AGRICULTURAL BIOLOGY,
2020, V. 55, № 2, pp. 378-393
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 636.934.57:619:616.993.192.1-07

doi: 10.15389/agrobiology.2020.2.378eng
doi: 10.15389/agrobiology.2020.2.378rus

PECULIARITIES OF DIAGNOSTICS AND PATHOMORPHOLOGY OF EIMERIIDOSES IN THE MINK FARMS OF THE NORTHWESTERN REGION OF THE RUSSIAN FEDERATION

Yu.E. KUZNETSOV[1], L.M. BELOVA[1], N.A. GAVRILOVA[1], M.E. MKRTCHYAN[1], K.V. SIDORENKO[1], A.B. MUROMTSEV[2]

[1]*Saint-Petersburg State Veterinary Academy*, 5, ul. Chernigovskaya, St. Petersburg, 196084 Russia, e-mail Fish2017@yandex.ru (✉ corresponding author), larissabelova2010@yandex.ru, nadezhda.gavrilova65@mail.ru, laulilitik@yandex.ru, capricorn26.12.94@yandex.ru;
[2]*Kaliningrad State Technical University,* 1, Sovetskii prosp., Kaliningrad Province, Kaliningrad, 236022 Russia, e-mail muromtsev.a@mail.ru
ORCID:
Kuznetsov Yu.E. orcid.org/0000-0001-9095-7049
Belova L.M. orcid.org/0000-0003-4473-1940
Mkrtchyan M.E. orcid.org/0000-0002-0615-5703
Gavrilova N.A. orcid.org/0000-0001-5651-5976
Sidorenko K.V. orcid.org/0000-0001-8987-9426
Muromtsev A.B. orcid.org/0000-0002-2456-7023
The authors declare no conflict of interests
Acknowledgements:
The authors thank colleagues from the Yakimov Department of Parasitology for help in identifying eimeriids and translation, the staff of the Department of Biology, Ecology, Histology (St. Petersburg State Academy of Veterinary Medicine) and TLVet Path International Consultants (Animal Eye Consultants of Iowa Laboratory, USA) for their assistance in tissue section preparation, histological pathomorphological analysis, and INC tests, and also we thank M.R. Kabilov (Gemomics SB RAS, Novosibirsk) for conducting molecular analyzes
Received September 12, 2019

Abstract

Parasitic diseases are widespread in fur-bearing animals, especially in minks. Coccidioses 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. The pathogenic effect of eimeriids on the body of fur-bearing animals consists of mechanical, toxic and inoculative effects. As a result, accumulations of mucus are found in the intestinal contents, sometimes with bloody patches. Subacute catarrhal hemorrhagic enteritis occurs, which is manifested by areas of hyperemia and edema of the mucous membrane of the small intestine, desquamation of the epithelium and is accompanied by a violation of the structure of the villi. In the presented work, in the fur bearing animal farms of the Northwestern region of the Russian Federation, the parasitic fauna, prevalence rates (PR) and invasion intensity (II) of minks were studied for the first time, the species composition of eimeriids was clarified by the molecular-genetic method, the clinical and biochemical composition of blood, as well as pathomorphological changes in intestines in animals with eimeriidosis were studied. *Isospora eversmanni* was discovered in the Kaliningrad region for the first time and we managed to discover ill mink puppies from 13 days of age. Even with low II in adult minks, pathomorphological changes in the small intestine were observed. With high II, all layers of the intestinal mucosa were affected and marked diffuse, subacute lymphoplasmacytic enteritis was noted. In ill mink, changes in the composition of the blood were revealed. The objective of our work was to develop an integrated approach to the diagnosis of mink eimeriidosis, including the study of the species composition of parasitic protozoa, the assessment of PR and II, the determination of the clinical and biochemical blood parameters of healthy and eimeriids infected animals, and the establishment of pathomorphological changes typical for eimeriosis and isosporosis that occur in chronic and asymptomatic form. In total, from 2013 to 2019, 6118 minks (*Mustela vison*, *M. lutreola* Linnaeus, 1761, *Neovison vison* Schreber, 1777) were studied in six fur-bearing animal farms of the North-Western region of the Russian Federation using the coprological method. Of these, 294 minks were studied intravitally (clinical study of animals, morphological and biochemical blood tests) and postmortem (autopsia of minks after euthanasia, histological and immunohistochemical tests). At the same time, parasitic fauna was studied in six animal farms of the North-West region of the Russian Federation, 2687 of the examined minks were infected, the prevalence rates (PR) were 43.92 %. It was found that two species of eimeria parasitize in minks, *Eimeria vison* and *E. furonis* and two isospores, *Isospora laidlawi* and *I. eversmanni*. The latter species was discovered by us in the Kaliningrad region of the Russian Federation for the

first time. A deep sequencing of the V4 region of the 18S rDNA gene and bioinformatics analysis were performed, which made it possible to determine OTUs (operational taxonomic units) and establish coccidia's taxonomic affiliation. Thus we were able to confirm the results of light microscopy and determine the taxonomic affiliation of the isolated oocysts. As a result of the analysis, it was found that the sequence of the *E. vison* DNA fragment of 383 bp is most similar (99.48 %) to the sequence of another species (*E. ictide*) found in the GenBank. Data on high morphological and genetic similarities raise the question of the taxonomic affiliation of these two species and require additional detailed study. Most often, eimeriidoses of minks proceeded in the form of mono infections (37.20 %), mixed infections with two parasites were 6.15 %, mixed infections with three protozoans made 0.57 % of cases. The peak of PR in young and adult minks occurred in the summer in the Northwestern region of the Russian Federation. In animals aged 1.5-6 months, eimeriosis and isosporosis proceeded mainly in acute and subacute forms, in minks older than 6 months — in subacute, chronic and latent. The content of hemoglobin and red blood cells in the blood of ill mink with eimeriidosis was significantly lower than in healthy minks, while the number of leukocytes, on the contrary, increased. Eosinophilia, segmented neutrophilia were also observed in ill animals, the number of basophils increased by 2 times, the content of stab neutrophils increased by 1.6 times. Proteinemia was observed in ill animals, the total bilirubin and creatinine content increased by 33.83 and 31.90 %, respectively, and the amount of urea decreased by 21.19 %. A histological examination of material from various parts of the intestine from animals infected with eimeriids revealed that at a low intensity of invasion (II) (in adult minks), although the disease was not clinically manifested in this group of animals, nevertheless, pathological changes in the histological level have already been recorded in small areas and were noted mainly only in the epithelial plate of the intestinal mucosa. With high II, damage to all layers of the intestinal mucosa was observed. Pronounced diffuse, subacute lymphoplasmacytic enteritis was discovered. The pathological processes caused by the parasitism of eimeriid in minks are often similar to those for various infectious diseases, such as the carnivorous plague virus, Aleutian mink disease and coronavirus born disease. To exclude the possibility of diagnostic errors, the material was sent to the laboratory for immunohistochemical studies (IHC), as a result of which antigens of the carnivorous plague virus, coronavirus and Aleutian mink disease were not detected. Nucleic acids of viruses were not detected in all studied samples; the result of IHC was negative in all samples.

Keywords: mink, eimeria, isospora, protozoa, pathogenesis, pathomorphology, histology, immunohistochemistry

Diseases caused by coccidian parasites are among the main reasons for growth slowdown and death of young minks, and deterioration of fur quality in adults [1-4]. *Eimeria* and *Isospora* parasites invade the epithelial cells of the intestinal mucosa and cause catarrhal hemorrhagic enteritis with hyperemia and edema of the small intestine mucosa, desquamation of the epithelium, and impaired villi structure [5]. Because of the pathogenic effect of the parasite, the intestinal mucosa is covered with viscous transparent mucus, thickens, and areas with point hemorrhages appear on it [6, 7]. Infestations may be chronic and asymptomatic, which complicates their timely diagnosis.

Coccidia significantly affect the morphobiological and immunological properties of mucus, which is important for the microbiota of vertebrates [8, 9]. Intestinal protozoa stimulate increased mucus production through the immune response of type 2 T-helpers (Th2), in which interleukins (IL)-13 and (IL)-22 (the cytokines involved in the regulation of inflammatory bowel reactions) control proliferation and goblet cell hyperplasia [10] during the immune response of a host organism trying to get rid of the parasite [6, 7].

Structural and chemical changes in mucin (glycoprotein, which forms the basis of mucus) especially often occur in case of simultaneous invasion of several parasitic protozoa species [6, 11]. Similar processes can occur in carnivores during infectious diseases [4]. Pathomorphological changes in the intestines of minks are mainly described in acute eimeridosis, while the chronic form of pathology is less studied. [12-15].

In the presented work, in the fur farms of the Northwestern region of the Russian Federation, the parasitic fauna, invasion extensity (IE) and invasion intensity (II) in minks were investigated for the first time, the species of eimeriids was specified by molecular methods, the blood clinical and biochemical analyses

were conducted, and pathomorphological changes in the intestine in animals with eimeriidosis were described. For the first time, *Isospora eversmanni* was discovered in the Kaliningrad region, and sick mink puppies were identified from 13 days of age. Pathomorphological changes in the small intestine in adult minks were observed even under low II value. With high II value, all layers of the intestinal mucosa were affected, and marked diffuse subacute lymphoplasmacytic enteritis was noted. Sick minks had changes in blood parameters.

The goal of our work was to suggest an integrated approach to the diagnosis of mink eimeriidosis, including detection of parasitic protozoa species, the assessment of the extensivity and intensity of invasion, the determination of the clinical and biochemical blood parameters of healthy and sick animals, and the pathomorphological changes upon chronic and asymptomatic eimeriosis and isosporosis.

Material and methods. From 2013 to 2019, 6118 invaded by eimeriids and intact minks (*Mustela vison*, *M. lutreola* Linnaeus, 1761, *Neovison vison* Schreber, 1777) were examined in six fur farms of the North-West region of the Russian Federation (Leningrad and Kaliningrad regions). In addition to intravital methods used (clinical observation, blood morphological and biochemical tests, coprological flotation method), pathoanatomic, histological and immunohistochemical studies were performed after euthanasia [16, 17].

For coprological examination, 10-20 g of feces per animal were hermetically packed in plastic bags and delivered to the laboratory at +4 °C. The Darling method using a universal flotation diagnostic fluid was applied [18]. The specimens were viewed with a Mikroton-200M light microscope (Petrolaser LLC, Russia) and a Primo Star microscope (Carl Zeiss, Germany) at 10×10, 10×20 and 10×40 magnification using the OMOM LOMO Micrometer nozzle (JSC LOMO, Russia). Image registration was carried out with a microscope camera and a smartphone camera Mi MIX 2 (Xiaomi, China). Invasion intensity (II) was assessed by counting eimeriid oocysts in 1 g of feces (a VIGIS counting chamber, VIGIS, USSR).

For DNA extraction, oocysts (10 specimens per sample) after a morphometry were frozen 3 times in liquid nitrogen (–196 °C) to destroy the walls and release sporocysts. Extraction and purification of genomic DNA from sporocysts was done as described [19, 20]. The DNA concentration in samples was measured (an SS2107 spectrophotometer, MEDIORA OY, Finland), and the obtained DNA preparations were stored at +4 or –20 °C.

Genotyping of each sample was carried out for two loci, the nuclear 18S rDNA (SSUrDNA) and subunit I of mitochondrial cytochrome oxidase (mt COI). Sequences of nu 18SSUrDNA and mtDNA Cytochrome Oxidase Subunit I (mt COI) were amplified by polymerase chain reaction (PCR) with the following primers: CYC1FE — 5′-TACCCAATGAAAACAGTTT-3′, CYC4RB — 5′-CGTCTTCAAACCCCCTACTG-3′ [21], Cocci 18S 595F 5′-CCGCGGTAATTCCAGCTCCAAT-3′, Cocci 18S 847R 5′-GCTGMAGTATTCAGGGCGACAA-3′, Lank 18S 224F 5′-TCATAGTAACCGAACGGATC-3′ [22], Api SSU 2733R 5′-CGGAATTAACCAGACAAATC-3′ [21-23]. Real-time PCR amplification was performed for all samples on Veriti® Thermal Cycler (Life Technologies, Inc., USA) in a 25 rl reaction mixture containing ~ 100 ng of genomic DNA, 1× PCR buffer, 1.5 mM MgCl2, 0.2 mM deoxyribonucleotide triphosphates (dNTP), 400 nM of each primer and 1 unit of Invitrogen Platinum TaqDNA polymerase (Thermo Fisher Scientific, Canada). For sequencing, PCR fragments were obtained using 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). PCR amplification products were separated electrophoretically in a 2% agarose gel, stained with ethidium bromide, visualized (a WUV-M10 ultraviolet transilluminator, DAIHAN Scientific, South Korea) and separated in agarose gel with fluorescence detection. DNA fragments were analyzed using a CEQ 8000 automatic sequencer (Beckman Coulter, USA) according to the manufacturer's recommendations. The error of the CEQ 8000 was no more than 5%. DNA was also extracted from 10 formalin-fixed and paraffin-embedded tissue samples (5-6 rm) (FFPE) using QIAamp DNA FFPE Tissue Kit (Qiagen, Germany) to detect genomic material of the carnivore plague virus, coronavirus and Aleutian mink disease virus by PCR according to the prescribed research protocol (TLVet Path International Consultants — Animal Eye Consultants of Iowa, USA).

Geneious database software (https://www.geneious.com/) was used for bioinformatics analysis, for taxonomic annotation of eimeriids, the BLAST search tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and nucleotide sequences published in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) were used.

The blood total protein, glucose, total bilirubin, urea, uric acid, creatinine, cholesterol, triglycerides, the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), acid phosphatase (AcP), α-amylase, creatine kinases (CK), lactate dehydrogenases (LDH) were quantified using a Sapphire-400 biochemistry analyzer (Tokyo Boeki Medisys Inc., Japan).

Died (n = 6) and forcedly euthanized (n = 20) animals were investigated by the Scriabin's method of incomplete helminthological autopsy. Mucosal scrapings of the affected areas of gastrointestinal tract membrane were collected, and the smears were prepared and stained according to Romanovsky-Giemsa method. A total of 20 intestines of minks spontaneously infected by eimeriids were examined. Samples from the central part of pathological foci (no more than 1 cm^3) and the area bordering the unaffected tissues were collected no later than 1 h after the death of animals.

Specimens were fixed for 7 days in a 10% buffer formalin solution (pH 6.8-7.0), the amount of which was 10-20 times the volume of the test sample. After washing in running water, 3-4 mm thick fragments cut out from fixed samples through the entire thickness of the tissue were dehydrated in alcohols of increasing concentration (50, 75, 90, 100, and 100%) and paraffin-embedded. An automatic carousel-type machine for histological processing STP-120 (MICROM International GmbH, Germany) was used for dehydration, a AP 280 station (MICROM International GmbH, Germany) was used for paraffin embedding. Slices 5-7 rm thick were made on a rotational microtome HM 320 E (MICROM International GmbH, Germany) with STS section transfer system (MICROM International GmbH, Germany). The slices were transferred to glass microscope slides and allowed to dry overnight. The deparaffining and staining with hematoxylin and eosin were carried out (an HMS 70 linear tissue staining machine, MICROM International GmbH, Germany).

To exclude infectious diseases, immunohistochemical (IHC) analysis was performed. For laboratory diagnostics, the REVEAL Biotin-Free Polyvalent DAB antigen (AG) detection system (Spring Bio Science, USA) was used as per the manufacturer's recommendations. IHC staining was carried out manually; to avoid unwanted evaporation of the liquid and to prevent slides from drying out, we used a special stand with a lid. The glass slides on the stand were covered with a Hydrogen Peroxide Block solution (Cell Marque Corporation, USA) to block endogenous peroxidase and allowed for 10 min at 18-25 °C, then the glasses were washed

3 times in a phosphate-buffered solution (FBI) [25]. To unmask AG, the slides were placed in cuvettes with citrate buffer (pH 6.0), which was heated in a water bath to 95 °C for at least 30-40 min, then cooled (the buffer was not poured out) and washed twice with distilled water. To block non-specific binding, a Protein Block DPB-125 solution (Spring Bio Science, USA) (pH 7.6) was applied to glass with tissue samples for 10 min at 18-25 °C. After removing Protein Block DPB-125 solution the sections were not washed. The slides were incubated with a set of primary antibodies (AT) against the carnivorous plague virus, coronavirus and Aleutian mink disease (Abcam, USA) at 18-25 °C for 25-30 min. The sections were covered with a solution of a conjugate of secondary antibodies with horseradish peroxidase and incubated for 15 min at 18-25 °C. The slides were washed 3 times with buffer solution and stained with a chromogen solution (0.020 cm^3 per 1 cm^3 DAB Substrate), which was applied to a tissue section. The slides were dark-incubated at 18-25 °C for 7-10 min, then washed 3 times with phosphate-buffered saline, stained with Mayer hematoxylin for 3-5 min at 18-25 °C. The dye was removed, and the slides were placed in distilled water for 3-5 min. Prior to coverslips were placed over the slides, tissue samples were dehydrated in 65% ethyl alcohol for 1-2 min, in 80% ethyl alcohol for 1-2 min, and 95% ethyl alcohol for 1-2 min, then placed in xylene for 1 min and air-dried for 10-15 min in a fume hood [24]. All tests were done in triplicate to detect AG of the carnivore plague virus, coronavirus and Aleutian mink disease virus. The results of IH tests were examined under a Primo Star microscope (Carl Zeiss, Germany; the magnification ×100 or ×400). IHC staining in negative control preparations ("tissue control" and "reaction control") was not allowed. The results of the IH tests were evaluated as follows: "–" — no AG of the virus was detected; "+" — single viral AG-positive focus; "++" — several viral AG-positive foci; "+++" — multiple viral AG-positive foci [24, 25].

Statistical processing was performed with Microsoft Excel 2013 and Primer of Biostatistics 4.03 for Windows. Variation statistics method was applied. A simple comparison of averages with the two-sided Student's *t*-test in Tippett's modification was used. Mean values (*M*) and standard error of mean (±SEM) were calculated for clinical and biochemical blood parameters. A p-value = 0.05 was deemed statistically significant; values were ranked by three levels of statistically significant differences: $p \leq 0.05$; $p \leq 0.01$; $p \leq 0.001$.

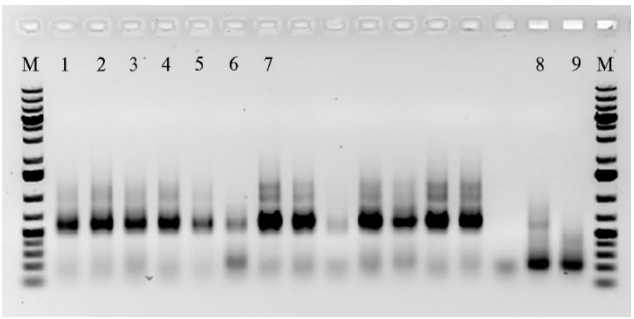

Fig. 1. An example of analysis of 18S rDNA libraries by gel electrophoresis when genotyping of eimeriids isolated form minks in fur farms of the Kaliningrad region in 2018. Samples (different DNA amounts): 1-3 — Nor1 (1, 2, 3 μl), 4, 5 — Nor5 (1, 0.5 мкл), 6, 7 — Nor5PC (1, 0.5 μl); 8, 9 — Ctrl_NegControl (in duplicates); M — molecular weight marker (GeneRuler 1 kb Plus DNA Ladder, Thermo Fisher, USA) (2 % agarose gel).

Results. In a survey of fur farms in the North-Western region of Russia we have established high rate of eimeriidosis. The causative agents were detected in 2687 animals out of 6118 tested, that is, the invasion extensity (IE) was 43.92% (Table 1).

Sequence analysis of 18S rDNA gene was performed in order to establish species affiliation of the isolated causative agents of mink eimeriidosis (Fig. 1).

The deep sequencing of the 18S rDNA gene V4 region and bioinformatics analysis made it possible to determine OTUs (operational taxonomic units) and establish their affiliation. The predominant species of parasitic protozoa were *Eimeria vison*, *E. furonis*, *Isospora laidlawi*, and *I. eversmanni*. In oocysts defined as *E. vison* a 383 bp fragment of 18S rDNA had the greatest (99.48%) similarity with the sequences of *E. ictide*. This suggests that more detailed molecular genetic studies of eimeriids are required. Moreover, high morphological and genetic similarities of *E. vison* and *E. ictidea* raise the question of possible synonymization of these two species.

The results of genotyping eimeriids by mt COI gene sequencing were similar to those for nuclear 18S rDNA.

1. Extensity of invasion (IE) **by eimeriids in minks form fur farms of the North-West region of the Russian Federation** (n = 6118, 2013-2019)

Eimeria and *Isospora* species	Number of invaded animals	IE, %
Eimeria vison	869	14.20
E. furonis	48	0.78
Eimeria invasion (in total)	917	14.99
Isospora laidlawi	1356	22.16
I. eversmanni	3	0.05
Isospora invasion (in total)	1359	22.21
Monoinvasions (in total)	2276	37.20
E. vison + *E. furonis*	34	0.56
E. vison + *I. laidlawi*	294	4.81
E. vison + *I. eversmanni*	2	0.03
E. furonis + *I. laidlawi*	34	0.56
E. furonis + *I. eversmanni*	1	0.02
I. laidlawi + *I. eversmanni*	11	0.18
Invasion with two parasites (in total)	376	6.15
E. vison + *I. laidlawi* + *I. eversmanni*	4	0.07
E. vison + *E. furonis* + *I. laidlawi*	31	0.51
Invasion with three parasites (in total)	35	0.57
Total	2687	43.92

In all farms, monoinvasions by eimeriids prevailed (37.2%), the rate of mixtinvasions with two parasites was 6.15%, mixtinvasions with three protozoa occurred in 0.57% of cases. The most common species in mono-invasions was *I. laidlawi* (see Table 1). IE for this parasite was 22.16%. It should be noted that rare species *I. eversmanni* were identified in one farm in the Kaliningrad region. IE for this parasite was insignificant (up to 0.05%). In this farm *I. eversmanni* was discovered for the first time and only in animals imported from the Stavropol Territory. Earlier, *I. eversmanni* was found in minks in Kazakhstan and Belarus in 1956 and 2006, respectively [26]. Among eimeria, the species *E. vison* prevailed (14.2%), while *E. furonis* was rare (0.78%). In all affected animals, IE was 50.5% for *I. laidlawi*, 32.3% for *E. vison*, and the association of these two protozoa was in third place (10.9%).

For all seasons during the observation years, with the exception of winter periods, IE in young minks was higher than in adults. E.g., in the winter 2016 in the Leningrad region the infection rate of eimeriids was 12.7% for young minks (n = 150) and 15.8% for adults (n = 120). The peak of invasion in both young and adult animals (50.7 and 43.3%, respectively) occurred in the summer. In spring and autumn, IE of young animals remained approximately the same, 38.0 and 36.0%, while in adults it was 30.8 and 24.2%. II also varied from season to season. Protozoa excretion from the body are known to occur with a certain periodicity, depending on the type of parasite, the internal conditions and external factors [2, 4, 12]. We found out that in adult animals from the Leningrad region, II value was the lower, the higher the IE. In the summer decade in the Leningrad region, II in females decreased to 2-58 oocysts, in males to 1-10 oocysts per sample. In autumn and winter, the number of oocysts per sample increased in females up to 480, in males to 240. In spring, II value decreased in females to 1-180, in males to 1-12 oocysts per sample. In young minks, the II reached up to 280 oocysts per sample.

In lab tests we managed for the first time to identify sick mink puppies, starting from 13 days of age (Leningrad region, 2016). In this group (n = 6), single eimeria oocysts were found, IE was 12%. A study of the monthly dynamics of invasion with eimeriids in puppies showed the peak to occur at 2 months of age (June-July), during this period the number of infected young animals reached 30 out of 50 examined, IE was 60%. We associate this increase in invasion in young animals with stress caused by weaning of puppies from nursing females on days 42-43 after birth. Having reached a peak, IE began to decline, reaching 56% in August and 50% in September. In winter, the number of sick animals decreased to a minimum, IE in February was only 6%, that is, 10 times less than in July. Therefore, by the age of 7-9 months, young minks infected with protozoa develop cell-mediated and humoral immunity the intensity of which depends on the type of pathogen and the physiological state of the animals. In 10- and 11-month-olds, a sharp increase in invasion was observed (on average up to 46-56% of the number of young minks examined), which we associate with a change in the of feeding, keeping conditions, grouping and assignment of females to males. However, as noted above, in the autumn-winter period, the II value increases in both young animals and adults.

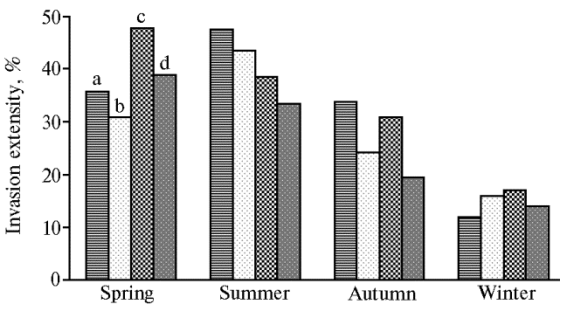

Fig. 2. Seasonal dynamics of eimeriid invasion of young (a, c) **and adult** (b, d) **minks in fur farms of the Leningrad** (a, b, 2016) **and Kaliningrad** (c, d, 2018**) regions.**

As per the publications [4, 26], young animals are more susceptible to eimeriidosis, so we compared the dynamics of the the disease that we identified in the Leningrad region with that in the Kaliningrad region, where the mink rutting occurs earlier.

The invasion peak in young minks and adults in the fur farms of the Kaliningrad region (2018) was in spring (May). IE value during this period was 47.7% in puppies, and 38.9% in animals of the main stock (Fig. 2). In the summer months, IE decreased in both groups to 38.5 and 33.3%, respectively. There was a relationship between animal IE and water supply. In farms with auto drinkers and unlimited access to water, IE was higher than in a farm where animals were supplied with water manually. We admit possible coincidence, however, these facts should be further examined. In autumn and winter, IE in animals from the Kaliningrad region decreased, as in the Leningrad region. However, in winter, in the Leningrad region the IE of minks was higher among adults (15.8%) than that of young animals (12.7%), while in the Kaliningrad region IE of young animals (16.9%) turned out to be higher than in adults (13.9%). A similar trend we observed throughout the year (see Fig. 2).

In both regions, a predominantly acute and subacute of eimeriosis and isosporosis was characteristic of minks from 1.5 to 6 months of age, in animals older than 6 months, a subacute, chronic and latent forms occurred. Animals with chronic form had a decreased activity and appetite, dull fur and diarrhea with an admixture of blood and mucus. The urge to defecate in sick minks was 2-4 times more often than in healthy minks. The body temperature in sick and healthy animals averaged 37.7±0.24 and 38.6±0.15 °C, respectively. Muscle tremor and photophobia were observed in eight sick individuals.

2. Blood parameters of healthy minks and minks infected with eimeriids (fur farms of the North-West region of the Russian Federation, 2017)

Indicator	Healthy minks (n = 12)		Sick minks (n = 40)		Test	
	M±SEM	Cv, %	M±SEM	Cv, %	$t_{cr.}$	p-value
Hemoglobin, g/l	170.00±5.50	10.7	147.00±4.80	20.7	3.151	0.0028
Red blood cells, $\times 10^{12}$/l	8.90±0.60	22.4	6.40±0.40	39.5	3.467	0.0011
Platelets, $\times 10^9$/l	447.80±15.10	11.2	417.00±19.60	29.7	1.245	0.219
White blood cells, $\times 10^9$/l	5.40±0.40	24.6	7.80±0.30	24.3	4.800	1.47×10^{-5}
Basophils, %	0.30±0.42	464.3	0.60±0.36	379.5	0.542	0.5900
Eosinophils, %	1.80±0.28	51.6	7.50±0.42	35.4	11.292	2.30×10^{-15}
Young neutrophils, %	0±0		0.36±0.40	702.7	0.900	0.3724
Band neutrophils, %	4.61±1.00	71.9	7.36±1.20	103.1	1.761	0.084
Segmented neutrophils, %	48.20±4.50	31.0	63.80±2.80	27.8	2.943	0.005
Lymphocytes, ×1012/l	42.99±3.90	30.1	17.44±4.60	166.8	4.237	9.73×10^{-5}
Monocytes, %	2.1±0.16	25.3	2.94±0.90	193.6	0.919	0.363
MID cells, $\times 10^9$/l	3.20±0.60	62.2	3.40±0.20	37.2	0.316	0.753
Total protein, g/l	74.46±3.42	15.2	64.70±2.14	20.9	2.419	0.019
Total bilirubin, rmol/l	7.08±0.32	15.0	10.70±0.90	53.2	3.790	0.0004
Glucose, mmol/l	9.10±0.51	18.6	5.63±0.84	94.4	3.531	0.001
Urea, mmol/l	6.18±1.13	60.6	4.87±0.36	46.8	1.105	0.275
Creatinine, rmol/l	47.80±1.87	13.0	70.20±2.41	21.7	7.343	1.74×10^{-9}
Uric Acid, rmol/l	48.10±2.54	17.5	54.60±1.62	18.8	2.158	0.036
Total lipids, mmol/l	6.71±0.06	3.0	7.14±0.13	11.5	3.003	0.0042
Cholesterol, mmol/l	6.53±0.58	29.5	8.70±1.40	101.8	1.432	0.1584
Triglycerides, mmol/l	1.01±0.05	16.4	1.34±0.07	33.0	3.836	0.0004

N o t e. The Student's t-test was used to compare the independent samples and assess the statistical significance of differences between the average values for each indicator. For groups, df = 50. At a significance level of p = 0.05, the value of Student's t-test $t_{cr.}$ = 2.009, with $t_{fact.} < t_{cr.}$ the difference in means is not statistically significant. The calculated p-value allow us to assess the differences in indicators in sick minks from those in healthy minks.

An analysis of hematological and biochemical parameters in clinically healthy and sick minks showed that the coefficient of variation for most values did not exceed 33% (Table 2), therefore, the analyzed set is homogeneous, and the data have small dispersion, which indicates a slight deviation of the observed values from the average values. The cellular components of blood in clinically healthy and sick minks differed (see Table 2). The hemoglobin content in sick animals was lower than in healthy ones, 147±4.8 vs. 170± 5.5 g/l, respectively ($p = 0.0028 < 0.05$), while the number of red blood cells was $(6.4\pm0.4)\times10^{12}$/l and (8.9 $0.6)\times10^{12}$/l ($p = 0.0011 < 0.05$). The counts of leukocytes were significantly greater in infected minks compared to healthy minks, $(7.8\pm0.3)\times10^9$/l vs. (5.4±0.4) Ч10^9/l ($p = 1.47\times10^{-5} < 0.05$). Sick animals showed a slight decrease in platelet count.

The leukogram (see Table 2) showed that most blood parameters in both healthy and sick animals remained within the reference values, with some exceptions. In infected minks, eosinophilia occurred, and the number of basophils was 2 times more. Young neutrophils appeared in the blood of infected minks, the number of lymphocytes decreased sharply, segmented neutrophilia was observed ($p = 0.005 < 0.05$), but this indicator remained within the reference values. The counts of stab neutrophils also increased 1.6 times.

Proteinemia was observed in sick animals. The total protein level was 13.1% less than in healthy ones ($p = 0.019 < 0.05$). Total bilirubin and creatinine in infected minks increased by 33.83 and 31.9% ($p = 0.0004 < 0.05$ and $p = 1.74\times10^9 < 0.05$, respectively), the urea concentration was 21.19% lower ($p = 0.036 < 0.05$).

Fat metabolism indicators (total lipids, cholesterol, triglycerides) which should be in focus upon invasion with eimeriids since the liver and intestinal mucosa are involved in the biosynthesis of these components were 6.0, 25.0, and 24.6% higher in sick minks than in healthy minks (see Table 2).

Postmortem pathomorphological examination of died and euthanized sick minks revealed a small amount of light-yellow liquid in the abdominal cavity.

Hemorrhagic inflammation was observed along the entire length of intestines. In duodenum, jejunum, cecum there were mucosal folds with spot and band-shaped hemorrhages (Fig. 3). Within the lumen of the small intestine there were gas and contents with streaks of blood and mucus. Meronts and merozoites of eimeriids were found in Romanovsky/Giemsa-stained smears of scrapings from the intestinal mucosa.

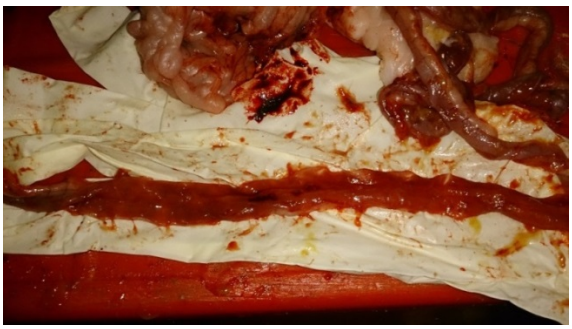

Fig. 3. Hemorrhagic inflammation of small intestine in a mink infected with eimeriids (a fur farm in the Leningrad region, 2016).

Despite the absence or weakly expressed clinical sings of eimeriidosis due to the low intensity of invasion in adults, the histological examination of the intestinal wall epithelium of sick minks revealed pathological processes. With a low degree of infection, the main pathomorphological changes were observed only in the epithelial plate of the intestinal mucosa. With high II, all the layers of the mucous membrane were involved in pathological process manifested by diffuse enteritis with a pronounced lymphoplasmacytic profile. Over the entire wall of the intestine, the vessels were moderately filled with blood, however, a well-defined vasculature were found in the small intestine (Fig. 4). In the own plate of the mucous membrane a polymorphic cell infiltration was revealed, which reached the muscle plate of the intestinal mucosa. At the same time, small-focal concentration of cell groups in the submucosa of the intestinal wall was noted.

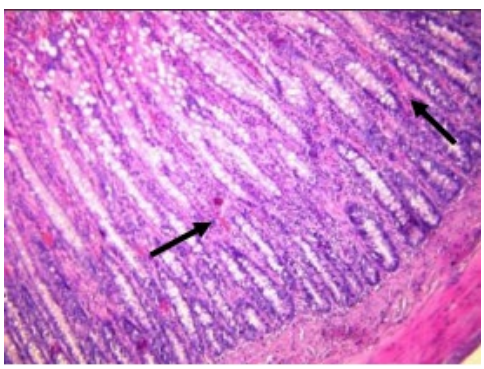

Fig. 4. The small intestine of a mink infected with eimeriids (see cell infiltration and vessels moderately filled with blood). The arrows indicate the areas of pronounced blood-filling of vessels in the own plate of the mucous membrane of the small intestine wall (staining with hematoxylin and eosin; microscope Mikroton-200M, LLC Petrolazer, Russia; magnification ×400) (a fur farm in the Kaliningrad region, 2018).

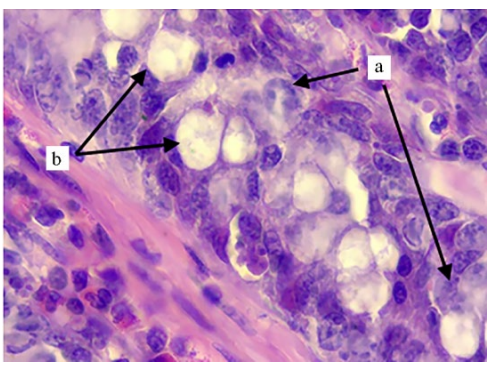

Fig. 5. Mitotic activity of enterocytes (a large number of cells in the late prophase of mitosis) (a) **and proliferation of goblet cells** (b) **(increase in their number and size) in the epithelial plate of small intestine mucous membrane in a mink infected with eimeriids** (staining with hematoxylin and eosin; microscope Mikroton-200M, LLC Petrolazer, Russia; magnification ×400) (a fur farm in the Kaliningrad region, 2018).

In the epithelial plate throughout the intestine with varying degrees of severity, the mitotic activity of enterocytes and the proliferation of goblet cells increased (Fig. 5). In part of the epithelial cells, necrotic processes took place, which was confirmed by the presence of picnotic, karyorectic and karyolytic nuclei. In the epithelium of the small intestine, there were uncharacteristic oval,

sometimes roundish formations with a diameter of 12-25 μm. They contain eimeria merozoites, which were stained with the alkaline dyes (hematoxylin) in blue-violet color. Using ultrastructural studies, multiple endozoites were found inside the meronts (Fig. 6).

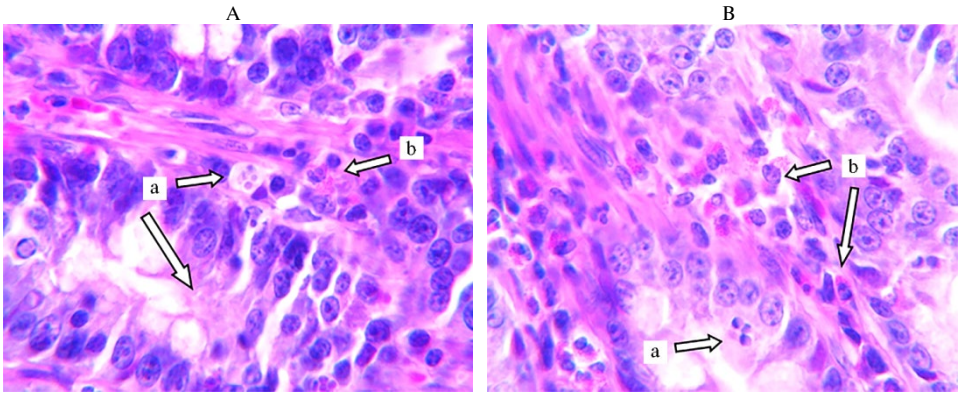

Рис. 6. Parasitophorous vacuole of coccidia merohogony (A) **and eosinophil** congestion **in the lesion caused by coccidia** (B) **in the small intestine of a mink infected with eimeriids:** a — meronts containing eimeria endozoites, b — eosinophils (staining with hematoxylin and eosin, microscope Primo Star, Carl Zeiss, Germany; magnification ×400) (a fur farm in the Kaliningrad region, 2018).

In the mucous membrane of the small intestine, necrotic changes in the cells and desquamation of the single-layer limbic epithelium were revealed, which spread into the crypt mucosal plate. A large number of cells of desquamated epithelium were found in the lumen of the intestine. The development of multicellular meronts (Fig. 7) and the further release of merozoites led to the destruction of surface epithelial cells and atrophy of the small intestine villi. Small sections of the small intestine were found in which the epithelial plate was completely absent. In the own plate, foci of infiltration by lymphocytes, plasmacytes, neutrophils, and eosinophils were present (Fig. 8). Particularly large aggregates of eosinophils surrounded the foci of localization of the endogenous stages of the eimeria.

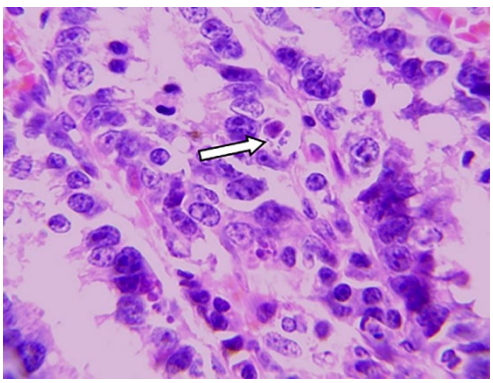

Fig. 7. Meront of coccidia containing coccidia endozoytes (marked by an arrow) **in the destroyed villi of the small intestine of a mink infected with eimeriids** (staining with hematoxylin and eosin; microscope Mikroton-200M, LLC Petrolazer, Russia; magnification ×400) (a fur farm in the Kaliningrad region, 2018).

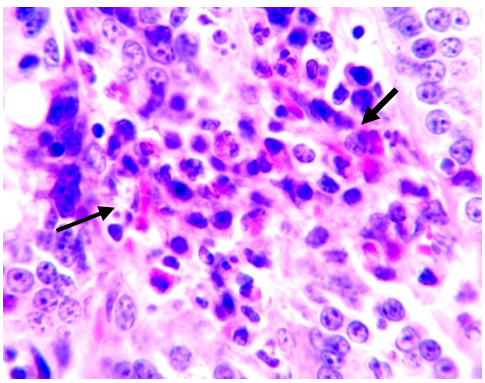

Fig. 8. Eosinophilia in the mucous membrane of the small intestine of a mink infected with eimeriids. The arrows indicate the eosinophil aggregates in the own plate of the intestinal mucosa (staining with hematoxylin and eosin; microscope Primo Star, Carl Zeiss, Germany; magnification ×400) (a fur farm in the Kaliningrad region, 2018).

The endogenous stages of coccidia in minks caused mild eosinophilic (see Fig. 6, Fig. 7) and lymphoplasmacytic enteritis, and in some cases were accompanied by necrosis of crypts. A small number of eosinophils, rare neutrophils,

plasmacytes, lymphocytes and multinucleated cells (see Fig. 8), indicating epithelial syncytium, were present in the own plate of the small intestine villi. Within the middle layer of the mucosa and intestinal crypts, individual necrotic epithelial cells were scattered. Intestinal crypts were occasionally replaced by residues of necrotic cells and a small number of degenerative neutrophils. At the same time, a moderately increased number of mitoses was observed in the remaining undamaged enterocytes of crypt cells. Goblet cell hyperplasia and severe lymphoid clusters were also observed.

Pathogens of plague of carnivores, Aleutian mink disease and coronavirus cause in minks pathomorphological processes similar to those caused by eimeriids. To exclude diagnostic errors, histological preparations of the small intestine (20 histosections) were stained with hematoxylin and eosin and immunohistochemically studied. Antigens of the plague virus, coronavirus and Aleutian mink disease virus were not detected in the studied histological preparations. Nucleic acids of these viruses were also not detected in any of the studied samples; the result of IHC in all samples turned out to be negative (–AG). Nevertheless, the eosinophilic component, together with detected parasitophorous vacuole, confirmed the presence of endoparasites (eimeriids) in the samples.

Our data on the taxonomic affiliation of eimeriids isolated from minks are consistent with the results of other researchers. In 2008-2017, *E. furonis* и *Isospora* (= *Cystoisospora*) *laydlaw* found in mink feces and during postmortem autopsies were studied in Canada [23]. The parasites were characterized using complete mitochondrial genome sequences and 18S rDNA nuclear sequences of *E. ictidea* and *E. furonis*; for comparison, *I.* (= *C.*) *laydlawi* was used [23]. DNA extraction from formalin-fixed paraffinized tissues and sequencing of PCR amplicons made it possible to identify coccidia in the studied samples with high reliability [23]. From 1990 to 2006, V.A. Gerasimchik studied the parasitic fauna of fur animals in the Republic of Belarus, and in 17.45% of animals in farms with different fur production technologies, he identified 4 species of eimeriids: two species of *E. vison* and *E. furonis* and two isospores, the *I. laidlawi* and *I. eversmanni* [26]. However, unlike our data, *E. vison* prevailed (57.03%) in all 24 examined animal farms, which may be due to different production conditions and diet. In the Republic of Karelia, according to V.S. Anikanova [1], only two eimeria species, *E. vison* and *E. furonis*, and one species of isospores, *I. laidlawi*, were identified. The latter species was more common than the rest [1], which is confirmed by findings on the fur parasitic fauna of animals in the Leningrad region, bordering Karelia. In the Republic of Kazakhstan, *E. vison* also prevailed among minks [2, 26]. Eumeria, in particular *E. vison*, were more common in the southern regions, and *I. laidlawi* remained the dominant species in the northern.

As per records in fur farms, breeding minks enter the Russian Federation mostly from Denmark, though also from other states. A number of researchers [13, 23, 27, 28] state that clinical eimeriosis is rarely found in Dutch and Danish mink farms, although coccidiidoses in association with other non-pathogenic or low pathogenic microorganisms are known to cause death of young mink [12, 23].

Analysis of previous studies [2, 3] and our data indicate that the species composition of emeriids is associated with animal age but does not depend on mink color and farm location. Gerasimchik also notes that the species composition of eimeria and isospores is independent of gender and color of minks [26].

It is known that parasitic infestations often occur in association with viral and bacterial infections, while complex relationships develop between animals and the entire complex of microparasitocenosis. In minks seropositive for Aleutian

disease (viral plasmacytosis), a wider variety of protozoan fauna and higher II have been established [26]. Perhaps this is due to a decrease in animal immunity, and the seropositivity of minks can play an important role in the investigation of their parasitic fauna, which is the opinion of many researchers [1, 13, 22].

It is known that the biochemical and physiological properties of coccidia parasitizing in different animal species are similar [1]; therefore, it seems interesting to us to compare the pathomorphology of eimeriidosis that we identified in minks with the course of coccidiosis infestations in other fur animals, in particular rabbits.

Thus, during 2013 to 2019, the eimeriids *Isospora laidlawi* with the invasion extensity of 22.16% were the most common in the surveyed fur farms of the North-West region of the Russian Federation. The species *Eimeria vison* (14.2%) prevailed among eimeria, and *E. furonis* was rare (0.78%). In one of the farms in the Kaliningrad region, *I. eversmanni* was found for the first time. Eimeriidosis of minks mostly occurred as a monoinvasions (37.2%), the rates of mixed invasions by two and three parasites were 6.15% and 0.57%, respectively. The peak of invasion extensity (IE) in the North-West region of the Russian Federation in young and adult minks occurred in the summer. Single oocysts of eimeriids in puppies were found from the 13-day age (IE = 12%). In minks aged 1.5-6 months, eimeriosis and isosporosis proceeded mainly in acute and subacute form, in animals older than 6 months the forms were subacute, chronic and latent. The blood level of hemoglobin and erythrocytes in the minks with eimeriidosis was significantly lower than in healthy minks, and the number of leukocytes, on the contrary, increased. Eosinophilia and segmented neutrophilia were also observed in sick animals, the number of basophils was 2 times higher, the counts of stab neutrophils were 1.6-fold. Proteinemia was characteristic of sick animals, the total bilirubin and creatinine levels increased by 33.83 and 31.9%, respectively, and the urea concentration decreased by 21.19%. Immunohistochemical studies did not detected antigens of the plague virus of carnivores, coronavirus and Aleutian mink disease virus. Mild eosinophilic and lymphoplasmacytic enteritis was detected, accompanied by rare crypt necrosis due to lesion caused by endogenous stages of coccidia. Coccidia infection as monoinvasion or parasite associations is accompanied by a violation of the integrity of the intestinal mucosa. At a high invasion rate, both the intrinsic and muscular plates and the submucosal base are involved in polymorphic cell infiltration. In this case, a syndrome of increased intestinal permeability arises, which is accompanied by a violation of the function of the gastrointestinal tract.

REFERENCES

1. Anikanova B.C. *Koktsidii zhivotnykh zverokhozyaistv Karelii. Avtoreferat kandidatskoi dissertatsii* [Coccidia of farm animals in Karelia. PhD Thesis]. Petrozavodsk, 1991 (in Russ.).
2. Kuznetsov Yu.E. *Sovremennaya nauka: aktual'nye problemy i puti ikh resheniya*, 2015, 1(14): 48-50 (in Russ.).
3. Kuznetsov Yu.E. *Kishechnye parazitozy pushnykh zverei v khozyaistvakh Leningradskoi oblasti. Kandidatskaya dissertatsiya* [Intestinal parasitoses of fur animals in farms of Leningrad region. PhD Thesis]. St. Petersburg, 2012 (in Russ.).
4. Safiullin R.T. *Rossiiskii parazitologicheskii zhurnal*, 2008, 2: 84-99 (in Russ.).
5. Kuznetsov Yu.E. *Mezhdunarodnyi vestnik veterinarii*, 2019, 4: 43-50 (in Russ.).
6. Hasnain S.Z., Gallagher A.L., Grencis R.K., Thornton D.J. A new role for mucins in immunity: insights from gastrointestinal nematode infection. *The International Journal of Biochemistry & Cell Biology*, 2013, 45(2): 364-374 (doi: 10.1016/j.biocel.2012.10.011).
7. Turner J.E., Stockinger B., Helmby H. IL-22 mediates goblet cell hyperplasia and worm expulsion in intestinal helminth infection. *PLoS Pathogens*, 2013, 9(10): e1003698 (doi: 10.1371/journal.ppat.1003698).
8. Chen T.-L., Chen S., Wu H.-W., Lee T.C., Lu Y.-Z., Wu L-.L., Ni Y.-H., Sun C.-H., Yu W.-H., Buret A.G., Yu L.C.-H. Persistent gut barrier damage and commensal bacterial influx following eradication of Giardia infection in mice. *Gut Pathogens*, 2013, 5(1): 26 (doi: 10.1186/1757-4749-5-26).

9. Kuznetsov Y.E., Engashev S.V., Engasheva E.S., Nikonov I.N., Kuznetsova N.V. Microbial community studying of the dogs' gastrointestinal tract by the T-RFLP molecular genetic method and assessing the natural resistance of animals. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 2018, 9(5): 1652-1660.
10. Broadhurst M.J., Leung J.M., Kashyap V., McCune J.M., Mahadevan U., McKerrow J.H., Loke P. IL-22^{+} CD4^{+} T cells are associated with therapeutic *Trichuris trichiura* infection in an ulcerative colitis patient. *Science Translational Medicine*, 2010, 2(60): 60ra88 (doi: 10.1126/scitranslmed.3001500).
11. Tsubokawa D., Ishiwata K., Goso Y., Yokoyama T., Kanuka H., Ishihara K., Nakamura T., Tsuji N. Induction of Sd^{a}-sialomucin and sulfated H-sulfomucin in mouse small intestinal mucosa by infection with parasitic helminth. *Experimental Parasitology*, 2015, 153: 165-173 (doi: 10.1016/j.exppara.2015.03.008).
12. Poloz S.V. *Aktual'nye problemy patologii sel'skokhozyaistvennykh zhivotnykh*, 2000, 2: 403-405 (in Russ.).
13. Jensen H.C. Coccidiose hos mink. *Dansk Pelsdyravl*, 1989, 8(52): 493.
14. Klopfer U., Neuman M. A note on coccidiosis in minks. *Refuah Veterinath*, 1970, 3(27): 122-124.
15. Heimesaat M.M., Bereswill S., Fischer A., Fuchs D., Struck D., Niebergall J., Jahn H.-K., Dunay I.R., Moter A., Gescher D.M., Schumann R.R., Göbel U.B., Liesenfeld O. Gram-negative bacteria aggravate murine small intestinal Th1-type immunopathology following oral infection with Toxoplasma gondii. *The Journal of Immunology*, 2006, 177(12): 8785-8795 (doi: 10.4049/jimmunol.177.12.8785).
16. *GOST 25383-82 (ST SEV 2547-80). Zhivotnye sel'skokhozyaistvennye. Metody laboratornoi diagnostiki koktsidioza* [GOST 25383-82 (ST SEV 2547-80). Farm animals. Methods of laboratory diagnosis of coccidiosis]. Moscow, 1982 (in Russ.).
17. MUK 4.2.3145-13. *Laboratornaya diagnostika gel'mintozov i protozoozov* [MUK 4.2.3145-13. Laboratory diagnosis of helminthiasis and protozoa]. Moscow, 2013 (in Russ.).
18. Belova L.M., Gavrilova N.A., Pudovkin D.N., Tokarev A.N., Kuznetsov Yu.E. *Zhidkost' dlya diagnostiki ootsist koktsidii, tsist balantidii i zhiardii, yaits gel'mintov raznykh klassov, kleshchei, nasekomykh, ikh otdel'nykh stadii razvitiya. Pat. RU 2472154 C2 (RF) MPK G01N33/48 A61D99/00. FGBOU VO «SPBGAVM» (RF). № 2010153464/13. Zayavl. 27.12.10. Opubl. 01.10.13. Byul. № 1* [A liquid for the diagnosis of coccidia oocysts, cysts of balantidia and giardia, helminth eggs of different classes, ticks, insects, their individual stages of development. Patent RU 2472154 C2 (RF) MPK G01N33/48 A61D99/00. FGBOU VO «SPBGAVM» (RF). № 2010153464/13. Appl. 27.12.10. Publ. 01.10.13. Bull. № 1] (in Russ.).
19. Carreno R.A., Schnitzler B.E., Jeffries A.C., Tenter A.M., Johnson A.M., Barta J.R. Phylogenetic analysis of coccidia based on 18s rDNA sequence comparison indicates that *Isospora* is most closely related to *Toxoplasma* and *Neospora*. *Journal of Eukaryotic Microbiology*, 1998, 45(2): 184-188 (doi: 10.1111/j.1550-7408.1998.tb04523.x).
20. Wilcox J.J.S.-Something for everyone: a review of «The biology and identification of the Coccidia (Apicomplexa) of carnivores of the world». *The American Midland Naturalist,* 2019, 181(1): 143-145 (doi: 10.1674/0003-0031-181.1.143).
21. Matsubayashi M., Takami K., Abe N., Kimata I., Tani H., Sasai K., Baba E. Molecular characterization of crane coccidia, *Eimeria gruis* and *E. reichenowi*, found in feces of migratory cranes. *Parasitology Research*, 2005, 97(1): 80-83 (doi: 10.1007/s00436-005-1404-9).
22. Ogedengbe J.D., Hanner R.H., Barta J.R. DNA barcoding identifies *Eimeria* species and **contributes** to the phylogenetics of coccidian parasites (Eimeriorina, Apicomplexa, Alveolata). *International Journal for Parasitology*, 2011, 41(8): 843-850 (doi: 10.1016/j.ijpara.2011.03.007).
23. Pastor A.R. *Investigating enteric coccidiosis in the black-footed (Mustela nigripes) and domestic ferret (Mustela putorius furo). PhD.* University of Guelph, 2017.
24. *Immunogistokhimicheskie metody* /Pod redaktsiei [Immunohistochemical methods. G.A. Frank, P.G. Mal'kov (eds.)]. Moscow, 2011 (in Russ.).
25. Kuznetsov Yu.E., Belova L.M., Gavrilova N.A., Kuznetsova N.V., Shiryaeva V.A., Loginova O.A., Roberman M.G. *Materialy III Mezhdunarodnogo parazitologicheskogo simpoziuma «Sovremennye problemy obshchei i chastnoi parazitologii»* [Mat. III Int. Parasitological Symp. «Modern problems of general and private parasitology»]. St. Petersburg, 2019: 164-168 (in Russ.).
26. Gerasimchik V.A. *Kishechnye parazitozy pushnykh zverei (etiologiya, epizootologiya, patogenez, diagnostika, terapiya i profilaktika). Avtoreferat kandidatskoi dissertatsii* [Intestinal parasitoses of fur animals (etiology, epizootology, pathogenesis, diagnosis, therapy and prevention). PhD Thesis]. Minsk, 2008 (in Russ.).
27. Duszynski D.W. Kvičerová J., Seville R.S. The biology and identification of the Coccidia (Apicomplexa) of Carnivores of the world. *Academic Press*, 2018.
28. Petersen H.H., Yang R., Chriél M., Hansen M.S., Ryan U.M. Morphological and molecular characterisation *of Eimeria vison*-like oocysts (Apicomplexa:Eimeriidae) in farmed mink (Neovison vison) in Denmark. *Parasitol. Res.*, 2018, 117(9): 2933-2939 (doi: 10.1007/s00436-018-5989-1).