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The Histological Structure of Pig Organs and Tissues during the Experimental Use of the Triazavirin Antiviral Drug

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Abstract

The authors have set the goal to experimentally study the effect of the Triazavirin antiviral drug on pigs, as well as the histological structure, the state, and abnormalities in several organs and tissues after the administration of the Triazavirin antiviral drug. For the experiment, an experimental group of 25 piglets has been formed. The animals have been kept in the same zoohygienic conditions, have had the same diet according to the age and the feeding technology. The piglets in the experimental group with symptoms of respiratory system impairment have received the Triazavirin antiviral drug as experimental etiological therapy. The drug has been given to the experimental animals with drinking in the form of an aqueous solution. After the slaughtering of the experimental animals at a meat processing factory, pieces of organs have been collected for histological examination — the trachea, the bronchi of the lungs and adjacent lymph nodes, the liver, the kidneys, the spleen, the heart, and the ovaries. During the experiment and administration of the Triazavirin antiviral drug in the indicated doses, no lethality has been observed in the experimental piglets. In the experimental group, the piglets' state has improved, the temperature has returned to normal, the appetite has improved, and there have been no clinical signs of respiratory impairment. As a result of the experiment with the piglets during the histological studies of several organs, no significant deviations from the norm have been revealed in their structure. The organs of the respiratory system have stabilized and have had a morpho-histological structure close to normal, which is a positive result of the experiment. Analyzing the data of the histological studies, it may be concluded that the Triazavirin antiviral drug does not cause changes in the morphology of the tissues of the studied internal organs. Based on the above, the authors recommend using the Triazavirin antiviral drug as part of comprehensive etiological therapy for the treatment of the respiratory system in pigs, including the treatment of viral diseases.

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Introduction

Intensive breeding of various species, including pigs, leads to the emergence of new unforeseen problems. The modern methods of keeping (closed keeping), breeding (technological stress), and using (high concentration of livestock) the animals lead to the emergence and active spread of several pathogenic agents, including viruses. The animals' impairment by viruses occurs both in pure form and in association with pathogenic microorganisms, which aggravate the course of the diseases. Active prevention and control of diseases of various etiologies are the basis for the high quality of the livestock and products obtained (Afonyushkin and Litvinov, 2017; Belkin et al., 2018; Maksimovich et al., 2019; Khlopitsky et al., 2018; Preobrazhensky and Shemanaev, 2019).

In pig breeding, several viral infections with clinical symptoms and respiratory system impairment are being actively prevented. The list of diseases is very long, these are classical (CSF) and African swine fever (ASF), Aujeszky's disease virus (ADV), parvoviral infection, porcine reproductive and respiratory syndrome virus (PRRS virus), influenza A virus (IAV), porcine circovirus type II (PCV-2), porcine respiratory coronavirus (PCVC), pseudo-rabies virus, etc. Besides, several opportunistic viral agents are not subject to active prevention but lead to many diseases in certain negative conditions (Afonyushkin and Litvinov, 2017; Maksimovich et al., 2019; Khlopitsky et al., 2018).

In this regard, it becomes necessary to use antiviral drugs that directly affect the viral agent in treating the animals (Solanki and Patel, 2019; Soboleva, et al., 2020; Atia, 2019; Khalil, et al., 2018). The choice of antiviral medication is not that wide when working with animals. Given this problem, the authors set the goal of performing an experimental study of the Triazavirin antiviral drug with pigs, revealing the presence of specific toxic effects upon administering the drug to the piglets and studying the histological structure, the state, and the abnormalities in several organs and tissues after the administration of the Triazavirin antiviral drug (Belkin et al., 2018; Gladkikh et al., 2016; Choi et al., 2015; Fujita et al., 2015; Reshetnikova et al., 2020; Reshetnikova and Zenkin, 2020).

Materials and Methods

The studies were performed at the Interfaculty Educational and Scientific Laboratory of Biotechnology of the Federal State Budgetary Institution of Higher Education Izhevsk State Agricultural Academy (FSBEI HE Izhevsk State Agricultural Academy) in 2016 and 2017. The experiment was performed at the Vostochny Pig-Breeding Complex LLC in the Republic of Udmurtia.

For the experiment, an experimental group of 25 pigs was formed. The animals were kept in the same zoohygienic conditions, had the same diet according to the age and the feeding technology; feeding was performed through the WEDA system.

The piglets from two to four months, 20 – 40 kg of live weight, were fed on complete feed compound KKS-5-256 DSM manufactured on January 29, 2017, sieve ø 4 mm, with the minimum exchange energy of 13.4 MJ/kg.

The feed compound included wheat, barley, soybean meal, sunflower meal, sunflower oil, table salt, feed yeast, monocalcium phosphate, lysine monochlorohydrate, limestone flour, beet pulp, Rovimix premix 1.5% 06568 for piglets growth, L-threonine, Agromix Plus, and biosorb. The indicators were the following, %: protein — 16.29, crude fiber — 3.89, lysine 1.16, methionine + cystine — 0.62, threonine — 0.72, tryptophan — 0.2, calcium — 0.5 – 0.6, phosphorus — 0.44 – 0.54, sodium chloride — 0.6, and humidity – 14. Additionally, biologically active substances were introduced into 1 kg of the feed compound in the amounts not less than the following values: Vitamin A — 9.0 thousand IU, Vitamin D3 — 1.9 thousand IU, Vitamin E — 60.0 mg, Vitamin K3 — 2.74 mg, Vitamin B1 — 1.4 mg, Vitamin B2 — 5.0 mg, Vitamin B3 — 17.0 mg, Vitamin B4 — 300.0 mg, Vitamin B5 — 26.0 mg, Vitamin B6 — 2.5 mg, Vitamin B12 — 0.03 mg, Vitamin Bc — 0.8 mg, Vitamin H — 0.15 mg, Magnesium — 255.0 mg, Iron — 75.0 mg, Copper — 165.0 mg, Zinc — 75.0 mg, Cobalt — 0.5 mg, Iodine — 0.7 mg, Selenium — 0.2 mg, and Manganese — 50.0 mg.

Scheduled treatments were carried out at the pig farm: on June 11, 2016, CSF (classical swine fever) vaccination, on September 12, 2016 — deworming, and on December 2, 2016 — erysipelas vaccination. The average air temperature in the room during the experiment was 23.1 °C.

The animals (piglets) were admitted to the farm on October 14, 2016, at the age of 77 days with an average weight of 31.6 kg. Upon admission, the group was formed, given the state and the age of the animals. The piglets with clinical signs of respiratory system impairment were selected for the experiment.

The experimental group (25 piglets), which included the animals with the symptoms of respiratory system impairment, received the Triazavirin antiviral drug as experimental etiological therapy.

The experiment involved the animals with clinical signs of respiratory impairment: increased body temperature, lethargy, nostril discharge, sneezing, snorting, wheezing, and rough breathing; the animals were passive and were mostly lying, refused to eat, the ears were lowered. The average body temperature of the piglets in the experimental group was 40.9 °C, in the reference group — 40.7 °C.

The drug was given to the experimental animals with drinking in the form of an aqueous solution. A 0.75 g capsule of the Triazavirin antiviral drug was opened; the contents were dissolved in 50 ml of saline, and given with drink once a day for five days (Table 1).

A set of studies was performed during the experiment. The experimental piglets were handed over to the Uvinsky Meat Processing Plant LLC in the period between January 19 and 23, 2017, according to the process plan for 174 – 178 days of the animals' life and fattening. At the meat processing plant, veterinary and sanitary examination of the carcass and the internal organs (the trachea, the bronchi of the lungs and adjacent lymph nodes, the liver, the spleen, and the ovaries) was performed.

Table 1. Experiment scheme.

Experiment No.	Dosage	Number of animals	Method of administration	Administration conditions
Experimental group	In sick animals, the Triazavirin antiviral drug used for treatment, 0.75 g of the drug dissolved in 50 ml of saline	25	Orally	Once a day for 5 days

After slaughtering the experimental animals at the meat processing factory (the Uvinsky Meat Processing Plant LLC), pieces of organs were collected for histological examination — the trachea, the bronchi of the lungs and adjacent lymph nodes, the liver, the kidneys, the spleen, the heart, and the ovaries.

For histological examination, the materials were selected following the classical method. The samples of the material were taken immediately after slaughtering and carcass cutting; 1 cm^3 piece was fixed in neutral formalin for 12 – 24 hours, and afterward washed for 24 hours. The material was passed through a group of alcohols with increasing concentrations, and then clarified through two or three portions of O-xylene and paraffin with a temperature of 37ºC. Subsequently, the preparations were embedded in paraffin at 56 ˚C and fixed on wooden blocks. Slices were prepared using a 5 µm thick microtome, staining was made with a group of hematoxylin-eosin dyes.

Microscopic examination and description of organ slides were performed using an Olympus CX43 light microscope with a 10x/20 eyepiece and a 10x/0.25 objective and with a 10x/20 eyepiece and a 40x/0.65 objective. The image was transmitted to a PC using the Video Zavr Standard video system, which was equipped with a video camera and appropriate software.

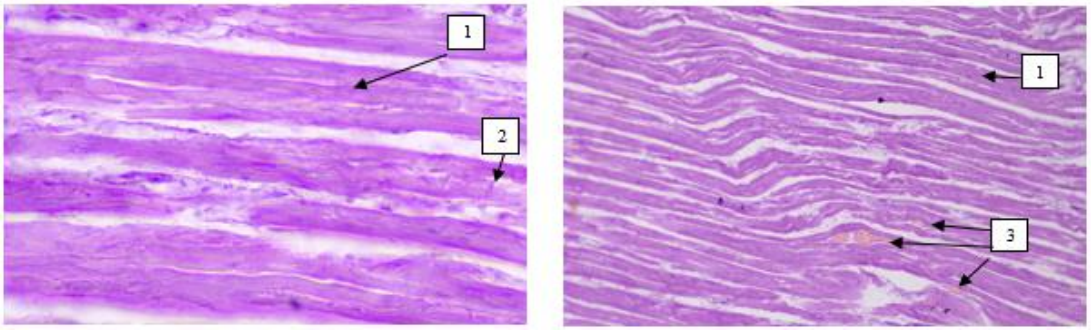
Results

During the experiment and administration of the Triazavirin antiviral drug in the indicated doses, no lethality was observed in the experimental piglets.

By day five of the experiment, all piglets in the experimental group showed normalized body temperature in the range of the physiological norms, it fluctuated within 37.5 – 38.9 ˚C. The animals were active, their ears were raised, the appetite returned to the norm, discharge from the nasal cavity stopped, there was no wheezing, the breathing was mixed, some animals were coughing.

The overall structure, components of the stroma, and parenchyma of these organs were preserved. The state of the blood and lymph vessels of the organs corresponded to the normal morphological and functional status.

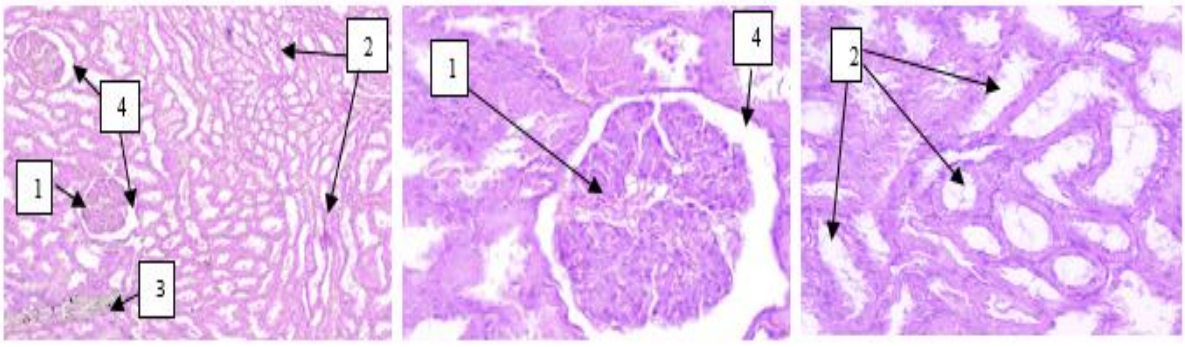
The heart was represented by muscle fibers with a significantly pronounced characteristic transverse striation. Cardiomyocytes (contractile, conducting) had clear contours, chromatin-rich nuclei, and eosinophilic cytoplasm. Loose intermuscular connective tissue was accordingly represented. The vessels were well expressed and full-blooded (Fig. 1. A, B).



(A) (B)

Figure 1. Histological slide of the pig myocardium. Staining with hematoxylin and eosin.
A — x400 magnification, B — x100 magnification.
1 – cardiomyocytes, 2 – intercalated discs, 3 – blood vessel.

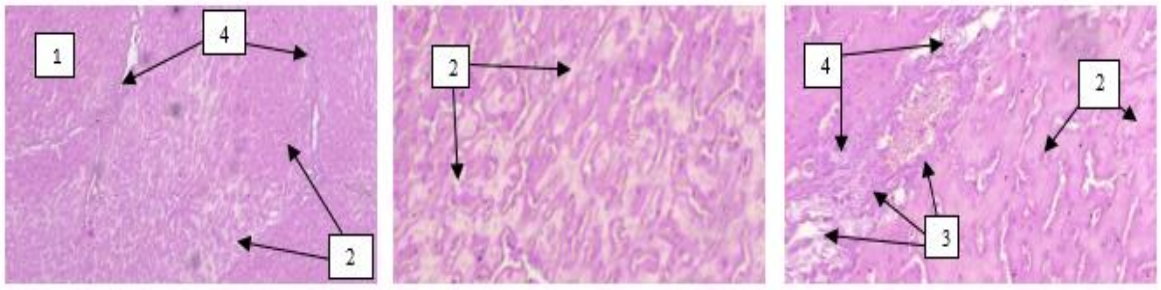
The kidneys contained renal corpuscles, which were evenly distributed in the cortical layer, not enlarged; all components of the nephrons were well visualized. The epithelium of straight and convoluted tubules had clear contours and classic shapes. The basement membranes were well defined. The stroma contained lymphohistiocytic cell elements and vessels of the medium filling (Fig. 2).



(A) (B) (C)

Figure 2. Pig kidney histological slide. Staining with hematoxylin and eosin.
A — x100 magnification, B — x400 magnification.
1 — renal corpuscles, nephrons, 2 — straight and convoluted pores, 3 — blood vessel, 4 — capsule.

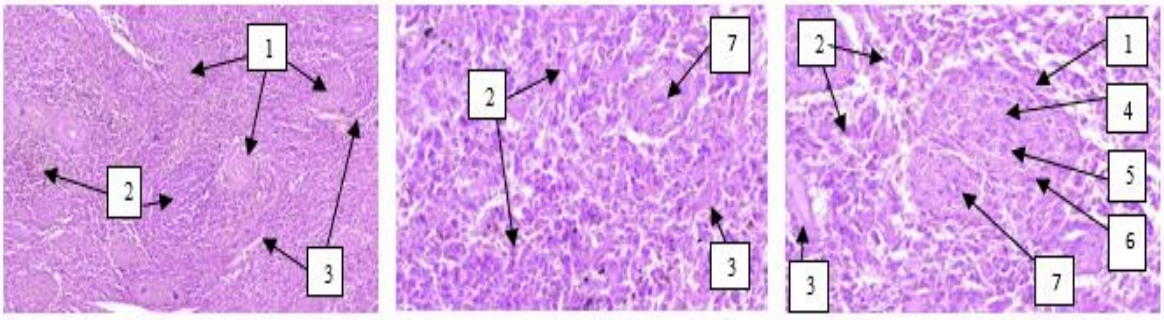
The liver had the classic lobular structure; the boundaries of the stroma and parenchyma were visible. In the histological slides, some structural and functional changes in the hepatobiliary system were revealed. The disturbances in the liver hepatocytes had the form of hydropic degeneration. The changes were focal. The hepatocytes formed lobules of irregular polygonal shapes, the structures of the liver triad were pronounced, the vessels were full-blooded, and the stagnant processes were noted in the bile ducts. The central veins and interbeam capillaries were moderately full-blooded. The liver was covered with an even, smooth capsule (Fig. 3).



(A) (B) (C)

Figure 3. Pig liver histological slide. Staining with hematoxylin and eosin.
A — x100 magnification, B — x400 magnification.
1 — liver lobules, 2 — hepatocytes with nuclei, 3 — liver triad (artery, vein, bile duct), 4 — connective tissue.

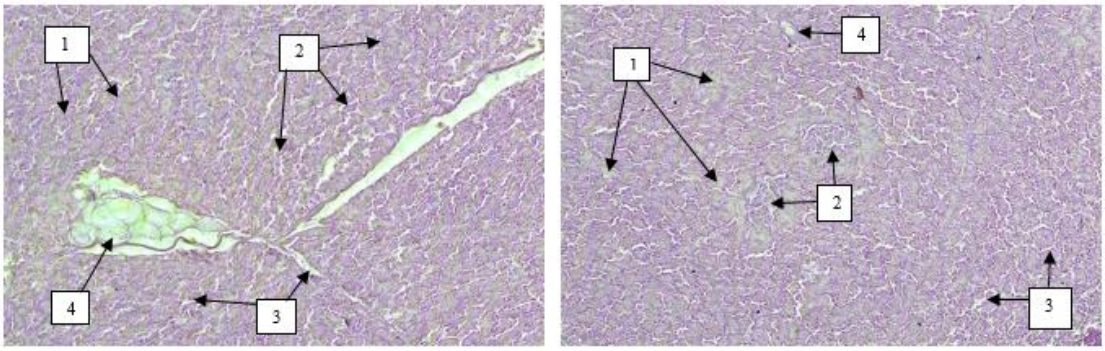
The spleen was covered with a capsule of connective tissue with few muscle cells. The internal structures were delimited by trabeculae. The splenic lymph follicles were well defined and formed the white pulp. The reticular tissue had rounded lymphatic follicles and was represented by the accumulation of lymphocytes. The red pulp actively filled the space between the capsule, the trabeculae, and the splenic lymph follicles. A normal ratio of the red and white pulp was observed on the histological slide. The red pulp was full-blooded. A network of venous sinusoidal capillaries also passed through the reticular tissue (Fig. 4).



(A) (B) (C)

Figure 4. Pig spleen histological slide. Staining with hematoxylin and eosin.
A — x100 magnification, B and C — x400 magnification.
1 — white pulp, 2 — red pulp, 3 — trabeculae, 4 – the lymphoid follicle germinal center, 5 — the mantle layer of the lymphoid follicle, 6 — the marginal layer of the lymphoid follicle, 7 — the central artery.

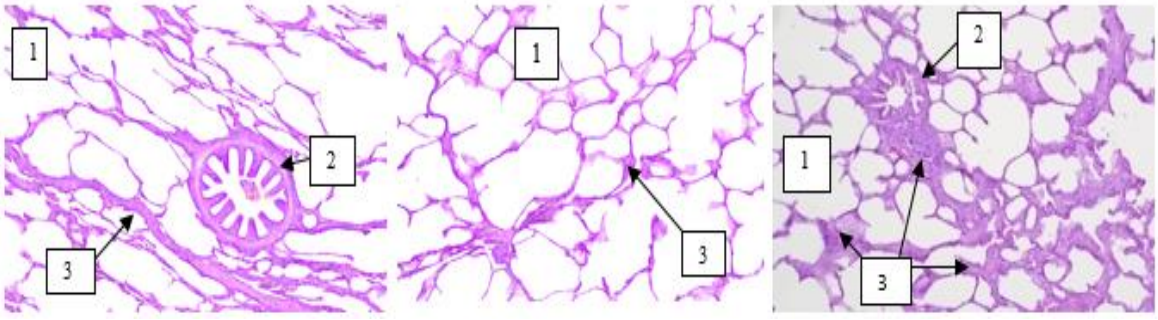
In the pancreas, the lobular structure was well represented. The lobules had classical sizes and were delimited by interlobular connective tissue. The cells had clear contours, most were conical or cube-shaped, with a pronounced basophilic cytoplasm. The Langerhans islets were well represented; they looked like rounded formations with clusters of lighter cells. The excretory ducts, both intralobular and interlobular, were well expressed. The blood vessels were medium-filled (Fig. 5).



(A) (B)

Figure 5. Pig pancreas histological slide. Staining with hematoxylin and eosin. A and B — x100 magnification.
1 — acinus, 2 — Langerhans islet, 3 — connective tissue, 4 — excretory duct.

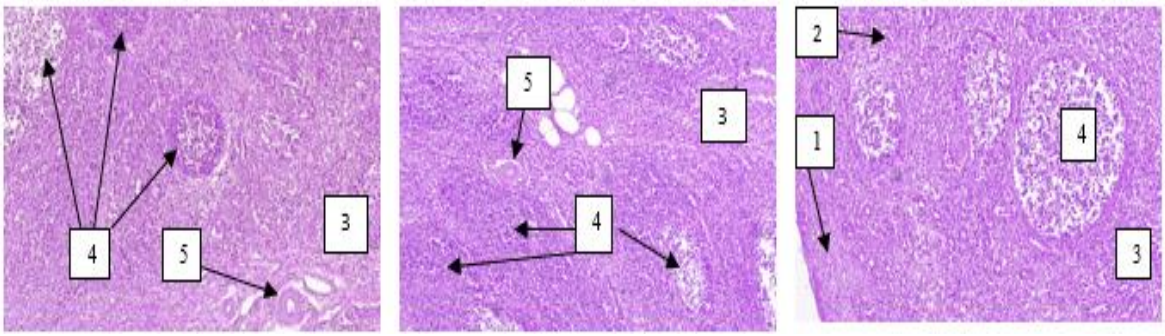
In the lungs, the lumen of the alveoli was not obstructed, the interalveolar septums were preserved and slightly thickened due to the edema of the interstitial tissue, and there was a small number of macrophages. In some areas of the lung, signs of emphysema were noted; the alveoli were dilated and thinned. The walls and structures of the bronchi were well-formed and clean, there was a small amount of mucous secretion in the lumen. In the lumen of the alveoli, there was no accumulation of desquamated epithelium and no presence of individual alveocytes and macrophages; therefore, there were no signs of inflammation (Fig. 6).



(A) (B) (C)

Figure 6. Pig lung histological slide. Staining with hematoxylin and eosin.
A, B, and C — x100 magnification.
1 — alveoli, 2 — small bronchi, 3 — the interstitial connective tissue of the lung.

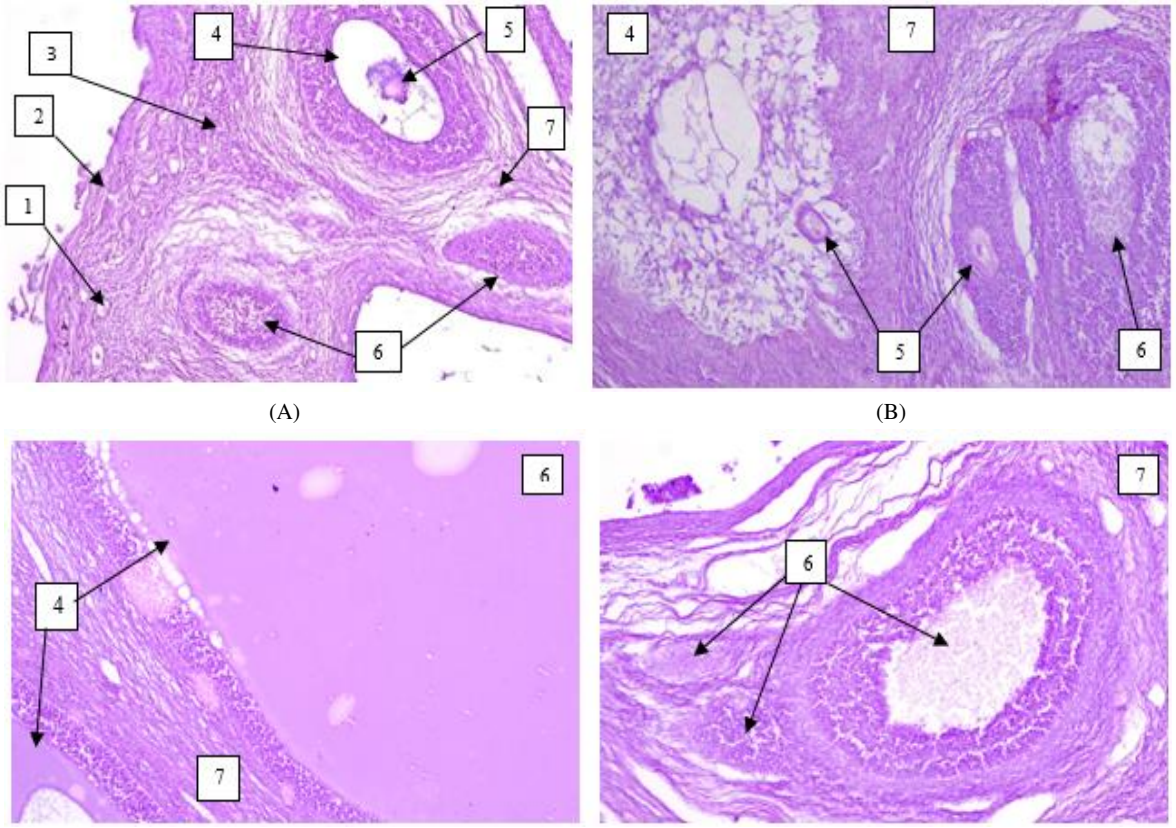
In the mediastinal lymph nodes (l/n) and the lymph nodes of the lungs, a dark cortical substance in the periphery and a light medulla in the center were detected. The reticular tissue of the cortical substance was densely filled with lymphocytes located in groups; the lymphocytes were at various stages of differentiation. Small lymphocytes in the medulla were represented by single cells, and in the peripheral zone, they were more numerous. Mitotic figures were present; no characteristic differences were found in the location and the number of dividing cells. In the experimental group, a slight decrease in the density of lymphocytes in the center of the follicle was noted. In the medulla, the medullary (pulp) cords and the central sinus were visible. The central zone contained macrophages. These morphological signs were the manifestations of the lymph nodes' dynamic state; no structural differences were found in the experimental animals (Fig. 7).



(A) (B) (C)

Figure 7. A histological slide of the lymph nodes in the mediastinum and the lung of a pig. Staining with hematoxylin and eosin. A, B, and C — x100 magnification.
1 — cortical substance, 2 — paracortex, 3 — medulla, 4 — lymphoid follicle of the cortical substance, 5 — vessels.

The pig ovary had a classic structure represented by cortical substance and medulla. Follicles with epithelial cells were located in the cortical zone, blood vessels and hilus cells were visible in the medulla. Follicles were presented at various stages of maturation (primordial, primary, secondary, tertiary follicles); there were many of them since pigs were multiparous mammals. In the structures of the Graafian follicles, the components were identified: the outer and inner shells, the follicle cavity with the fluid, the cumulus, the ovum (the oocyte), they radiate crown, and the follicular cells. The sheath was formed (Fig. 8).



(A) (B)

(C) (D)

Figure 8. Pig ovary histological slide. Staining with hematoxylin and eosin.
A, B, C, D — x100 magnification.
1 — primary follicles, 2 — albuginea, 3 — cortical substance, 4 — follicle cavity,
5 – ovum, 6 – vesicular follicle, the cumulus, and the oocyte did not get in the slide,
7 — medulla.

Discussion

The use of the Triazavirin drug in the indicated doses for the treatment of the piglets with clinical symptoms of respiratory system impairment does not cause a lethal outcome.

The histological examination of the organs and tissues of the piglets used in the experiment revealed no pathological and morphofunctional changes in the state of all components and mucous membranes of the above organs. No transformation of any specific and connective tissue structures of the parenchymal organs (the heart, the liver, the kidneys, the spleen, the pancreas, the ovaries, the lymph nodes, and the lungs) was found (Balabanova and Kudryashov, 2020; Nikalova, 2020).

The organs of the respiratory system, the lungs, and organ lymph nodes, in particular, had some deviations. This histological pattern was quite expected and was considered a positive result. This was because the experiment involved the piglets with an impaired respiratory system, lungs in particular. The use of the Triazavirin antiviral drug for the treatment of respiratory diseases had a significant positive and therapeutic effect.

The histological slides of the liver showed some structural and functional changes in the hepatobiliary system, signs of vacuolar dystrophy in particular. These changes indicated a serious load on the organ during disease development and drug administration.

The histological slides showed residual processes of the disease and stabilization of the organs.

Conclusion

1. During the experiment and administration of the Triazavirin antiviral drug in the indicated doses, no lethality has been observed in the experimental piglets.
2. In the experimental group, after the administration of the Triazavirin antiviral drug for five days in the indicated doses, an improved state of the pigs has been observed; their temperature has normalized, the appetite has improved, and there have been no clinical signs of respiratory impairment. This picture allows concluding the viral agents' participation in the occurrence of respiratory tract pathologies.
3. As a result of the experiment with the piglets, during the histological studies of several organs no significant deviations from the norm have been revealed in their

structure. The organs of the respiratory system have stabilized and have had a morphohistological structure close to normal, which has been a positive result of the experiment. Disorders have been revealed in hepatocytes of the liver; they have had the form of vacuolar degeneration and have been focal. Analyzing the data of the histological studies, it may be concluded that the Triazavirin antiviral drug does not cause changes in the morphology of the tissues of the studied internal organs.

Based on the above, the authors recommend using the Triazavirin antiviral drug as part of comprehensive etiological therapy for the treatment of the respiratory system in pigs, including the treatment of viral diseases.

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