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PATHOHISTOLOGICAL CHANGES IN PIGS WITH MYCOPLASMOSIS

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Abstract. *A pathological autopsy was performed on 6 corpses of piglets during the first week of their life who died from mycoplasmosis. Examination of the visible mucous membranes revealed hyperemia of the mucous membrane of the nasal cavity and thymus. Simultaneous lesions of the pharyngeal, parotid, cervical and mandibular lymph nodes were noted. They were slightly enlarged, from dark pink to dark red. The heart is irregularly shaped due to the expansion of the right ventricle or the diffuse expansion of all departments. Lungs have doughy consistency, uneven color. In some cases, there are diffuse red areas covering the entire lobe of the lungs, in other cases, there are lesions of small areas. The liver has a smooth surface, soft or pasty consistency, the parenchyma pattern is slightly smoothed in section. The color of the liver is different: dark red areas without clear boundaries turn into creamy-clay. A characteristic feature was flatulence of the stomach and intestines. Catarrhal enteritis was registered in animals, which manifested itself in the form of moderate hyperemia of the intestinal mucosa and serous membranes.*

Microscopically, there is a significant blood supply to the vessels in the lungs. Alveoli are half fall down, in the form of slit-like lumens. In areas of tissue infiltration by inflammatory infiltrate, the alveolar wall is thickened, alveolocytes are in a state of turbid swelling and vacuolar dystrophy, they are impregnated with erythrocytes. Peribronchial pneumonia of lymphocytic character is observed. The liver is in a state of acute venous hyperemia. The central and intraparticle capillaries are sharply dilated and filled with blood in some lobes, and the hepatic beams are compressed accordingly. In the center of other lobes, diffuse infiltration of liver tissue by erythrocytes as a consequence of diapedesis is noted. Hepatocytes are in a state of granular dystrophy. Destructive changes are strongly expressed in the mucous membrane of the small intestine: desquamation of the epithelium, necrosis of epitheliocytes and villi, destruction of crypts. In the brain tissue, there is dilation of the lumens of large and small blood vessels, extracellular and perivascular edema, areas of reactive necrosis.

Keywords: *mycoplasmosis, pathomorphological changes, pigs, lungs, pneumonia*

Introduction

Mycoplasma infection in pigs is widespread in countries with developed pig farming and is one of the reasons for economic losses in meat production. Losses from the disease include treatment costs, decrease in production indicators in piglets for rearing and fattening, lower market cost of carcasses for sale. The introduction of pathogenic mycoplasmas into farms never reporting the disease occurs mainly with infected livestock and semen under violation of quarantine measures and requirements for supplier farms (Rusalesv et al., 2006; Arsenakis et al., 2016).

Analysis of recent researches and publications

Enzootic pneumonia of pigs (mycoplasma pneumonia, respiratory mycoplasmosis, mycoplasmosis of pigs (pneumonia enzootica suum)) is a chronic infectious disease characterized by inflammation of the lungs, serous membranes, and impaired reproductive function of sows.

In 1957, Whittlestone (England), and then Goodwin et al. (1965) showed that the causative agent of porcine enzootic pneumonia, which was previously described as flu, influenza, or viral pneumonia, are microorganisms of the Mycoplasmataceae family. The etiological role of mycoplasmas in porcine enzootic pneumonia was later proved by many researchers.

Most of the mycoplasmas that infect animals are superficial, so-called "membrane parasites", which are firmly attached to the epithelial membranes of the respiratory and urogenital tract. Mycoplasmas have no cell wall. They cannot synthesize peptidoglycan precursors (muramic and diaminopimelic acids) and are bounded only by a thin three-layer membrane. They were allocated to a special division Tenericutes, the Mollicutes class (mollis – soft, tender; cutis – "delicate skin"), the order Mycoplasmatales. The last one includes several families, particularly Mycoplasmataceae, which are pathogenic mycoplasmas, opportunistic (asymptomatic carriers of which are often cell cultures) and Saprophytic Mycoplasma (Ferrarini et al., 2016; Frey et al., 2016; Liu et al., 2016; Xiong et al., 2016).

Piglets can get infected in the womb either by direct contact or by aerosol (vertical transmission) (Calsamiglia & Pijoan, 2000; Vicca et al., 2002).

In modern pig breeding, porcine enzootic pneumonia does not have a pronounced seasonality – the disease occurs any time of the year.

The manifestation of mycoplasma infections is usually associated with a violation of the symbiotic system due to the massive interaction of endo- and exogenous (including environmental) factors.

Mycoplasma infections are a kind of indicator of stress in animal. The severity of the course depends on the general health of pigs, the presence of helminths, as well as on the conditions of animal keeping. In farms where animals are kept in poorly ventilated cold rooms and have an unbalanced diet with an insignificant content of the necessary vitamins and minerals, the incidence of diseases in the livestock can reach 40–85%, moreover, the difference in conditions of keeping can reach about 15%. The disease may not show clinical signs, while the productivity of sick pigs is reduced by about 20% (Samuylenko et al., 2006; Giacomini et al., 2016; Kolych, 2016).

Materials and methods of research

The research was carried out at a private enterprise for pigs raising and fattening in the Poltava region. The material for the study was the pathological material taken during the pathological necropsy of the carcasses of dead piglets (n = 6) at the age of 7 days, in which the causative agent of mycoplasmosis was diagnosed and identified during life by laboratory methods.

The main method used in the study was a histological examination, during which microstructural changes in organs and tissues were recorded and described. After sampling, the pathological material was fixed in a 10% aqueous solution of neutral formalin, followed by embedding in paraffin. The prepared histological sections were stained with hematoxylin and eosin according to standard prescriptions (Goralskyi et al., 2011).

The general histological structure and microstructural changes in histological sections were studied under a light microscope (MC 100LED, Micros Austria).

Results of the research and their discussion

In several cases, the corpses of piglets of the first week of life had a cyanotic coloration of the dewlap, limbs, ears, which we associate with cardiac disorders in animals shortly before death.

Examination of visible mucous membranes revealed hyperemia of the nasal mucosa, thymus, anemic mucous membranes of the trachea, esophagus, and pharynx.

The heart had an irregular shape due to enlargement of the right ventricle or diffuse enlargements of all parts. In some animals, hemorrhages were observed in the pericardium along large vessels, which were filled with blood significantly. In some cases, the epicardium had an unevenly gray-pink color, moderately wet, in others, it was diffusely clay color, anemic. The myocardium was flabby, clay-colored.

Simultaneous damage of the pharyngeal lymph nodes, parotid and cervical, mandibular ones (slightly enlarged, from dark pink to dull red color). The lymph nodes of the chest cavity acquired pronounced or indistinct pronounced tuberosity of the surface, uneven color (pink, cyanotic, dark red areas, the cut surface was significantly wet, scrape was not available).

Insignificant enlargement in the lymph nodes of the middle and posterior parts of the digestive tract (abdominal lymphatic center: gastric, pancreatic-duodenal; cranial mesenteric lymphatic center: jejunal, ileocolic; lymph nodes of the caudal mesenteric lymphatic center). Lymph nodes are pale pink with light red areas, in some animals, there was significant enlargement, intense red color (Fig. 1).

In young boars, changes in the lymph nodes of the iliofemoral and inguinal-femoral lymphatic centers were observed, especially there was a significant enlargement in the superficial inguinal lymph nodes, which were lumpy, dense, had irregular red-brown or red-pink color.

The lungs had doughy consistency, uneven color. In some cases, there were diffuse red areas covering the entire lobules of the lungs, in other cases, small areas were affected. A large amount of blood was released from the vessels on the cut surface and a frothy liquid was registered in the alveoli. In some areas, crepitus foci were recorded. A mucous mass was released from the bronchi.

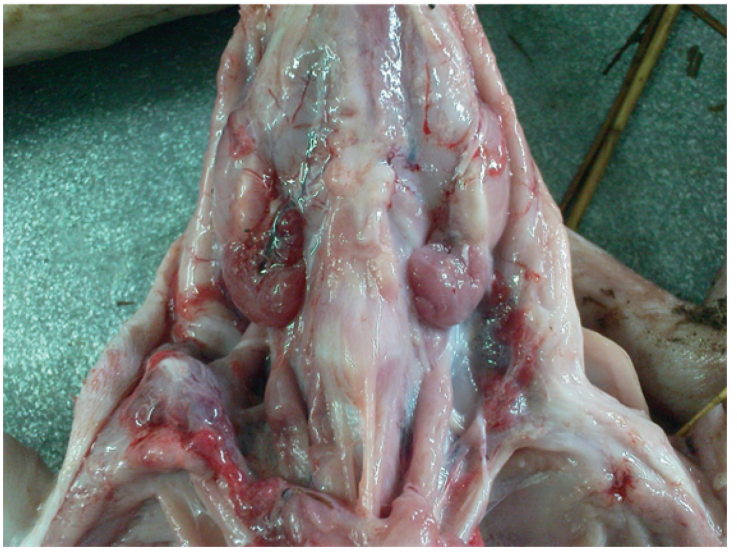

Fig. 1. Hyperemia and enlargement of the mandibular lymph nodes

The spleen had a regular, elongated-oval shape, pink with a bluish tinge of color. The cranial edge of the organ was often much wider than the caudal one, dark red hemorrhages were traced in parenchyma thickness. In some cases, hemorrhages in the form of black small cells were located along the edge of the organ.

Different levels of flatulence severity of stomach and intestines were characteristic. The animals' stomach was filled moderately, contained milk clots, the mucous membrane was moderately hyperemic. Catarrhal enteritis was registered in animals manifested as moderate hyperemia of the mucous and serous membranes of the intestine, accumulation of the insignificant amount of cloudy gray mucus on the inner lining of the intestine.

Uneven color of the liver occurs in piglets: dark red areas without clear boundaries changed into creamy clay ones.

The liver had a smooth surface, soft or doughy consistency, parenchyma pattern on the cut surface was smoothed insignificantly. In areas of dark red color on the cut surface, a large amount of dark red blood was released when pressed.

The organ contained small single striped or punctate hemorrhages. Small gray-white cells were registered under the liver capsule, which are barely visible against the general background of the organ. the gallbladder was enlarged by 2–3 times in up to 50% of cases, bile was yellow and had liquid consistency.

In the majority of the studied cases of piglet death, uneven color of the kidneys was observed, indistinctly delineated diffuse rounded areas of darker (up to bluish-red) or, conversely, lighter (up to creamy-pink) color were observed. The capsule was transparent and removed easily. The renal cortex was clay-colored, the renal medulla varied from pink to dark red color (due to hyperemia of the renal medulla). The cut surface was

smooth, the parenchyma was significantly wet.

In the reproductive system of females, changes were not expressed at the macroscopic level. The vaginal tunic of the testes of aborted male fetuses was translucent and insignificantly thickened. Vessels in the thickness of the tunic were full-blooded with small punctate hemorrhages. Testes had a bluish tint due to full-blooded vessels.

The brain had milky color, often with a pale pink tinge, blood vessels were filled with blood.

In the lungs, on the histological level, both large and small vessels were full-blooded significantly. In large areas, the alveoli were semicollapsed, in the form of slit-like gaps. In areas of the tissue infiltrated with inflammatory infiltrate, the alveolar wall was thickened, the alveoli were semicollapsed, alveolocytes were in a state of cloudy swelling and vacuolar dystrophy, they were saturated with erythrocytes (Fig. 2).

Peribronchial pneumonia of a lymphocytic nature was noted. The walls of blood vessels were in a state of mucoid swelling, hydropic degeneration of endothelial cells, and perivascular edema was also observed.

Thickening of the interlobular connective tissue fibers was noted. There were registered areas, in which the lumens of the alveoli were enlarged and contain a homogeneous pale pink mass in the field of view.

In small bronchi and alveoli, there were a large number of lymphocytes, single leukocytes, a significant part of which were in a state of decay and necrosis. Among these cells, there were desquamated (destroyed) cells of the epithelium of the bronchi and erythrocytes. Connective tissue and the wall of the bronchi were infiltrated with lymphocytes and monocytes. Alveoli with elongated lumens and thinned walls often with cystic formations can be noted along the periphery of such areas. There

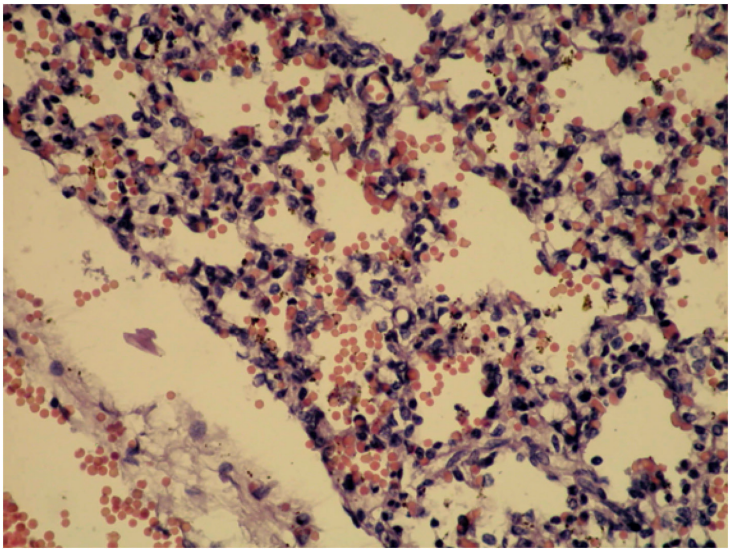

Fig. 2. Thickened walls of alveolocytes and their infiltration with erythrocytes

were single hemorrhages in the alveoli and stroma of the organ.

The heart. Cardiomyocytes were in a state of cloudy swelling, thickened, the contours of the nuclei were not clear, sometimes did not register at all. The cytoplasm was not transparent, pink-colored. The vessels were filled with blood, their walls were thickened and also in a state of mucoid swelling (Fig. 3).

The pancreas. The histoarchitecture of the pancreas was preserved, the blood vessels had moderate blood filling, the lumens contained concentrated eosinophilic secretions, and the pancreocytes were mostly without visible changes. In some areas (with clear edema) parenchyma was with signs of necrosis. Destruction of the glands occurred, and shapeless mass was observed in the field of view, in which fragments of cells can be noticed.

In the spleen, there was a thickening of the capsule, trabeculae, and the walls of the blood vessels of the white and red pulp, also insignificant fiber dissociation was observed as well as increased volume of the cells' nuclei of connective tissue, but blood vessels were average blood-filled. Severe edema of the reticular tissue was registered. Lymph nodules were paler than normal, lack of leukocytes, single small foci of necrosis were visible in the field of view. In some areas, the structure of the trabecula fibers was not clear, there was thickening and homogenization of the intima of the blood vessels and narrowing of their lumen. In some areas of the organ, in areas with edema, foci of unreactive necrosis, which did not have clear contours, were recorded.

In the thymus of piglets, vessels were blood-filled, pronounced perivascular edema of the tissue was observed. In the cortical zone of the thymus lobule, conglomerates of destroyed lymphocytes were formed, acquiring basophilic properties; scattered single erythrocytes were also observed between thymocytes. In the medulla of the thymus lobules, lymphocytes in most animals were scattered, the nuclei of individual lymphocytes were in a state of karyorrhexis,

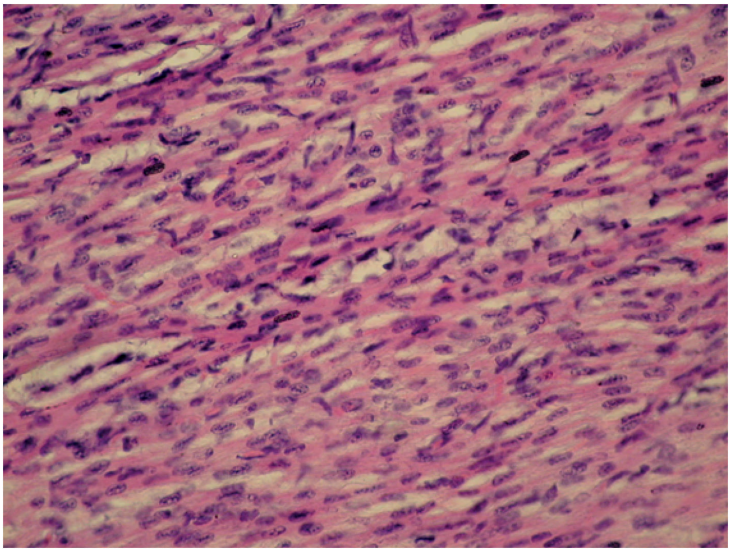

Fig. 3. Histological slide of the myocardium of a 3-days-old piglet with signs of protein myocardosis. Staining with hematoxylin and eosin, ×200

and some of the nuclei had an irregular shape. There were a large number of eosinophils between thymocytes.

In addition, pink-red colored cells were observed in significant areas, while the contours of such cells were barely traced and nuclei were not expressed.

Lymph nodes (mesenteric) contained focal hemorrhages, hyperemia of blood vessels, mucoid and fibrinoid swelling and single areas of necrosis of their walls were revealed. The lymph nodes of the abdominal cavity contained small areas of necrosis and stromal proliferation.

In the lymph nodes (chest cavity), there were hyperemia, serous edema of the sinuses and parenchyma, increase of lymph nodules' size, but the lymphocytes in them were sparsely located, also accumulation of plasmacytes, macrophages, and lymphocytes in the cortex and medulla were observed.

The liver was in a state of acute venous hyperemia. At a low magnification of the microscope, in some lobules, pronouncedly enlarged and blood-filled central and intralobular capillaries were found, and the hepatic plates were respectively compressed. In the center of other lobules, there was a diffuse infiltration of liver tissue with erythrocytes, as a result of diapedesis. In such places, protein and fatty degeneration were observed in hepatocytes. There were often areas with hepatocytes in a state of decomposition (phanerosis). At the same time, the cells had a reticular structure, larger than normal nuclei were located in the center but in many cases, they were destroyed (fatty decomposition).

In some areas, there were foci where the cytoplasm of hepatocytes contained large vacuoles, the nucleus was displaced to the periphery – ring-shaped cells (fatty infiltration). Fatty infiltration occurred much less frequently than decomposition.

There were often areas of tissue in a state of paranecrosis in the field of view: foci in which histological structure of the organ was barely noticed, also fragments of nuclei, swelling of individual nuclei, contours of individual cells could be detected. Small blood vessels were filled with blood, erythrocytes were registered between the plates.

At the periphery of the lobule, the structure of the cells remains normal. The plates structure of hepatocytes was damaged, which had the form of conglomerates without clear boundaries that merged. Up to 40% of parenchyma cells did not contain nuclei. In all other cases, the nuclei did not have clear contours. They had increased volume, up to 10% of them were in a state of lysis. Hepatocytes were in a state of granular dystrophy, in their cytoplasm a large number of grains of protein nature were registered. The interlobular connected tissue is poorly noticed. In some cases, significant swelling of the intralobular connective tissue was observed. Fatty extracellular degeneration was also registered (fatty vacuoles were located in the interlobular connective tissue). There were single hepatocytes with signs of fatty infiltration. Fat in such cells in the form of one large drop pushed the nucleus and cytoplasm to the periphery, so the liver cells looked like typical fat cells (ring-shaped cells). Such cells were increased in their volume (Fig. 4).

In some areas, hepatic lobules had different sizes, interlobular connective tissue was infiltrated with lymphoid, plasma cells, and fibroblasts. Around the central veins of the hepatic lobules plate structure of the parenchyma was not registered, single hepatocytes with signs of dystrophy and necrosis were observed, the vessels contain a small number of erythrocytes.

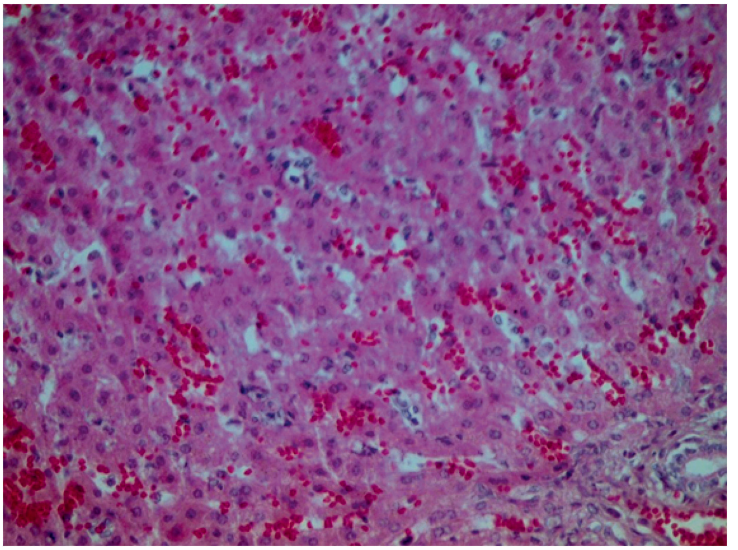

Fig. 4. The liver of a 2-days-old piglet: blood-filled sinusoids and granular degeneration of hepatocytes. Staining with hematoxylin and eosin; ×200

The wall of the stomach and small intestine. Destructive changes were strongly expressed in the mucosa (desquamation of the epithelium, necrosis of epithelial cells and villi) that resulted in the partial absence of epithelial cover, the crypts were half-destroyed.

On the surface of the mucous membrane, a pale pink mass (mucus containing proteins) with an admixture of desquamated epithelial cells, a small number of polymorphonuclear leukocytes, and single erythrocytes. In large areas, the destruction of the villi up to crypts was observed. The submucosa was thickened, the blood vessels were filled with blood, moderate infiltration of the connective tissue by cells of the inflammatory infiltrate was registered – mainly lymphocytes and macrophages, which were most pronounced in the tissue around the blood vessels, where small (diapedesis) hemorrhages were also visible.

In the kidneys, there was a lesion of the parenchyma (straight and convoluted tubules), blood-filled of the renal medulla vessels. Epithelium of the convoluted tubules in some areas was in a state of hydropic dystrophy, the cells were enlarged, rounded, protrude into the lumen of the tubules, often desquamated, the nucleus and cytoplasm were dissolved, only the cell membranes were visible. The structure of epithelial cells was absent in some areas. In cell cytoplasm, fine acidophilic granularity was noticed. Some cells had foamy cytoplasm, some others were separated from the basal membrane and each other. Desquamated cells were registered in the lumen of the tubules.

In addition, a large number of areas were recorded with epithelial cells of the tubules (mainly convoluted ones) increased in the volume containing a different number of small pink grains. Boundaries of tubules' epithelial cells were not clearly visible; lumens of the vessels were narrowed with different diameters due to the cells increased volume. In some tu-

bules, lumens were well noticed, in others – they looked like gaps, or were not visible at all. The cytoplasm was cloudy, dull, the nuclei were barely visible. In the most affected cells nuclei were in a state of karyolysis or not registered at all. In the general background, areas with complete blockage of the tubule's lumens by the protein were well noticed.

In some areas around the vessels, glomeruli, and between the tubules, there was an accumulation of a significant amount of proliferating tissue (lymphoid, epithelioid cells, and histiocytes). Between the tubules there were cells that looked like a coarse-grained deep mass of dark red color, in which fragments of epithelial cells were registered with nuclei forming pale blue homogeneous spheres.

In the cortical layer, blood-filled vessels of the capillary network of the glomeruli were observed. Glomeruli were increased in volume and occupied the entire lumen of the Bowman's capsule. There were up to 40% of such glomeruli. Twenty % of the Bowman's capsule in the glomeruli contained serous exudate. There were also single glomeruli with the destroyed walls. In these cases, there was bleeding into the lumen of the capsule.

The blood-filled vessels of the medulla were clearly visible. In most cases, up to 60–70%, the lumens of the straight tubules were not registered due to increased volume of epithelial cells, which were in a state of hydropic dystrophy. Their cytoplasm was vacuolated; in some cases, the cell nuclei were in a state of lysis. One large vacuole occupied almost the entire cell, and the nucleus and the remnants of the cytoplasm were pushed out to the periphery and compressed. The cells looked like a balloon. Up to 40% of the tubules had dilated spherical lumen.

The testicles contained punctate diffuse hemorrhages. The parenchyma looked like a single-layer spermatogenic epithelium (Fig. 5).

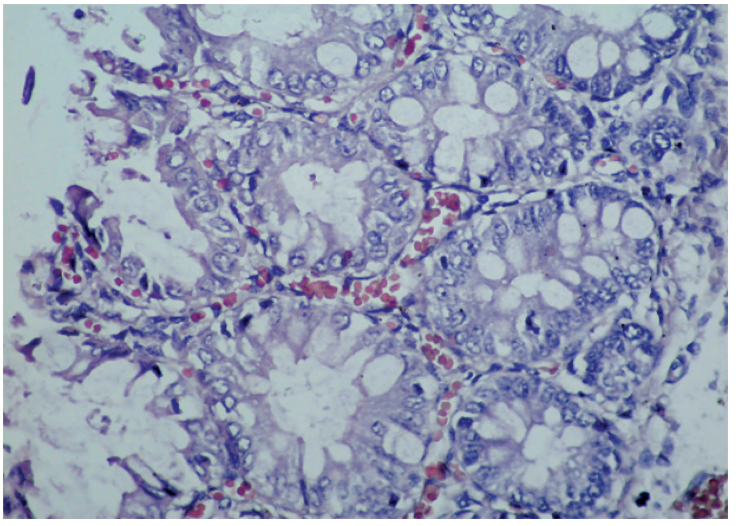

Fig. 5. Histological section of testes of a 5 days-old piglet: blood-filled vessels, hydropic dystrophy and epithelial necrosis. Staining with hematoxylin and eosin; ×400

Distention of the lumens of large and small blood vessels was registered in brain tissue, a "sludge" phenomenon in individual vessels. Around the blood vessels of neurons, there were clearing zones (extracellular and perivascular edema), areas of paranecrosis, and unreactive necrosis (Fig. 6).

In the spinal cord, individual neurons were increased in volume, cytoplasm opacity and its intense pink color, nucleus enlarged in size that did not have clear contours. Individual neurons were in a state of decay (both the cell and the nucleus had an irregular shape, the nucleus was shrunken, colored intensely blue-violet, and in other cases, it was enlarged, with no clear boundaries, smoky colored). The blood vessels contained a large number of erythrocytes as a homogeneous mass.

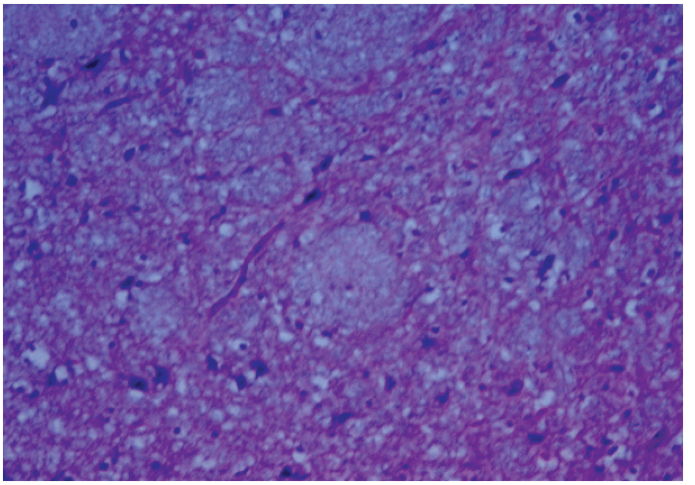

Fig. 6. Brain tissue of a piglet of the first week of life: blood-filled capillaries and extracellular edema. Staining with hematoxylin and eosin; ×100

Conclusions and future prospects

Mycoplasmosis in piglets under 7 days of age is characterized by hyperemia of the upper respiratory tract and exudative bronchopneumonia. Damage of the digestive tract manifested as catarrhal and serous-catarrhal gastroenteritis.

A histological study found that in the piglets with mycoplasmosis, there was a violation of the general protein metabolism, which manifested by granular degeneration of the liver, heart muscle, and kidneys.

There was unreactive necrosis of the parenchyma of the liver, kidneys, pancreas, and brain, necrosis of the intestinal villi up to the crypt cells.

In the organs of immunopoiesis (thymus, spleen), edema, areas of unreactive necrosis, and mucoid swelling of stromal elements were registered.

The study of the mycoplasma pneumonia issue in pigs indicates the need for further continuous monitoring in pig farms in Ukraine. Correct and timely diagnostics is important in the control of the disease; therefore, it is imperative to learn modern and develop new methods for studying mycoplasmosis.

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Анотація. *Проведено патологоанатомічний розтин 6 трупів поросят першого тижня життя, які загинули від мікоплазмозу. Під час дослідження видимих слизових оболонок встановлено гіперемію слизової оболонки носової порожнини й тимуса. Відмічали одночасне ураження заглоткових, привушних, шийних і нижньощелепних лімфатичних вузлів. Вони були незначно збільшені, від темно-рожевого до темно-червоного кольору. Серце неправильної форми внаслідок розширення правого шлуночка або дифузного розширення всіх відділів. Легені тістуватої консистенції, нерівномірного забарвлення. В одних випадках спостерігаються дифузні червоні ділянки, що охоплюють цілі долі легень, в інших випадках відбувається ураження невеликих ділянок. Печінка має гладку поверхню, м'якої чи тістуватої консистенції, на розрізі малюнок паренхіми дещо згладжений. Забарвлення печінки різне: темно-червоні ділянки, без чітких меж переходять у кремово-глинисті. Характерною ознакою був метеоризм шлунку та кишечнику. У тварин зареєстровано катаральний ентерит, який проявлявся у вигляді помірно вираженої гіперемії слизової та серозної оболонок кишечника.*

На мікроскопічному рівні в легенях спостерігається значне кровонаповнення судин. Альвеоли напівспалі, у вигляді щілеподібних просвітів. У ділянках інфільтрації тканини запальним інфільтратом стінка альвеол потовщена, альвеоцити були в стані мутного набухання та вакуольної дистрофії, вони просочені еритроцитами. Спостерігається перибронхіальна пневмонія лімфоцитарного характеру. Печінка була в стані гострої венозної гіперемії. В одних часточках знаходять різко розширені й заповнені кров'ю центральні і внутрішньочасточкові капіляри, а печінкові балки відповідно стиснуті. В інших часточках у центрі відмічається дифузна інфільтрація тканини печінки еритроцитами, як наслідок діапедезу. Гепатоцити були в стані зернистої дистрофії. У слизовій оболонці тонкого кишечника сильно виражені деструктивні зміни: десквамація епітелію, некроз епітеліоцитів та ворсинок, руйнування крипт. У тканині головного мозку спостерігається розширення просвітів великих і дрібних кровоносних судин, екстрацелюлярний та периваскулярний набряк, ділянки ареактивного некрозу.

Ключові слова: *мікоплазмоз, патоморфологічні зміни, свині, легені, пневмонія*