farms are often exposed to various infectious and invasive diseases [1-3]. Captive carnivores, especially those kept in cages, are often infested with intestinal protozoa. Analysis of research letters shows that in Russia

and other countries, mink are infected mainly with protozoans of the family Eimeriidae Munchin, 1903 [4-6]. Researchers note the most significant vulnerability to eimeriosis of young mink 2-3 months of age, among which the infection reaches 68% [7-9].

Diseases of parasitic etiology cause tangible economic damage to global fur farming [3, 10-12]. In the pathogenesis of eimeriosis, the solution of continuity of the integrity of the intestinal mucosa in sick mink, which is due to the endogenous stage of the parasite (merogony) biological cycle, takes an important place, resulting in pathological changes in the morphology of the small intestine mucosa. The inflammatory dendrite that develops during epithelial desquamation and the protozoa metabolic by-product create conditions for penetration and reproduction of secondary microflora. Due to catarrhal and hemorrhagic enteritis and intestinal necrosis, the body does not absorb nutrients, animals lose weight, which may be lethal [13-15]. *Eimeria* are strictly specific parasites and parasitize in animals of different systematic groups [1, 5, 8].

Studies conducted by a research team on Danish farms between April and October 2016 showed that out of 4,140 animals examined, 108 were infected; hence a prevalence (P) was 2.6%. Morphological analysis of sporulated oocysts ($n = 20$), carried out by light microscopy, allowed establishing their species membership to the genus *Eimeria*. The size of the oocysts was 21.0×13.8 μm with a length-to-width ratio (L/W) of 1.5. Until today, the species membership of *Eimeria* is debatable in some cases. Only molecular genetic studies and phylogenetic analysis of 18S rRNA sequences (1221 bps) of samples obtained from infected minks allowed establishing that *E. vison* is the species with the most significant genetic similarity with *Eimeria* sp. first isolated in the black-striped field mouse (*Apodemus agrarius*) in the Czech Republic. The shorter 18S rRNA site (531 bps) showed that the *E. vison* genome sequences had 97.7% similarity with another species, *E. furonis* [16]. The findings of Petersen et al. [16] may indicate that *E. vison* and *E. ictidea* are probably the same species because they have a high morphological and genetic similarity. Thus, it is possible to solve the question of the taxonomic independence of this or that species of Eimeridae only with a comprehensive molecular genetic study [16, 17].

The epizootic situation of eimeriosis within the regions of Russia is not the same, and in some regions, it requires additional study [7, 18]. Analysis of specialized literature showed that eimeriosis of fur animals within the Kaliningrad Region had not been studied sufficiently; in particular, there is no information on the infection extensiveness and prevalence and intensity, age dynamics of mink eimeriosis in fur farms [19].

This paper presents the epizootic situation monitoring protozoosis in fur farms of the Kaliningrad Region for the first time in many years. The infection extensiveness and prevalence by coccidia in mink of different age groups were determined. Using sequencing and analysis of the small subunit ribosomal RNA gene (SSUrDNA), the parasitic fauna of minks was identified.

The work purpose is to study the epizootic situation of mink eimeriosis in fur farms of the Kaliningrad Region.

Materials and methods. Studies were conducted from 2018 to 2020 in three fur farms of the Kaliningrad Region on mink (*Mustela vison* Linnaeus, 1761, *Neovison vison* Schreber, 1777) (young animals 5-6 months old and adult stock of females and males aged 1 to 2 years). Animals' age, sex, and feeding and housing conditions were considered when selecting research methods and analyzing the data obtained.

A total of 561 mink (*Eimeriidae*-infected and intact), including 267 adult animals (115 males and 152 females) and young animals (294 individuals), were

examined during the observation period. The sampling at fur farm No. 1 was 273 animals, including 198 juveniles and 75 adults (33 males and 42 females); at fur farm No. 2 — 160 minks, including 68 juveniles and 92 adults (44 males and 48 females); at fur farm No. 3 — 128 minks, including 28 juveniles and 100 adults (44 males and 56 females).

Freshly isolated fecal mass samples (10-20 g each) from the examined minks were placed in individual containers, labeled, and transported (at +2-8 °C) to the laboratory for analysis.

Eimeriidae oocysts were isolated from fecal masses by the Darling method using a universal flotation diagnostic fluid [20]. The obtained material was viewed by a light microscope Microton-200M (OOO Petrolaser, Russia), using a Micrometer OMP LOMO (AO LOMO, Russia) tip and a Primo Star (Carl Zeiss, Germany) microscope with visualization at 10×10, 10×20, and 10×40 magnifications. Photorecording was performed using a camera microscope and a Mi MIX 2 smartphone (Xiaomi, China). When determining the parasite species, attention was paid to the oocysts' size, shape, color, shell thickness, the presence or absence of micropyle, clear globule, polar granule, and the shape index was determined (length to width ratio).

Eimeriidae oocysts were cultured according to Arnastauskene's method (1985) using a 2% potassium dichromate solution [3, 20]. After concentrating and washing, the oocysts were placed in advanced Petri dishes and incubated in an incubator at 25-28 °C [21], viewing daily under a microscope under magnification (10×10 and 10×40) [9, 18] to the sporulation timing.

The infestation intensity was assessed by counting oocysts of *Eimeriidae* in 1 g of feces using a VIGIS counting chamber (VIGIS, USSR). The protozoa taxonomic affiliation was determined according to the description of their morphological characteristics in the monograph by Pellerdy [22]. According to Darling, the prevalence was assessed by a flotation test of fecal samples as the percentage of infested samples from the number of animals examined.

The sequencing method was used to confirm the coccidia species composition, and phylogenetic analysis of the small subunit ribosomal RNA gene (SSUrDNA) was performed.

Ten fecal samples from different animals were used for DNA isolation. The washed oocysts were morphometric and subjected to 3-fold freezing in liquid nitrogen (–196 °C) to destroy the walls and release sporocysts. Genomic DNA was extracted and purified as described [23-25]. The concentration of the obtained DNA was evaluated on a spectrophotometer SS2107 (MEDIORA OY, Finland), the preparations were stored at +4 or –20 °C [23].

Each sample was genotyped at two loci, nuclear 18S rDNA (SSUrDNA) [26] and mitochondrial cytochrome oxidase subunit I (mt COI) [27]. Sites from nu 18SSUrDNA and mitochondrial cytochrome oxidase subunit I (mt COI) DNA were amplified using polymerase chain reaction (PCR). For all samples, real-time PCR amplification was performed on a Veriti® Thermal Cycler (Life Technologies, Inc., USA) in a 25 rl volume containing ~100 ng of genomic DNA, 1× PCR buffer, 1.5 mM MgCl2, 0.2 mM dNTPs, 1 unit TaqDNA Invitrogen Platinum polymerase (Thermo Fisher Scientific, Canada), and 400 nM of each primer [28, 29]. For sequencing, PCR fragments were obtained in a Bio-Rad T100 thermal cycler (Bio-Rad Laboratories, Singapore). Amplification mode: 3 min at 95 °C; 30 s at 94 °C, 30 s at 56–62 °C, 30–75 s at 72 °C (35 cycles); 7 min at 72 °C (final elongation). The obtained amplification products were separated by electrophoresis in 2% agarose gel, stained with ethidium bromide, visualized on a WUV-M10 ultraviolet transilluminator (DAIHAN Scientific, South Korea), and separated by

electrophoresis in agarose gel with fluorescent detection. According to the manufacturer's recommendations, they were then analyzed using a CEQ 8000 automatic sequencer (Beckman Coulter, USA). The instrumental error of the CEQ 8000 was 5% or less [23]. The results were counted by peak size and area using a program unit in the Geneious database (https://www.geneious.com/); taxonomic annotation of *Eimeriidae* was performed by BLAST searches (https://blast.ncbi.nlm.nih.gov/Blast.cgi) using nucleotide sequences published in GenBank (https://www.ncbi.nlm.nih.gov/gen-bank/) [23-25, 28]. Phylogenetic analysis was performed using the Molecular Evolutionary Genetics Analysis (MEGA v. 7.1) program [30, 31].

Statistical processing of the results was performed using Microsoft Excel 2013 and Primer of Biostatistics 4.03 for Windows.

Results. Monitoring the invasion and parasitic fauna of minks is vital to determine their essential role in transmitting parasitic zoonoses [32]. In animal farm No. 1 (Bagrationovskiy District, Kaliningrad Region), *Eimeriidae* infestation was found in 153 minks out of 273 examined (P = 56%). The research team found two species of eimeria, *E. furonis* and *E. vison* (Fig. 1, a), and two species of isospores, *I. laidlawi* and *I. eversmanni* (see Fig. 1, b).

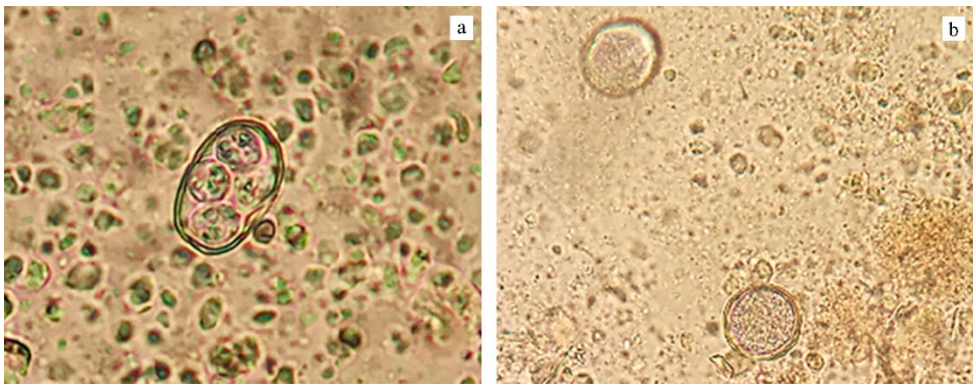

Fig. 1. Light microscopy of oocysts of *Eimeriidae* isolated from minks (*Mustela vison*) in fur farms (Kaliningrad Province, 2018-2020): a — sporulated oocyst of *Eimeria vison* (magnification ×1480), b — non-sporulated oocysts of *Isospora laidlawi* + *I. eversmanni* (magnification ×1280) (Microton-200M (OOO Petrolaser, Russia).

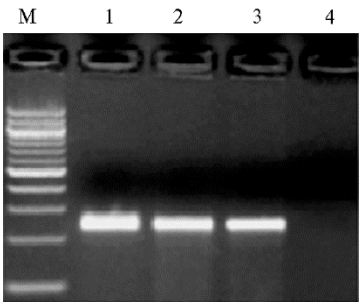

Fig. 2. Electrophoretic analysis of 18S rDNA libraries in genotyping of *Eimeriidae* isolated from minks in fur farms (Kaliningrad Province, 2018) (an example): M — molecular weight-size marker (GeneRuler 1 kb Plus DNA Ladder, Thermo Fisher, USA), 1-3 — Nor1 sample (*Eimeria vison*), different amounts of DNA (1, 2, 3 μl), 4 — Ctrl_NegControl (2% agarose gel).

The species composition of protozoa in the examined minks was confirmed by metagenomic sequencing (Fig. 2) with the following primers (Table 1) [28].

1. Primer pairs for amplification of nuclear and mitochondrial loci [24-26] **used for genotyping of *Eimeriidae* isolated from minks (*Neovison vison*) in fur farms** (Kaliningrad Province, 2018-2020)

Locus	Primer	Sequence (5′→3′)	Author
18S rRNA	V4F	CCAGCASCYGCGGTAATTCC	S. Balzano et all. (2015)
	V4RB	ACTTTCGTTCTTGATYRR	
mt COI	COI_10F	GGWDSWGGWRYWGGWTGGAC	J.D. Ogedengbe et all. (2011)
	COI_500R	CATRTGRTGDGCCCAWAC	

Molecular analysis of the nucleotide sequence of the ribonucleic acid gene of the small ribosomal subunit determined the species identity of the isolated eimeriosis pathogens. Deep sequencing of the V4 site of the 18S rRNA gene and bioinformatic analysis made it possible to identify operational taxonomic units and establish their affiliation. According to the genotyping results, the predominant protozoan species parasitizing minks were *E. vison, E. furonis, and I. laidlawi*, and a rarely encountered species, *I. eversmanni* [25]. The sequence of a 383-bp 18S rDNA fragment detected in the oocysts assigned to the *E. vison* species by light microscopy had the highest (99.48%) similarity to the sequences of *E. ictidea* [25]. The latter species was not previously detected by the authors during coproovoscopy under a light microscope in any of the surveyed fur farms. This result suggests that more detailed molecular genetic studies of Eimeriidae using a more extended nucleotide sequence are required. The high morphological and genetic similarity between *E. vison* and *E. ictidea* raises the question of the possible need to synonymize these two species [9]. At the same time, the conducted phylogenetic analysis and the construction of a combined tree based on the comparison of a 383-bp 18S rDNA fragment sequence of sample OTU 213 indicate that *E. ictidea* is still most likely an independent species of Eimeridae, which differs from *E. vison*, judging by the nucleotide sequences published in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) (Fig. 3).

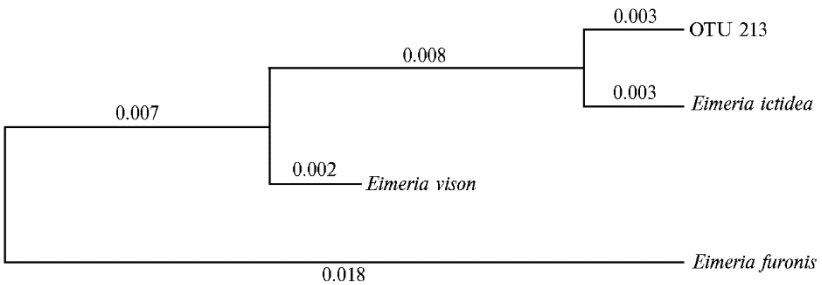

Fig. 3. Phylogenetic analysis (combined tree) **of *Eimeriidae* species based on the nucleotide sequence of a 383-bp 18S rDNA fragment.** OTU 213 was isolated from minks in a fur farm in the Kaliningrad region in 2019; nucleotide sequences of *Eimeria vison*, *E. furonis* and *E. ichdea* are published in Gen-Bank (https://www.ncbi.nlm.nih.gov/genbank/).

The results of genotyping based on the analysis of nuclear 18S rDNA SSUrDNA (with confirmation by mt COI) also indicated the circulation in the surveyed farms of two *Eimeria* species, *E. furonis* and *E. vison* and two isospores species, *I. laidlawi* and *I. eversmanni*, which were detected by light microscopy. It should be noted that in *E. vison* Kingscote, 1934, oocysts have morphological similarity with another species of *Eimeria*, *E. ichdea* Hoare, 1927, that is, it is difficult to differentiate them only by light microscopy (morphometrically). According to the morphometric description we performed in *E. vison* species, the size of the oocysts is 16.3-27.7 μm (length)×11.6-18.54 μm (width), on average 22.0×15.07 μm, and shape index 1.46. There is no micropyle. The zygote is fine-grained, globular-extended, and centered. At one pole, there is a polar granule between the wall and the germ mass. Gerasimchik [3] given a similar description, indicating a maximum oocyst size of 27.72×15.86 μm and a minimum size of 17.71×11.17 μm, with a shape index of 1.18-2.01, averaging 1.59 [3]. Despite the slight variation in measurements, the authors in other studies identified the species they found as *E. vison* by the same morphological characters.

According to Pastor [29], the oocysts identified as the *E. ichdea* species had the following morphometric parameters: length 23.98 μm (18.59-30.57 μm), width 18.55 μm (13.73-23.83 μm), and the shape index 1.01-1.60 (mean 1.30).

The oocysts were elliptic in shape, had a colorless double shell, and contained four sporocysts, each with two sporozoites. The sporocysts were ovoid and contained Stieda bodies and a clear globule [29]. At the same time, Pastor points out a rather large dispersion and variability in the measurements of oocysts (Fig. 3) [29].

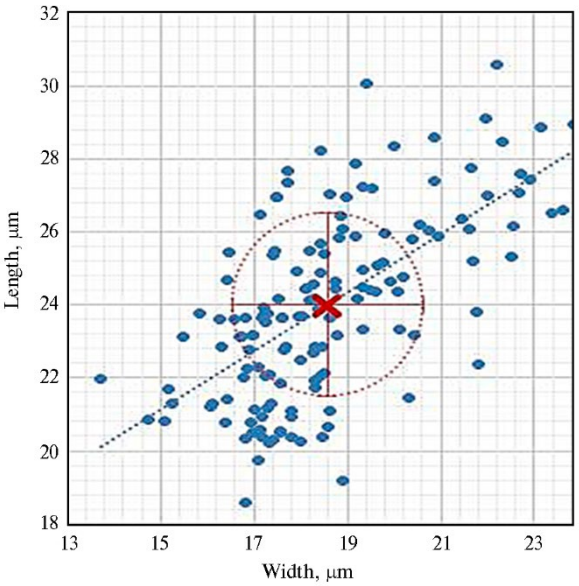

Fig. 4. Measurements of shape, length and width of *Eimeria ictidea* sporulated oocysts in black-footed ferrets (*Mustela nigripes*) [26]. The check (×) marks the mean value, the dashed oval covers one standard deviation from the mean.

Thus, there are two morphologically very similar species with relatively similar shapes, oocyst size, differing only in the presence or absence of the polar granule, which is sometimes difficult to detect under light microscopy. Consequently, molecular and genetic studies are necessary to determine the genus and species of oocysts, especially of unsporulated oocysts. It should be noted that no *E. ictidea* species were detected by light microscopy.

In fur farm No. 1, 47.0% of examined animals wer found to be monoinfested with coccidia. Co-infestation caused by the association of two parasites was noted in 39.0%, three — in 14.0% of the number of infected. *E. vison*, the predominant species of *Eimeridae* causing monoinfestation, was found in 37 minks. At the same time, P among animals infested with this species was 24.20%. The second most common isospore was *I. laidlawi* (17.60%) (Table 2).

2. Protozoa prevalence (P) **in minks of the surveyed fur farms** (Kaliningrad Province)

Protozoa species	Number of animals		P, %
	surveyed	infested	
Fur farm No. 1 (Bagrationovsky District, 2018)			
Eimeria vison	273	37	13.55
E. furonis	273	4	1.47
Isospora laidlawi	273	27	9.89
I. eversmanni	273	3	1.10
Mono invasions in total	273	71	26.01
E. vison + *E. furonis*	273	3	1.10
E. vison + *I. laidlawi*	273	33	12.09
E. vison + *I. eversmanni*	273	2	1.10
E. furonis + *I. laidlawi*	273	10	3.66
E. furonis + *I. eversmanni*	273	1	0.37
I. laidlawi + *I. eversmanni*	273	11	4.03
Associations of two parasites in total	273	60	21.98
E. vison + *I. laidlawi* + *I. eversmanni*	273	4	1.47
E. vison + *E. furonis* + *I. laidlawi*	273	18	6.59
Association of three parasites in total	273	22	8.06
Total number of invasions	273	153	56.04
Fur farm No. 2 (Gur'evsky District, 2019)			
E. vison	160	45	28.13
I. laidlawi	160	33	20.63
Mono invasions in total	160	78	48.75
E. vison + *I. laidlawi*	160	6	3.75
Associations of two parasites in total	160	6	3.75
Total number of invasions	160	84	52.50

Continued Table 2

Fur farm No. 3 (Zelenogradsky District, 2019)

E. vison	128	4	3.13
I. laidlawi	128	41	32.03
Итого моноинвазий:	128	45	35.16
E. vison + *I. laidlawi*	128	1	0.78
Associations of two parasites in total	128	1	0.78
Total number of invasions	128	46	35.94

Monoinvasions caused by *E. furonis* (2.6%) and *I. eversmanni* (2.0%) were less common. Previously, the authors reported that the species *I. eversmanni* was first found in mink within the Kaliningrad Region [25]. According to the literature, Svanbaev in 1956 [6] first discovered and described this species in the steppe polecat in Kazakhstan. Later, Gerasimchik [3] discovered this species in minks in the Republic of Belarus. The *I. eversmanni* species distribution in the Northwestern region has not been studied before.

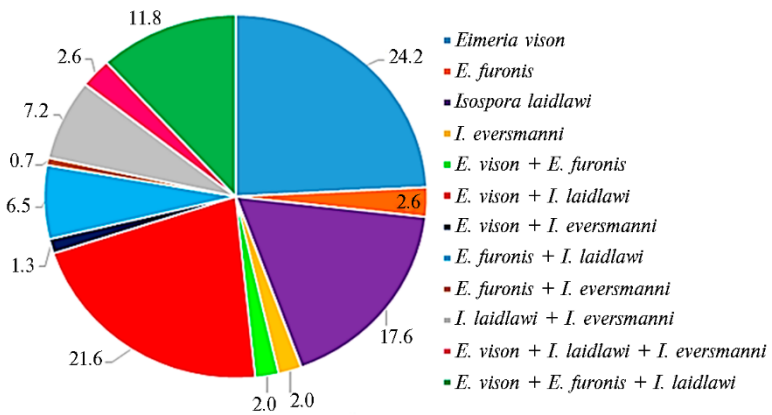

Fig. 5. Parasitic fauna structure in mink infested with *Eimeriidae* in fur farm No. 1 (Kaliningrad Region, 2018).

Co-infestation with two species of parasites in fur farm No. 1 occurred in 39.0% of infected animals and was represented by associations of different protozoan species. Co-infestation with *E. vison* + *I. laidlawi* was found in 33 animals, which amounted to 21.6% of the examined animals. The remaining parasite associations ranged from 0.7% (*E. furonis* + *I. eversmanni*) to 7.2% (*I. laidlawi* + *I. eversmanni*) (see Table 2, Fig. 5). Co-infestation with three species of parasites was recorded in 14.0% of the infected minks. The most frequent association (in 18 animals) of *E. vison* + *E. furonis* + *I. laidlawi* (11.8%) was observed. In 2.6% of infected mink, simultaneous parasitization of *E. vison* + *I. laidlawi* + *I. eversmanni*.

In fur farm No. 1, *Eimeriidae* infestation was found in 23 of 42 examined females (P = 54.76%). Of the 33 males examined, 13, or 39.39%, were infected with protozoa. It was found that in males, as in females, the species *E. vison* was predominant, and to a lesser extent, they were infested by *I. laidlawi*. Co-infestation was less common in adult mink than in juveniles. Juveniles of the current year of birth turned out to be the most infested with *Eimeriidae*, with a P of 76.50%.

In fur farm No. 2, out of 160 examined minks, 84 were infected, which amounted to 52.50% (see Table 2). The coccidia species detected in the examined minks are *E. vison* and *I. laidlawi* (P of 28.13% and 20.63%, respectively). Eimeriosis in this farm was represented mainly by monoinvasions (48.75% of the number of examined minks). Co-infestation with the association of two coccidia species was detected in 3.75% of animals. The ratio of coccidia species within this farm among infected individuals was 53.57% for *E. vison*, 39.29% for *I. laidlawi*, and 7.14% for association of these species.

Within fur farm No. 2, P was 51.85% in females, 44.70% in males, and 57.35% in juveniles of the current year of birth.

Within fur farm No. 3, P for coccidia in minks was 36.0% (see Table 2). In this farm, coccidiosis was predominantly monoinfastation. Among the isospores, the species *I. laidlawi* was found in 41 animals (32.0%). Parasitization of *E. vison* was noted in 3.13% of minks. The association of protozoa was also represented by these two species. Co-infestation was found in one animal (0.78% of the total number of examined animals) (see Table 2). In the parasitofauna structure within this farm, the species *I. laidlawi* (89.13%), *E. vison* (8.70%) was found less frequently in sick animals, and the association of these parasites was rare (2.17% of the number of infested animals).

Note that most of the 128 minks examined by the authors within fur farm No. 3 (see Table 2) were not infested with protozoans (82 animals). Out of 56 adult females, only 6 animals were infested (P = 10.71%), and only one species was detected in them – *I. laidlawi*. Of the 44 males examined, 27, or 61.36%, were infested with protozoa. It was found that in males, as in females, the species *I. laidlawi* was predominant, and to a lesser extent, they were infested by *I. laidlawi*. Co-infestation has not been observed in the adult mink population. Juveniles of the current year of birth were the most infested with coccidia (P = 46.42%). Juveniles were predominantly infested with *I. laidlawi,* and the species *E. vison* was less common. This species structure of the parasitofauna was common for both juvenile and adult males. Co-infestation in juveniles was diagnosed only in one animal (*E. vison* + *I. laidlawi*).

Within all three surveyed farms, P in juveniles was high. Within fur farms No. 1 and No. 2, it was higher than in other groups of animals (76.50% and 46.40%, respectively). Within fur farm No. 3, this characteristic was 28.30% and significantly lower than in adult males with P = 58.70%. Perhaps, the reason for the widespread infestation was the unsatisfactory sanitary condition of the sheds in which the males are kept. In all farms, predominant infestation with eimeriosis in females compared to males was observed.

Infestation intensity in animals in all three farms was low. The infestation intensity ranged from 1 to 50 in females, 1 to 80 in males, and 1 to 180 oocysts in juveniles.

Thus, in all surveyed farms within the Kaliningrad region, protozoans of the family *Eimeriidae* were detected in mink. According to the authors' observations, the widespread eimeriosis is promoted by their asymptomatic course, making timely diagnosis difficult. Molecular genetic studies showed that the sequence of a 383-bp 18S rDNA fragment isolated from samples of oocysts identified by light microscopy as *E. vison* had the most significant (99.48%) similarity with the sequences of *E. ictidea*. However, coproovoscopy did not detect *E. ictidea* in any of the surveyed fur farms. This indicates the need for a more detailed molecular genetic study of Eimeriidae, including the development of methods for their detection and identification to diagnose eimeriosis. The high morphological and genetic similarity between *E. vison* and *E. ictidea* leaves open the discussion of the possible need to synonymize these two species [9].

Within fur farm No. 1, the highest P (56.00%) and the widest variety of coccidia species (*E. vison*, *E. furonis*, *I. laidlawi, I. eversmanni*) were found with the predominance of monoinvastations (47.00% of examined animals). Mink were less infested within farm No. 2 (52.50%), and also with the predominance of monoinvasions (48.75%). In the examined minks, the species of *E. vison* and *I. laidlawi* have been identified. Within fur farm No. 3, the lowest P was set at 35.94%.

In the surveyed fur farms of the Kaliningrad region, differences both in

invasive load and in the species composition of protozoa were revealed. The parasitofauna was more diverse at the breeding fur farm (fur farm No. 1), where the breeding stock is annually renewed by purchasing animals from other farms both in the Russian Federation (Stavropol Territory) and abroad (Denmark). Lack of strict veterinary control at the border or when moving fur animals from one region of Russia to another leads to the spread of protozoosis pathogens. The asymptomatic eimeriosis course makes them difficult to diagnose. Veterinary control is significant when minks come from regions where monitoring for protozoan infestation is not mandatory. The animals were not imported to other examined farms; therefore, in the authors' opinion, two species of parasites, *E. vison* and *I. laidlawi*, can be considered as endemics of mink within the Kaliningrad Province.

Thus, in all surveyed farms, eimeriosis occurs in the form of both mono- and co-infestations. On the whole, monoinfestations account for 68.55% of cases, co-infestations with two parasite species account for 23.67%, and three species account for 7.77%. Two species of *Eimeria* (*Eimeria vison*, *E. furonis*) and two species of isospores (*Isospora laidlawi*, *I. eversmanni*, with *I. laidlawi* dominated) predominate. Young minks are more susceptible to eimeriosis than adult animals. In animals of the current year of birth, there are *E. vison* (18.36%), *I. laidlawi* (16.32%), *E. vison* + *I. laidlawi* (11.90%). These monitoring results allow the development of regulations for treatment and prophylactic measures in the Kaliningrad Region fur farms, taking into account the species composition and biology of *Eimeriidae*.

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