DOI: 10.29326/2304-196X-2021-2-37-103-112
UDC 619:616.98:578.834.1-091:636.934.57

Post-mortem and histological lesions in the European minks (*Mustela lutreola*) induced by spontaneous infection with coronavirus SARS-CoV-2

I. A. Subbotina[1], I. N. Gromov[2]
Educational Establishment "Vitebsk State Awarded the "Badge of Honour" Order Veterinary Medicine Academy" (EE "VSAVM"), Vitebsk, Republic of Belarus
[1] *ORCID ID 0000-0001-8346-2988, e-mail: irin150680@mail.ru*
[2] *ORCID ID 0000-0001-8065-5661, e-mail: gromov_igor@list.ru*

SUMMARY
The paper contains data on registration of the SARS-CoV-2 virus in the European mink population. Profound and detailed studies of the virus circulation in the European mink population and the clinical manifestations of the disease, comprehensive approaches to the disease diagnosis, including epidemiological studies, clinical and post-mortem examinations, molecular genetic laboratory diagnostics (polymerase chain reaction and full-genome sequencing) contributed to better understanding of the disease features. The paper presents the data on post-mortem and histological lesions in the European minks infected with the new coronavirus SARS-CoV-2 obtained during the research. All the animals from which the pathological material was collected were infected with SARS-CoV-2, and the diagnosis was made using polymerase chain reaction (RT-PCR). The obtained and presented in the paper data reveal the features and dynamics of pathological processes in the body of infected animals (European mink), demonstrate the characteristics of the lesions in organs and tissues in case of acute and chronic disease, explain the clinical and post-mortem disease pattern and indicate the causes of animal deaths. All this together will allow veterinary specialists not only to quickly and timely diagnose the disease in the population of fur animals (European mink), but also to take necessary therapeutic and preventive measures in a timely manner, to select the most effective means for symptomatic and pathogenetic therapy as well as the most rational and effective substances and disinfection procedures.

Keywords: European mink, coronavirus, SARS-CoV-2, post-mortem lesions, histological examination.

Acknowledgements: The research was carried out at the expense of the republican budget of the Educational Institution Vitebsk State Awarded the "Badge of Honour" Order Veterinary Medicine Academy as part of the research work.

For citation: Subbotina I. A., Gromov I. N. Post-mortem and histological lesions in the European minks (*Mustela lutreola*) induced by spontaneous infection with coronavirus SARS-CoV-2. *Veterinary Science Today*. 2021; 2 (37): 103–112. DOI: 10.29326/2304-196X-2021-2-37-103-112.

Conflict of interest: The authors declare no conflict of interest.

For correspondence: Irina A. Subbotina, Candidate of Science (Veterinary Medicine), Associate Professor, Department of Epizootology and Infectious Diseases, EE "VSAVM", 210026, Republic of Belarus, Vitebsk, 1st Dovator str., 7/11, *e-mail: irin150680@mail.ru.*

УДК 619:616.98:578.834.1-091:636.934.57

Патологоанатомические и гистологические изменения у норки европейской (*Mustela lutreola*) при спонтанном инфицировании коронавирусом SARS-CoV-2

И. А. Субботина[1], И. Н. Громов[2]
УО «Витебская ордена «Знак Почета» государственная академия ветеринарной медицины» (УО «ВГАВМ»), г. Витебск, Республика Беларусь
[1] *ORCID ID 0000-0001-8346-2988, e-mail: irin150680@mail.ru*
[2] *ORCID ID 0000-0001-8065-5661, e-mail: gromov_igor@list.ru*

РЕЗЮМЕ
Представлены данные о регистрации вируса SARS-CoV-2 в популяции норки европейской. Проведение глубоких и детальных исследований циркуляции данного вируса в популяции норки европейской, изучение клинического проявления болезни, комплексные подходы в диагностике данной болезни, включающие эпизоотологические, клинические и патологоанатомические исследования, молекулярно-генетическую лабораторную диагностику (полимеразную цепную реакцию и полногеномное секвенирование) позволили более детально понять ряд особенностей данной болезни. В статье приведены данные собственных исследований об особенностях патологоанатомической картины и гистологических изменений у норки европейской при инфицировании новым коронавирусом SARS-CoV-2. Все животные, от которых отбирался патологический материал, были инфицированы SARS-CoV-2, диагноз ставился с использованием полимеразной цепной реакции (RT-PCR). Полученные и приведенные в исследовании данные широко раскрывают особенности и динамику патологических процессов в организме инфицированных животных (норки европейской), показывают особенности происходящих

изменений в органах и тканях как при остром, так и при хроническом течении болезни, объясняют клиническую и патологоанатомическую картину болезни и указывают на причины летальных исходов у животных. Учитывая очевидную высокую информативность результатов гистологического исследования по сравнению с неспецифичностью патологоанатомических изменений, считаем проведение его обязательным при постановке предварительного диагноза на COVID-19 у норок. Все это в совокупности позволит ветеринарным специалистам не только быстро и вовремя диагностировать данную патологию в популяции пушных животных (норки европейской), но и своевременно провести необходимые лечебно-профилактические мероприятия, подобрать наиболее эффективные средства для симптоматической и патогенетической терапии, подобрать наиболее рациональные и эффективные вещества и режимы для дезинфекции.

Ключевые слова: Норка европейская, коронавирус, SARS-CoV-2, патологоанатомические изменения, гистологическое исследование.

Благодарность: Работа выполнена за счет средств республиканского бюджета УО «Витебская ордена «Знак Почета» государственная академия ветеринарной медицины» в рамках проведения научно-исследовательских работ.

Для цитирования: Субботина И. А., Громов И. Н. Патологоанатомические и гистологические изменения у норки европейской (*Mustela lutreola*) при спонтанном инфицировании коронавирусом SARS-CoV-2. *Ветеринария сегодня*. 2021; 2 (37): 103–112. DOI: 10.29326/2304-196X-2021-2-37-103-112.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Субботина Ирина Анатольевна, кандидат ветеринарных наук, доцент кафедры эпизоотологии и инфекционных болезней, УО «ВГАВМ», 210026, Республика Беларусь, г. Витебск, ул. 1-я Доватора, 7/11, *e-mail: irin150680@mail.ru.*

INTRODUCTION

The coronavirus infection COVID-19 caused by SARS-CoV-2 virus has prevented humanity from returning to a normal active life and communication in all spheres for nearly two years already. Strict quarantine measures are still in place in many countries, in some countries lockdown have been implemented, some countries closed their borders for foreigners and imposed restrictions on leaving the country, the trade, political and social relations between countries have been impeded. And to think that at the beginning when the infection occurred and the agent was identified the humanity neglected the situation and compared it with "common seasonal influenza". But unfortunately the disease appeared to be more severe than influenza and for today more than two million people have become its victims. The coronavirus infection can, of course, be compared to the Spanish flu based on many characteristics. However, one shouldn't forget the time when the Spanish flu, having claimed about 100 million lives, occurred and healthcare level of that time. And now, take into consideration the COVID-19 impact in the century of high technologies and advanced medicine – the comparison is not in our favour. Many doctors, virologists and researchers consider that this is just the beginning of the journey of the relatively aggressive and deadly virus.

One more characteristic of the new coronavirus raises concern among scientists and epidemiologists in the world – absence of distinct species specificity. Originally it was proved that COVID-19 – is a zoonosis transmitted from an animal to a human. The sources of the virus were determined and bat is considered to be one of the basic sources. However, there is some controversy as to whether pangolins and snakes were involved in the process of mutation and agent transmission to the human. In the first days and months of the pandemics the disease was reported and studied only in humans, but currently the situation has drastically changed [1–3].

According to the official data of the World Organization for Animal Health (OIE), Food and Agricultural Organization of the United Nations (FAO), Centers for Disease Control and Prevention (CDC), World Health Organization (WHO), American Veterinary Medical Association and some other international organizations SARS-CoV-2 has currently been recovered from quite a huge number of animals. Moreover, the virus not only circulated in the body of animals but causes the disease development and death in some species. The clinical disease manifestations are similar to those in humans: common symptoms are depression, feed refusal, coughing, fever, labored breathing, dyspnea, sometimes diarrhea [4].

So far, the disease has been registered and the clinical picture in representatives of the *Felidae* family (cats, lions, leopards, tigers and pumas) as well as in fur animals (European mink, tinker) has been partially described. Multiple positive results of COVID-19 diagnostics in representatives of the *Felidae* family were obtained in the USA, some European countries (Italy, France, Belgium, Lithuania, etc), in the Russian Federation, Ukraine and China. All animals had contacts with sick people (COVID-19 positive). The infected animals had damaged respiratory organs, the major disease symptoms were nasal discharge, rapid and shallow breathing, coughing. In several cases gastrointestinal disorders were observed (diarrhea). The Chinese researches performed an experiment and proved animal-to-animal SARS-CoV-2 transmission within the cat population. Italian researchers conducted a comprehensive examination of cats and dogs in most COVID-19 affected Italian regions and detected a relatively high percent of animals with SARS-CoV-2 antibodies (dogs – more than 30%, cats – more than 40% of all tested animals), which is indicative of susceptibility of these animal species to the new virus [4, 5].

Besides data on the virus spread among pets (cats and dogs) there are data on possible virus persistence and its transmission within tinker population (a perfect biological model for studying SARS-CoV-2). The virus circulation in raccoon dogs and rabbits is also possible. There is evidence of the possibility of infecting laboratory animals (white

mice, golden Syrian hamster and guinea pig), badger, pig (with experimental infection), small ruminants. There have been published data on the possible (theoretical) infection of about 400 animal species due to the receptor protein ACE2 they have [5, 6].

Within the last months there have been reports from some zoos about infection of gorillas (San-Diego). AVMA has also provided data on profound studies of SARS-CoV-2 circulation in different animal species (more than 2000), as a result of which the virus was recovered in samples collected from civet cats, dolphins, armadillos, and anteaters. The researchers determined that 80% of the tested pets (cats and dogs) were infected. The data on SARS-CoV-2 circulation in populations of different animal species are constantly updated [4–7].

The new coronavirus circulation in the mink population and the pathological condition induced by it are worth mentioning. As the latest OIE, FAO, CDC, WHO data demonstrate minks are one of the potential (and the only confirmed as for today) source of the backward virus transmission to humans and one of the animal species in whose body the virus is likely to mutate. Denmark published information about destruction of the entire mink population (about 17 million animals) because of the possible SARS-CoV-2 virus mutation in their body and its probable transmission to humans (12 people). Later the strain with a similar mutation was detected in more than 200 people (according to official data of the National Danish Institute for Infectious diseases). However, there was no any direct proof that the infection was transmitted from minks, it was only a hypothesis. Previously, the Netherlands informed about possible infection of two humans by minks. To date, data on the infection of minks have already been published by many countries around the world (Poland, Lithuania, Denmark, the Netherlands, the USA, etc.), in a number of countries it was decided to destroy the mink population due to the threat of the virus mutation and its transmission to the human population [8–13].

The issue of the animal's role in SARS-CoV-2 spread and its persistence in their populations and the virus influence on the animal's body is considered to be practically unstudied. The research in this field is just taking shape in several countries.

Based on the above, the circulation of SARS-CoV-2, which causes COVID-19, in populations of various animal species, as well as the features of the clinical and pathological disease manifestation, histological lesions during infection with this virus are poorly understood and relevant.

The aim of the research was to identify the post-mortem and histological lesions of the European mink (*Mustela lutreola*) during SARS-CoV-2 infection.

MATERIALS AND METHODS

The carcasses of culled European minks (young animals, parent stock) kept in industrial fur farms were used as material for research. According to anamnestic data, in May – June 2020, on a number of farms, there was an increased morbidity and mortality of animals with signs of respiratory tract and cardiovascular lesions.

The PCR tests (RT-PCR) of the biological material (swabs from the mucous membranes of the oral, nasal cavities and rectum) from sick and dead animals using test systems for the detection of SARS-CoV-2 virus RNA (ArtBioTech LLC, Republic of Belarus) revealed positive results.

During autopsy of mink carcasses the nature and severity of pathomorphological lesions were taken into account, a pathological diagnosis was made [14], macro photography was performed using natural light. The autopsy was carried out in specially equipped rooms in compliance with personal and biological safety rules followed by the neutralization and disposal of biomaterial, disinfection of the room and tools, preventing contamination of rooms and equipment.

Pieces of the lungs, liver, kidneys, heart, pancreas, and spleen were taken for histological examination [15]. The resulting material was fixed in a 10% solution of neutral formalin and compressed by paraffin embedding process in accordance with the generally accepted method [16]. Dehydration and paraffinization of organ pieces was performed using an spin tissue processor with carousel system MICROM STP 120 (Germany). An automatic tissue embedding centre MICROM EC 350 was used to embed the pieces and prepare the paraffin blocks. Histological samples of the organ pieces embedded in paraffin were prepared using a rotary (pendulum) microtome MICROM HM 340 E. De-embedding and staining of histological sections with hematoxylin – eosin was performed using an automatic station MICROM HMS 70. Histological examination was performed using a light microscope "Biomed-6" (Russia). The data obtained were documented by microphotography using the DSM-510 digital reading and video input system, as well as the ScopePhoto image input and preprocessing software. Structural changes in the parenchyma and in the stroma were taken into account according to the guidelines [17, 18].

RESULTS AND DISCUSSION

The major post-mortem lesions during the acute course of the disease were characterized by the predominance of hemodynamic disorders and serious disorders of the cardiovascular and respiratory systems.

Macroscopic changes in the lungs were characterized by the simultaneous development of a number of interrelated processes, among which three main combinations were distinguished.

In the first case, severe acute venous hyperemia, serous or serous-hemorrhagic edema, alveolar emphysema were observed in the lungs (Fig. 1). Macroscopically, the lungs did not collapse, their shape was not changed, the color was dark red (almost black-red), the consistency was soft. Jell-like clots or red foam were observed in incised primary bronchi, and well – formed blood clots in dissected large arteries and incised veins. The pattern of the lobular structure is poorly visible. These processes are most likely due to the presence of fibrin in the transudate, which was further confirmed by the results of histological examination. An important sign indicating the absence of classical pneumonia is the buoyancy of the pieces in the water. On a dark red background of the lung parenchyma, the areas of emphysema were clearly visualized in the form of poorly shaped, slightly elevated foci of gray-white color. The fact of the development of this pathological process is important for the disease diagnosis, and we consider it a significant pathognomonic sign. However, when performing an autopsy of dead minks, it can be easily confused with post-mortem processes: redistribution of blood flow, cadaveric autolysis, cadaveric emphysema. In this regard, the results of histological examination are of decisive importance.

In the second case, a combination of acute serous pulmonary edema with areas of alveolar emphysema was observed. The lungs were not collapsed, with a doughy consistency, classic for edema, pink-red, light-red ("carmine" lungs), their shape was not changed. The pattern of the lobules is not pronounced, the pieces of the organ float, completely immersed in the water.

In the third case, the following combination was observed: acute serous edema, areas of alveolar emphysema, small-focal pneumonia with localization in the anterior, middle and caudal lobes of the lungs (Fig. 2–5). Against the background of the processes described above (serous edema, alveolar emphysema), the presence of small-focal pneumonia was observed. The lesions were small (up to 5–6 mm), dark red, localized subcapsularly in various parts of the lungs (caudal, middle, anterior lobes). Pieces of the affected lungs did not drown, but floated, completely submerged in the water. In this regard, this process can be easily confused with spotted hemorrhages. The small size of the inflamed areas does not allow to determine the nature of pneumonia: serous, catarrhal, fibrinous or interstitial. In this case, it is necessary to conduct a histological examination of the lungs.

When studying the heart, three variants of pathological processes were also identified, indicating the development of acute cardiac and concomitant cardiopulmonary failure.

In the first case, in our opinion the most severe and irreversible, there was an acute expansion of all cardiac cavities with the development of a classic round heart, sometimes with severe acute venous hyperemia of the myocardium (Fig. 1, 2, 4). In the second case, the blood content of the heart muscle was less pronounced, however, the characteristic signs of fatty degeneration of the myocardium with its staining in a light yellow color came to the fore (Fig. 3). Fatty degeneration of the myocardium, liver and kidneys is the morphological equivalent of acute intoxication of the body. In the third case, signs of asphyxia prevailed – acute expansion of the right ventricle and atrium, blood stagnation in the pulmonary circulation system (Fig. 5).

Considering the deep structural changes in the lungs, the development of signs of asphyxia, viremia, infectious shock, the formation of pronounced post-mortem blood coagulation looks paradoxical not only in the cavities of the heart and large arteries, but also in veins of various sizes (Fig. 6). In our opinion, this is due to a systemic imbalance in the blood coagulation and anticoagulation systems towards thrombus formation, which plays an important role in the COVID-19 pathogenesis in humans and animals.

In addition, the autopsy of mink carcasses revealed morphological signs of acute heart failure – cyanosis of the mucous membranes, especially of such organs of the oral cavity as the tongue and gums (Fig. 7), skin, skeletal muscles, acute venous hyperemia of the liver and kidneys. Most animals showed punctate hemorrhages in the renal cortex (Fig. 8), and in some specimens, serous or hemorrhagic splenitis (Fig. 9).

In some cases, these processes developed concurrently with chronic feed toxicosis, the characteristic morphological sign of which was fatty liver and interstitial hepatitis (Fig. 1). At the same time, the liver was not enlarged, the shape was not changed, the consistency was elastic, dense, yellow, the pattern of the lobular structure in the section was more distinct.

Six months after recovering from COVID-19, minks killed for diagnostic purposes showed characteristic pathological changes indicating a chronic course of the disease, long-term persistence of the virus in susceptible livestock. As in the beginning of the outbreak, the major processes were observed in the lungs, cardiovascular system and blood. Morphological signs of regeneration of the structure of previously affected organs and tissues were not recorded.

Lung examination revealed alveolar emphysema, edema, hemorrhages, small- and large-focal interstitial pneu-

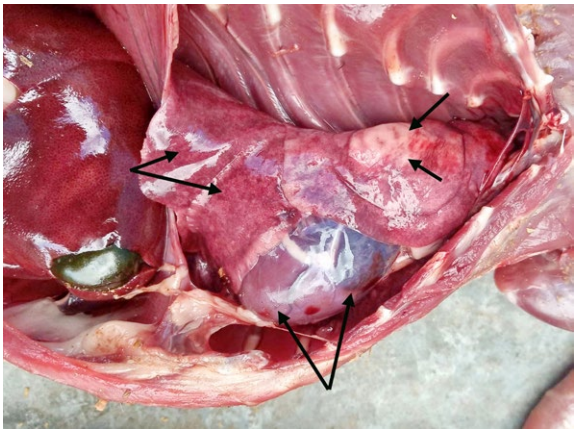

Fig. 1. Post-mortem lesions in a 6-month-old mink infected with COVID-19: acute venous hyperemia (arrows on the left), pulmonary emphysema (arrows on the right), acute cardiac dilatation (arrows below). Background processes: interstitial hepatitis, gallbladder expansion (left)

Рис. 1. Патологоанатомические изменения у 6-месячной норки при COVID-19: острая венозная гиперемия (стрелки слева), эмфизема легких (стрелки справа), острое расширение сердца (стрелки внизу). Фоновые процессы: интерстициальный гепатит, расширение желчного пузыря (слева)

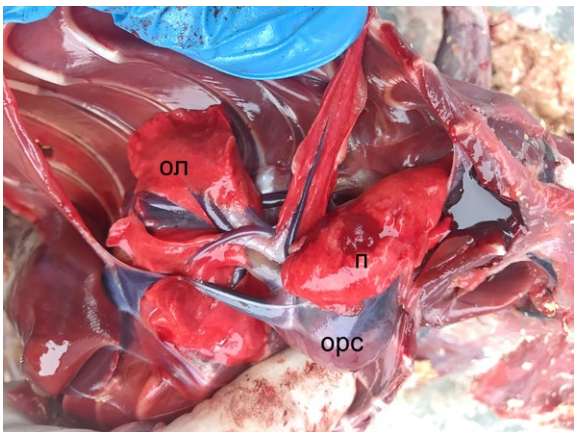

Fig. 2. Post-mortem lesions in a 6-month-old mink infected with coronavirus SARS-CoV-2: pulmonary edema (ОЛ), pneumonia sites (П), acute cardiac dilatation (ОРС)

Рис. 2. Патологоанатомические изменения у 6-месячной норки, инфицированной коронавирусом SARS-CoV-2: отек легких (ОЛ), участки пневмонии (П), острое расширение сердца (ОРС)

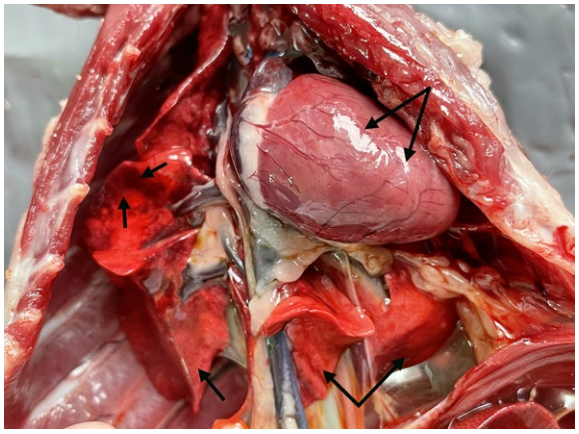

Fig. 3. Post-mortem lesions in a 6-month-old mink infected with COVID-19: pulmonary edema (arrows below), pneumonia sites (arrows on the left), acute cardiac dilatation, myocardial lipidosis (arrows on the left)

Рис. 3. Патологоанатомические изменения у 6-месячной норки при COVID-19: отек легких (стрелки внизу), участки пневмонии (стрелки слева), острое расширение сердца, жировая дистрофия миокарда (стрелки справа)

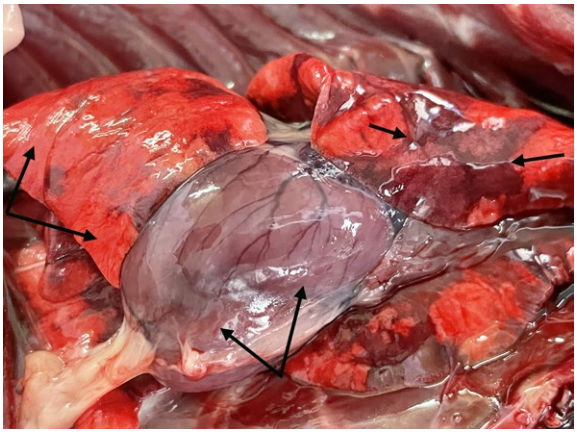

Fig. 4. Lungs of a 6-month-old mink infected with coronavirus SARS-CoV-2: pulmonary edema (arrows on the left), pneumonia and emphysema sites (arrows on the right), round heart (arrows below)

Рис. 4. Легкие 6-месячной норки, инфицированной коронавирусом SARS-CoV-2: отек легких (стрелки слева), участки пневмонии и эмфиземы (стрелки справа), круглое сердце (стрелки внизу)

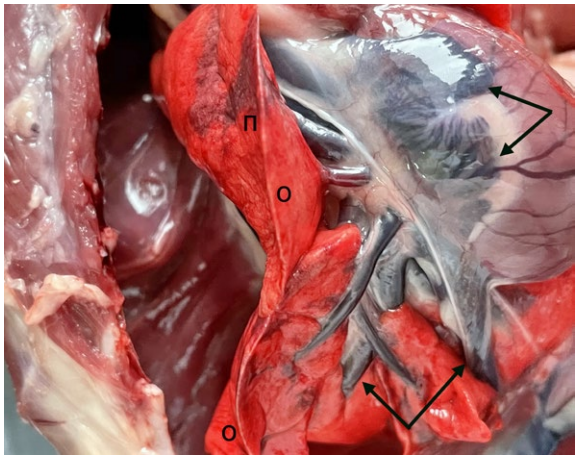

Fig. 5. Pulmonary edema (O) with pneumonia sites (П) in a 6-month-old mink. Enlargement of the left artrium and pulmonary veins (arrows)

Рис. 5. Отек легких (О) с участками пневмонии (П) у 6-месячной норки. Острое расширение левого предсердия и системы легочных вен (стрелки)

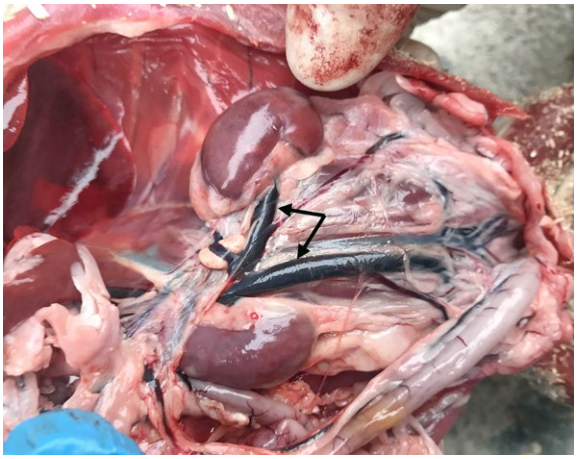

Fig. 6. Severe blood clot formation in the caudal vena cava of a 6-month-old mink infected with coronavirus SARS-CoV-2

Рис. 6. Выраженное формирование посмертных свертков крови в каудальной полой вене 6-месячной норки, инфицированной коронавирусом SARS-CoV-2

monia (Fig. 10, 11). The lungs were not collapsed, their shape was not changed, the consistency was soft with a doughy feel. The background color, as during the acute disease, was pink-red, carmine. Subcapsular, well-defined hemorrhages were common. There were also regions of classic interstitial pneumonia with compaction of the parenchyma, staining it in a "fleshy", red-brown color, as well as a more distinct pattern of the lobular structure. In all cases, the buoyancy of the affected pieces of the lungs was preserved. In some animals, an aggravating process was noted – focal chronic fibrinous pleurisy.

In the heart there were signs of acute expansion, fatty degeneration, but without venous hyperemia of the myocardium (Fig. 10, 11). In the cavities of the heart, the lumen of large arteries and veins, as in the acute course, post-mortem blood clots were detected. The renal cortex showed multiple punctate hemorrhages and brownish pigment spots, which are "old" hemorrhages (Fig. 12).

The spleen was enlarged in size, had elastic consistency, the parenchyma was red-brown in color with a steel tint. The scraping of the pulp with the back of the knife is insignificant.

Post-mortem diagnosis:

acute disease

1. Severe acute venous hyperemia, serous or serous-hemorrhagic pulmonary edema, areas of alveolar emphysema.

or

Serous pulmonary edema (carmine lungs), areas of alveolar emphysema.

Fig. 7. Acute venous hyperemia of the mucous membrane of the tongue in a 6-month-old mink

Рис. 7. Острая венозная гиперемия слизистой оболочки языка у 6-месячной норки

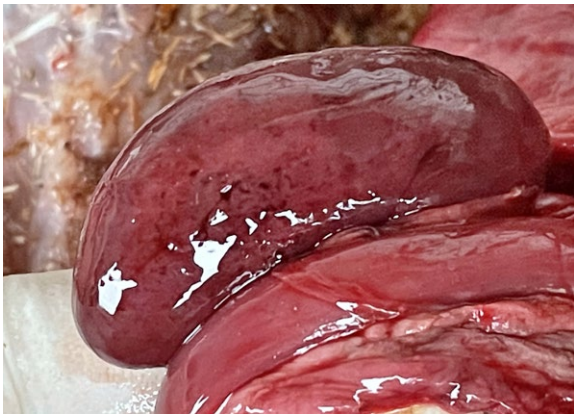

Fig. 8. Hemorrhages in the renal cortex in a 6-month-old mink infected with the coronavirus SARS-CoV-2

Рис. 8. Кровоизлияния в корковом веществе почки у 6-месячной норки, инфицированной коронавирусом SARS-CoV-2

Fig. 9. Acute serous splenitis in a 6-month-old mink

Рис. 9. Острый серозный спленит у 6-месячной норки

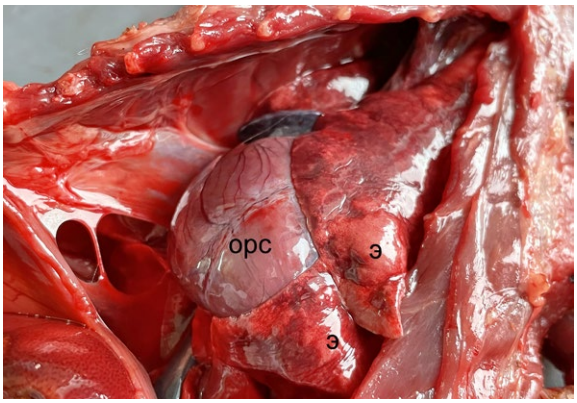

Fig. 10. Post-mortem lesions in an 18-month-old mink infected with the SARS-CoV-2 coronavirus: interstitial pneumonia, sites of emphysema (Э), acute cardiac dilatation (ОРС)

Рис. 10. Патологоанатомические изменения у 18-месячной норки, инфицированной коронавирусом SARS-CoV-2: интерстициальная пневмония, участки эмфиземы (Э), острое расширение сердца (ОРС)

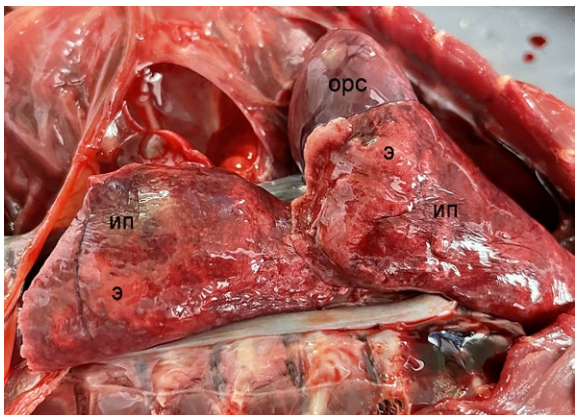

Fig. 11. Interstitial pneumonia (ИП) with emphysema sites (Э) in an 18-month-old mink. acute cardiac dilatation (ОРС), acute venous hyperemia of the myocardium

Рис. 11. Интерстициальная пневмония (ИП) с участками эмфиземы (Э) у 18-месячной норки. Острое расширение сердца (ОРС), острая венозная гиперемия миокарда

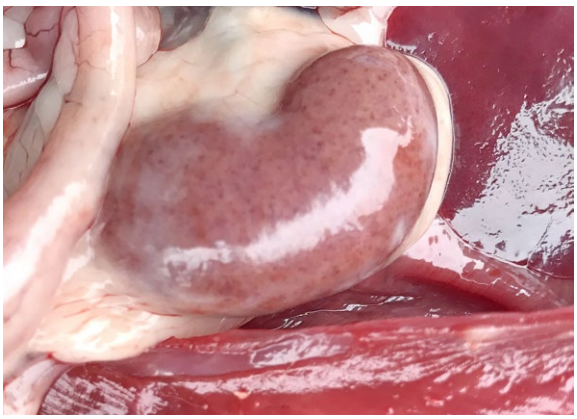

Fig. 12. Kidney of an 18-month-old mink infected with the coronavirus SARS-CoV-2. Pigmented spots in the cortex (old hemorrhages)

Рис. 12. Почка 18-месячной норки, инфицированной коронавирусом SARS-CoV-2. Пигментные пятна в корковом веществе (старые кровоизлияния)

or

Serous pulmonary edema, areas of alveolar emphysema, small focal pneumonia with localization in the anterior, middle and caudal lobes of the lungs.

2. Acute heart enlargement (round heart), acute venous myocardial hyperemia.

or

Acute heart enlargement (round heart), myocardial fatty degeneration.

or

Acute enlargement of artrium and right ventricle (cor pulmonale, asphyxia), pulmonary veins.

3. Severe post-mortem blood coagulation in the cavities of the heart, large arteries and veins.

4. Acute venous hyperemia as well as fatty liver and kidneys. Pinpoint hemorrhages in the renal cortex.

5. Acute venous hyperemia of mucous membranes, skin and skeletal muscles.

6. Acute serous or hemorrhagic splenitis (not always).

chronic disease

1. Alveolar emphysema, edema (carmine lungs) and hemorrhages in the lungs, focal interstitial pneumonia.

2. Focal fibrinous pleurisy (complication).

3. Acute heart enlargement (round heart).

4. Severe post-mortem blood coagulation in the cavities of the heart, large arteries and veins.

5. Hemorrhages and pigment spots (old hemorrhages in the kidneys).

6. Splenomegaly (hyperplasia of the spleen).

Histological diagnosis:

acute disease

• **Lungs** (Fig. 13–16) – vascular hyperemia of the microvasculature, thrombosis of arterioles, venules, interalveolar capillaries (disseminated intravascular coagulation syndrome, shock lungs), severe serous, serous-hemorrhagic edema of interstitial tissue and parenchyma, necrosis and desquamation of alveolar epithelium fibrin threads in the form of a mesh, fragments of necrotic epithelium, hemolyzed erythrocytes and eosinophilic hyaline membranes, extensive lymphoid-macrophage peribronchitis and perivasculitis, focal proliferation of fibroblasts, alveolar emphysema.

• **Liver** (Fig. 17) – acute venous hyperemia, serous edema, thrombosis of the central veins of the hepatic lobules and sinusoidal capillaries (disseminated intravascular coagulation, shock liver), multiple hemorrhages, hemosiderin deposition (hemosiderosis), total droplet fatty degeneration, areas of necrobiosis and parenchymal necrosis.

• **Pancreas –** venous hyperemia, hemostasis (especially in the area of the islets of Langerhans), vacuolar degeneration of individual acini epithelial cells.

• **Kidneys** (Fig. 18) **–** acute venous hyperemia, edema, extensive hemorrhages, large-droplet fatty degeneration of the epithelium of the urinary tubules.

• **Spleen –** focal lymphoid-macrophage infiltrates in the red pulp, hyperemia of sinusoidal capillaries.

chronic disease

• **Lungs** (Fig. 19–21) – a pronounced proliferation of interlobular and interalveolar connective tissue, lymphoid-macrophage peribronchitis and perivasculitis, the formation of nodular lymphoid tissue, chronic venous hyperemia, blood stasis in the vessels of the microvasculature, multiple hemorrhages with RBC hemolysis and hemosiderin accumulation, large regions of alveolar emphysema, atrophy or absence of alveolar epithelium.

• **Liver –** chronic venous hyperemia, hemosiderin deposition (hemosiderosis).

• **Kidneys** (Fig. 22) – venous hyperemia, serous edema of the glomeruli and intertubular connective tissue, hemosiderosis, deposition of uric acid salts in the lumen of individual urinary tubules.

• **Heart –** serous myocardial edema.

• **Spleen** (Fig. 23, 24) – ubiquitous proliferation of connective tissue (sclerotization), severe lymphoid hyperplasia of the white pulp, deposition of hemosiderin granules in the red pulp.

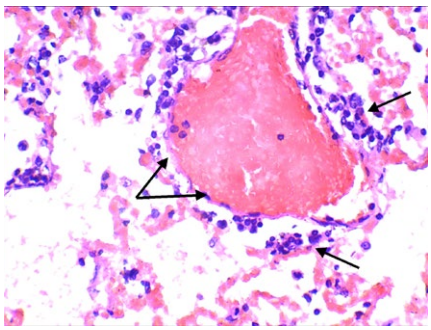

Fig. 13. Lungs of a 6-month-old mink: venous thrombosis (arrows on the left), lymphoid-macrophage perivasculitis (arrows on the right), alveolar rupture. Hematoxylin-eosin staining, magnification ×480

Рис. 13. Легкие 6-месячной норки: тромбоз венулы (стрелки слева), лимфоидно-макрофагальный периваскулит (стрелки справа), разрыв альвеол. Окраска гематоксилином и эозином, увеличение ×480

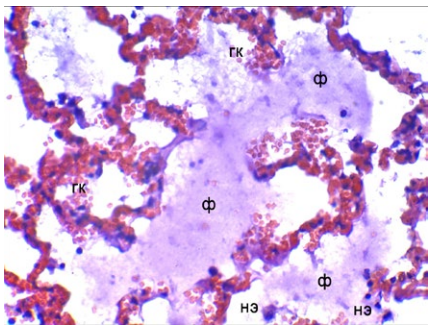

Fig. 14. Lungs of a 6-month-old mink: hyperemia of capillaries (ГК), fibrin (Ф) and necrotic epithelium (НЭ) in the alveolar lumen. Hematoxylin-eosin staining, magnification ×480

Рис. 14. Легкие 6-месячной норки: гиперемия капилляров (ГК), фибрин (Ф) и некротизированный эпителий (НЭ) в просвете альвеол. Окраска гематоксилином и эозином, увеличение ×480

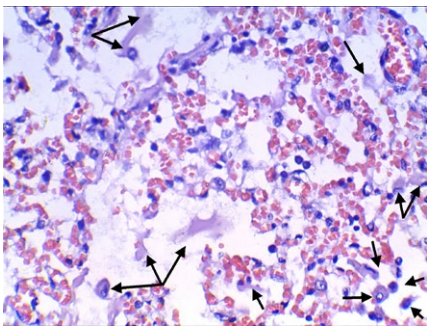

Fig. 15. Desquamated epithelium, fibrin filaments, hyaline membranes in the lumen of the lung alveoli of a 6-month-old mink. Hematoxylin-eosin staining, magnification ×480

Рис. 15. Слущенный эпителий, нити фибрина, гиалиновые мембраны в просвете альвеол легких 6-месячной норки. Окраска гематоксилином и эозином, увеличение ×480

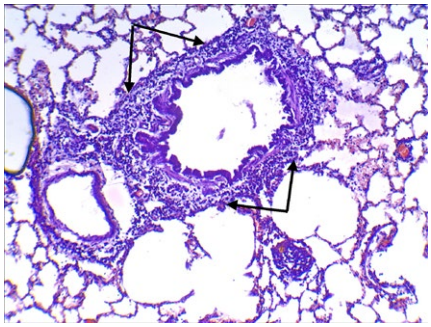

Fig. 16. Lungs of a 6-month-old mink: lymphoid-macrophage peribronchitis (arrows), emphysema. Hematoxylin-eosin staining, magnification ×120

Рис. 16. Легкие 6-месячной норки: лимфоидно-макрофагальные перибронхиты (стрелки), эмфизема. Окраска гематоксилином и эозином, увеличение ×120

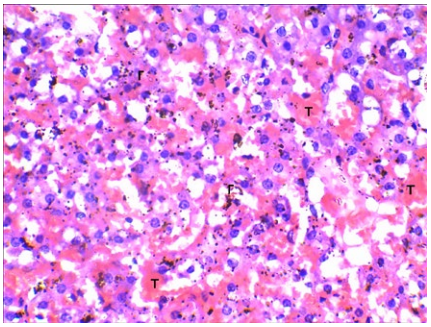

Fig. 17. Deposition of hemosiderin granules (Г) in the shock liver of a 6-month-old mink, thrombosis (Т) of sinusoidal capillaries. Hematoxylin-eosin staining, magnification ×480

Рис. 17. Отложение гранул гемосидерина (Г) в шоковой печени 6-месячной норки, тромбоз (Т) синусоидных капилляров. Окраска гематоксилином и эозином, увеличение ×480

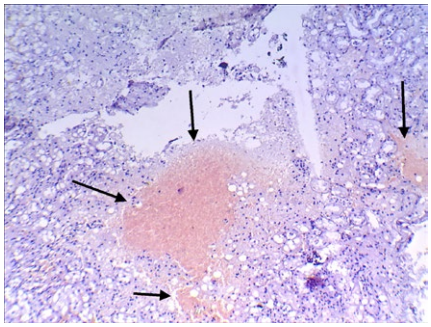

Fig. 18. Hemorrhages in the kidney of a 6-month-old mink (arrows). Hematoxylin-eosin staining, magnification ×120

Рис. 18. Кровоизлияния в почке 6-месячной норки (стрелки). Окраска гематоксилином и эозином, увеличение ×120

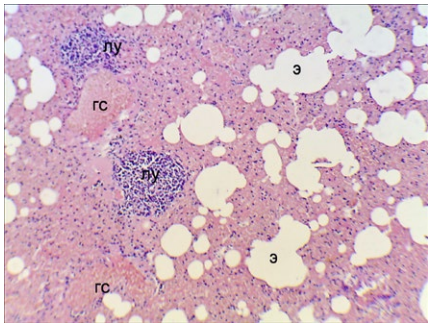

Fig. 19. Interstitial pneumonia in an 18-month-old mink. Hemostasis (ГС), lymphoid nodules (ЛУ), emphysema (Э). Hematoxylin-eosin staining, magnification ×120

Рис. 19. Интерстициальная пневмония у 18-месячной норки. Гемостаз (ГС), лимфоидные узелки (ЛУ), эмфизема (Э). Окраска гематоксилином и эозином, увеличение ×120

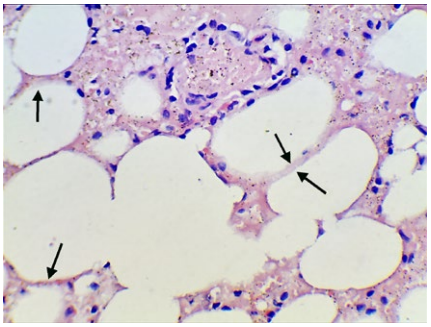

Fig. 20. Lungs of an 18-month-old mink: sclerotization, emphysema, hemosiderosis. The alveolar epithelium is atrophied or absent (arrows). Hematoxylin-eosin staining, magnification ×480

Рис. 20. Легкие 18-месячной норки: склеротизация, эмфизема, гемосидероз. Альвеолярный эпителий атрофирован или отсутствует (стрелки). Окраска гематоксилином и эозином, увеличение ×480

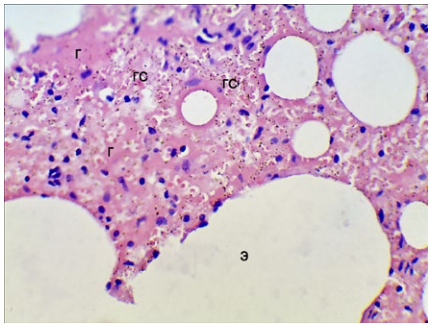

Fig. 21. Lungs of an 18-month-old mink: Sclerotization, emphysema (Э), hemorrhages with hemolysis (Г) of erythrocytes, hemosiderin granules (ГС). Hematoxylin-eosin staining, magnification ×480

Рис. 21. Легкие 18-месячной норки: Склеротизация, эмфизема (Э), кровоизлияния с гемолизом (Г) эритроцитов, гранулы гемосидерина (ГС). Окраска гематоксилином и эозином, увеличение ×480

CONCLUSION

Post-mortem lesions during the acute course of COVID-19 in minks are characterized by the predominance of hemodynamic disorders, serious disorders of the cardiovascular and respiratory systems i.e. development of acute venous hyperemia, pulmanory emphysema and microfocal pneumonia in various combinations, acute expansion of the heart or its right cavities, acute venous hyperemia of the myocardium, liver, kidneys, skin and mucous membranes, hemorrhages in the kidneys, pronounced post-mortem blood coagulation in the heart, arterial lumens and veins of various diameters. The development of serous and hemorrhagic splenitis, in our opinion, cannot be considered as a diagnostic marker of this disease. In case of chronic disease, the nature of the pathological picture remains, which is apparently associated with the long-term persistence of the pathogen in susceptible minks. Morphological signs of a long-term viral infection are focal interstitial pneumonia, lymphoid hyperplasia of the spleen (splenomegaly), as well as the formation of pigment spots at the site of hemorrhages due to the appearance of hemoglobinogenic pigments.

Histological lesions in minks during the acute course of COVID-19 are characterized by acute interstitial pneumonia and alveolitis complicated by respiratory distress syndrome and alveolar emphysema. The immediate cause of death is membranogenic pulmonary edema. Deep and irreversible changes in the vessels of the microvasculature of the internal organs (disseminated intravascular coagulation syndrome, disseminated intravascular coagulation syndrome) are a sign of shock development (infectious-toxic, septic, etc.).

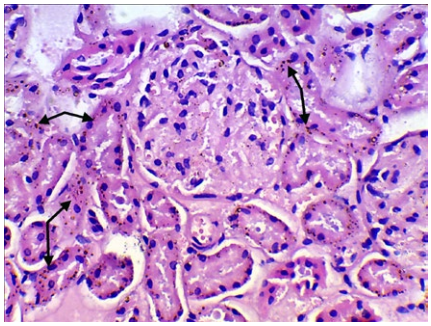

Fig. 22. Hemosiderosis of the kidney of an 18-month-old mink. Hematoxylin-eosin staining, magnification ×480

Рис. 22. Гемосидероз почки 18-месячной норки. Окраска гематоксилином и эозином, увеличение ×480

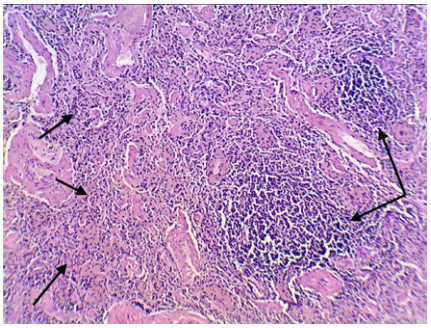

Fig. 23. Spleen of an 18-month-old mink: proliferation of a connective tissue (arrows on the left), hyperplasia of lymphoid nodules (arrows on the right). Hematoxylin-eosin staining, magnification ×120

Рис. 23. Селезенка 18-месячной норки: разрастание соединительной ткани (стрелки слева), гиперплазия лимфоидных узелков (стрелки справа). Окраска гематоксилином и эозином, увеличение ×120

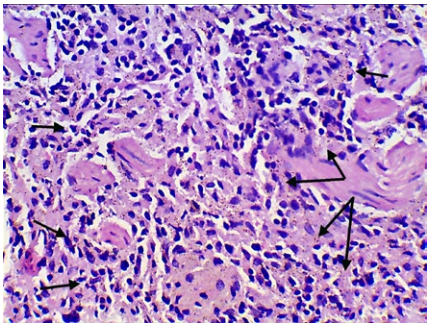

Fig. 24. Spleen of an 18-month-old mink: proliferation of a connective tissue (arrows on the left), deposition of hemosiderin granules (arrows on the right). Hematoxylin-eosin staining, magnification ×480

Рис. 24. Селезенка 18-месячной норки: разрастание соединительной ткани (стрелки слева), отложение гранул гемосидерина (стрелки справа). Окраска гематоксилином и эозином, увеличение ×480

Structural lung disorders of minks with a long course of the disease are characteristic of chronic interstitial pneumonia, complicated by alveolar emphysema, combined with profound lesions in the microvasculature (venous hyperemia, hemostasis, hemorrhages, local hemosiderosis). An aggravating process caused by prolonged pulmonary and heart failure is chronic venous hyperemia of internal organs (liver, kidneys). General hemosiderosis is a concomitant process, probably associated with prolonged intravascular hemolysis of erythrocytes. Lymphoid hyperplasia and sclerotization of the spleen are signs of a viral infection that is systemic in nature and accompanied by prolonged viremia.

Taking into account the relatively nonspecific pathological changes and the obvious high information content of the results of histological examination, we consider it's mandatory to perform it when making a presumptive diagnosis of COVID-19 in minks.

REFERENCES

1. Nikiforov V. V., Suranova T. G., Mironov A. Yu., Zabozlaev F. G. New coronavirus infection (COVID-19): Etiology, epidemiology, clinic, diagnosis, treatment and prevention. M.; 2020. 48 p. Available at: http://www.medprofedu.ru/upload-files/koronoviruc20.pdf. (in Russian)

2. Coronavirus Disease 2019 (COVID-19). Epidemiology, Pathogenesis, Diagnosis, and Therapeutics. Ed. S. K. Saxena. Singapore: Springer; 2020. 213 p. DOI: 10.1007/978-981-15-4814-7.

3. Ahn D. G, Shin H. J., Kim M. H., Lee S., Kim H. S., Myoung J., et al. Current status of epidemiology, diagnosis, therapeutics, and vaccines for novel coronavirus disease 2019 (COVID-19). *J. Microbiol. Biotechnol*. 2020; 30 (3): 313–324. DOI: 10.4014/jmb.2003.03011.

4. OIE. Technical Factsheet: Infection with SARS-CoV-2 in Animals. June 2020. Available at: https://www.oie.int/fileadmin/Home/eng/Our_scientific_expertise/docs/pdf/COV-19/A_Factsheet_SARS-CoV-2.pdf.

5. CDC. COVID-19 and Animals. Updated March 25, 2021. Available at: https://www.cdc.gov/coronavirus/2019-ncov/daily-life-coping/animals.html.

6. USDA. Confirmed cases of SARS-CoV-2 in Animals in the United States. September 2020. Available at: https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/sa_one_health/sars-cov-2-animals-us.

7. AVMA. SARS-CoV-2 in Animals. Available at: https://www.avma.org/resources-tools/animal-health-and-welfare/covid-19/sars-cov-2-animals-including-pets.

8. USDA, CDC. Response and Containment Guidelines: Interim Guidance for Animal Health and Public Health Officials Managing Farmed Mink and other Farmed Mustelids with SARS-CoV-2. 2020. Available at: https://www.aphis.usda.gov/publications/animal_health/sars-cov-2-mink-guidance.pdf.

9. USDA. National Agricultural Statistics Service. Mink. July 2020. Available at: https://furcommission.com/wp-content/uploads/2020/07/USDAmink2020.pdf.

10. COVID-19 mink variants discovered in humans in seven countries. *The Guardian*. 2020. Available at: https://www.theguardian.com/environment/2020/nov/18/covid-19-mink-variants-discovered-in-humans-inseven-countries.

11. USDA. Interim SARS-CoV-2 Guidance and Recommendations for Farmed Mink and Other Mustelids. Available at: https://www.aphis.usda.gov/animal_health/one_health/downloads/sars-cov-2-guidance-for-farmed-mink.pdf.

12. CDC. Steps to Prevent COVID-19 on Mink Farms. 2020. Available at: http://furcommission.com/wp-content/uploads/2020/11/Mink-Training-Presentation_4Nov2020. pdf.

13. Oreshkova N., Molenaar R. J., Vreman S., Harders F., Oude Munnink B. B., Hakze-van der Honing R. W., et al. SARS-CoV-2 infection in farmed minks, the Netherlands, April and May 2020. *Euro Surveill*. 2020; 25 (23):2001005. DOI: 10.2807/1560-7917.ES.2020.25.23.2001005.

14. Prudnikov V. S., Malashko V. V., Zhukov A. I., German S. P., Gromov I. N. Pathological anatomy of farm animals. Practical course [Patologicheskaya anatomiya sel'skohozyajstvennyh zhivotnyh. Praktikum]. Ed. V. S. Prudnikov. Minsk: IVC Minfina; 2018; 6–11. eLIBRARY ID: 35316191. (in Russian)

15. Gromov I. N., Prudnikov V. S., Krasochko P. A., Motuzko N. S., Zhurov D. O. Sampling for laboratory diagnosis of

bacterial and viral animal diseases [Otbor obrazcov dlya laboratornoj diagnostiki bakterial'nyh i virusnyh boleznej zhivotnyh]: study guide. Vitebsk: VSAVM; 2020. 64 p. (in Russian)

16. Microscopic technique: Guide [Mikroskopicheskaya tekhnika: rukovodstvo]. Ed by D. S. Sarkisov, Yu. L. Petrov. M.: Medicina; 1996; 14–25, 36–50. (in Russian)

17. Shuravin P. V. Description of histological specimens: guide [Opisanie gistologicheskih preparatov: rukovodstvo]. M.: Simple Pathology; 2020; 5–14, 32–34, 64–70, 73–74, 77–82, 128, 135–136, 139. (in Russian)

18. Zairatyants O. V., Samsonova M. V., Mikhaleva L. M., Chernyaev A. L., Mishnev O. D., Krupnov N. M., Kalinin D. V. Pathological Anatomy of COVID-19: Atlas. Under general ed. O. V. Zairatyants. M.: Health Organization GSI "NPCSR"; 2020. 140 p. Available at: https://mosgorzdrav.ru/uploads/imperavi/ru-RU/Патанатомический%20атлас%2023.06.2020%20-%202.pdf. (in Russian)

Received on 30.03.2021
Approved for publication on 30.04.2021

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Irina A. Subbotina, Candidate of Science (Veterinary Medicine), Associate Professor, Department of Epizootology and Infectious Diseases, EE "VSAVM", Vitebsk, Republic of Belarus.

Igor N. Gromov, Doctor of Science (Veterinary Medicine), Head of the Department of Pathological Anatomy and Histology, EE "VSAVM", Vitebsk, Republic of Belarus.

Субботина Ирина Анатольевна, кандидат ветеринарных наук, доцент кафедры эпизоотологии и инфекционных болезней, УО «ВГАВМ», г. Витебск, Республика Беларусь.

Громов Игорь Николаевич, доктор ветеринарных наук, заведующий кафедрой патологической анатомии и гистологии, УО «ВГАВМ», г. Витебск, Республика Беларусь.