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ORIGINAL ARTICLE

Pathomorphological changes in laying hens' organs in case of infection by a field strain of the Marek's disease virus

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In small private farms, Marek's disease is one of the causes of laying hens' death, especially in case of a spontaneous infection by a field strain of the virus. For several decades, the disease has been successfully prevented using vaccination. However, the vaccines' effectiveness decreases over time due to the emergence of more virulent strains. Economic losses from the disease are caused by the high mortality rate, reduced poultry productivity, and the additional financial burden on implementing veterinary measures. Marek's disease is still being registered in all countries worldwide that have the developed poultry farming industry.

The article highlights the results of the autopsy and histopathological studies of the organs of laying hens of the Leghorn breed in various age groups (120 to 350 days) affected by the field strain of Marek's disease virus. During the disease period, sick fowl lost its productivity sharply, and the mortality rate accounted for 35% (150 hens). The main clinical signs were exhaustion, paresis of the wings, atypical gait, and neck curvature (in some cases). The fowl with the feet oriented in different directions was not detected. The autopsy results revealed tumors on the liver, heart, kidneys, spleen, and ovaries in most hens. Macroscopically, affected organs are enlarged, with miliary proliferates, especially the spleen. Histologically, diffuse massive infiltration and proliferation of pleomorphic lymphoid cells into the liver's parenchyma, heart muscle, lungs, spleen, pancreas, kidney, and glandular stomach were detected. Morphological changes in the spleen were characterized by lymphocyte pleomorphism and necrosis of the reactive centers of lymphoid nodules. Massive diffuse foci of lymphoblast proliferation were found in the glandular stomach. Areas of lymphoblast proliferation with significant damage to the parenchyma, hemorrhage, and stasis were found in the liver and kidneys. Characteristic lymphoid infiltrations were also discovered in the peripheral nerves of some laying hens.
Keywords: splenomegaly, necrosis, perivascular clutches, proliferation, edema, lymphoblasts.

Introduction

Neurolymphomatosis, or Marek's disease, is a lymphoproliferative disease that damages the peripheral nervous system and is accompanied by visceral lymphoma development in parenchymal organs, the liver, kidneys, and ovaries, rarely infiltration of mononuclear cells in the iris and brain. The etiological factor is a DNA-containing virus with a hexagonal nucleocapsid, with a diameter of 85 to 100 nm, and a shell with 150 to 160 nm. This airborne virus that can also be transmitted with poultry slaughter products belongs to *Herpesviridae*, subfamily *Mardivirus*. It remains resilient on the eggshells, making it possible to infect chickens in the incubator (Payne & Biggs, 1967; Witter, 1997; Frank, 2001). The fowl that was once affected by the disease remains a carrier of the virus throughout its whole life, constantly supplying the virus into the environment. Virions of Marek's disease virus commonly replicate in cell nuclei and less frequently in the cytoplasm or extracellular space (Lawn & Watson, 1982; Osterrieder et al. l., 2006).

The history of this disease began with Dr. József Marek, a prominent veterinarian and pathologist, head of the Department of Veterinary Medicine at the Royal Hungarian Veterinary School in Budapest. In 1907, he described some cases with paresis' clinical manifestations and paralysis of four adult roosters' feet and wings. After detailed morphological studies, thickening of the sacral nerve plexuses and massive proliferates of the spinal nerves by mononuclear cells were found. This made it possible to separate neurolymphomatosis from the diseases with similar symptoms and publish the first report on the sickness, which was later called Marek's disease (Nazerian et al., 1968; Biggs et al,1972; Calnek, 2001).

In 1914, similar changes were recorded and described by veterinarians from the United States and the Netherlands. They discovered that not only the nervous system was affected, but visceral lymphomas developed too, which gave the disease a new name – neurolymphomatosis (*Neurolymphomatosis gallinarum*). In 1924, van der Walle and Winkler-Junius first proved the infection's viral origin (Payne & Venugopal, 2010).

In the 1960s, the first experimental infections of poultry with Marek's disease virus were carried out. However, the disease got complicated by bacterial infections, which did not allow us to study all the organism changes. In 1967, British and American scientists independently discovered alpha-herpesvirus, the etiological agent that caused the disease. Shortly after this, a detailed study of the pathogenesis, immunological aspects of the disease, and the production of a vaccine started (Calnek & Witter, 1985; Biggs, 2001; Biggs & Nair, 2012). At the end of the 1970s, the losses from Marek's disease among the vaccinated poultry reached 60%, caused by the emergence of a new highly virulent strain. A bivalent vaccine made from SB-1 and HVT (FC-126) strains had already been developed, but the desired effect was not achieved. Therefore, the world leaders in poultry vaccines are still working

hard to improve existing vaccines and develop new ones, including those against Marek's disease (Witter et al.,1980; Witter, 2001; Davison & Nair, 2005; Suma et al., 2017).

Several authors analyzed the clinical signs and pathological changes that developed in the poultry's organism affected by the Marek's disease virus and identified two forms of the disease - classical and acute. The classical form was characterized by the enlargement and thickening of the peripheral nerves with pronounced symptoms of paresis and paralysis. Acute form progressed with the formation of lymphoid tumors in the internal organs. Morphologically, damage to peripheral nerves without visible macroscopic changes was also detected in the acute form (Simu et al., 1980; Witter et al., 2005; Othman & Aklilu, 2019; Stamilla, 2020).

In Ukraine's industrial poultry breeding, Marek's disease is successfully controlled through vaccination. Nevertheless, small farms often suffer from its outbreaks. This happens mainly due to untimely vaccinations, ineffective vaccines, or the emergence of more virulent strains of the virus (Witter, 1997; Pejović et ll, 2007; Baaten et al., 2004). Therefore, poultry deaths, especially laying hens, have become more frequent among private farms in recent years. Commonly, detecting Marek's disease is based on the clinical picture analysis, pathological changes, isolating and defining the pathogen by PCR diagnosis and histopathological studies (Cui et al., 2005; Kezawa et al., 2010).

Our research aimed to study pathomorphological and pathohistological changes in the organs of laying hens of the Leghorn breed affected by a field strain of Marek's disease virus.

Materials and methods

The study's material was the carcasses of laying hens of the Leghorn breed in various age groups (120 to 350 days), kept in a small private farm in the Lviv region. Chickens were vaccinated on their first day of life in an incubator with MAREK-VAC BIVALENT FROZEN.

Mass deaths of laying hens were observed in the spring. The sick poultry lost weight abruptly, the signs of wing paresis and neck curvature appeared. The poultry with the orientation of feet in different directions was not found. The mortality rate reached 35% (150 hens). The pathological autopsy was performed in the prosector chamber of the Department of Normal and Pathological Morphology and Forensic Veterinary Medicine (Lviv National University of Veterinary Medicine and Biotechnology named after S.Z. Gzhytsky). The material was sent for PCR studies to confirm the diagnosis.

Fragments of internal organs were taken for histological examination and fixed in 10% aqueous neutral formalin solution. The tissues fixed in the formalin solution were washed and dehydrated in an ascending row of alcohols, followed by pouring into paraffin according to the conventional method. Histocuts with a thickness of 7 μm were made from paraffin blocks using a sled microtome MS-2. Paraffin sections were stained with Mayer's hematoxylin and eosin to perform light optical microscopy (Kiceli, 1962; Merkulov, 1969). Light microscopy and macrophotography of the obtained histological preparations were performed using Leica DM-2500 microscope, Leica DFC 450C camera, and Leica Application Suite Version 4.4.

Results and discussion

Changes of various kinds were revealed during the pathological autopsy of laying hens. The sick fowl was exhausted, with the anemic crest and earrings, atrophied muscles, clearly visible keel bone, and absent subcutaneous fat (Fig. 1). Some laying hens had enlarged liver of dark cherry color with small spot hemorrhages under the capsule and miliary foci; the edges were rounded (Fig. 2). Other hens' liver was dark brown, dense consistency with pronounced tumors of gray and white color and different size. The spleen was enlarged several times, of dark cherry color with gray spots in the parenchyma; the capsule is tense, and marbling is visible on the section (Fig. 3). The glandular stomach wall is thickened, swollen, the glands were sharply filled with secretions. The kidneys were of cherry color, blood-filled, flabby, enlarged with diffuse lesions of light gray shade (Fig. 4). Small gray tumors were also found on the heart surface and in the ovaries (Fig. 5). Lesions of the bursal sac and thymus were characterized by atrophic changes in the majority of fowl.

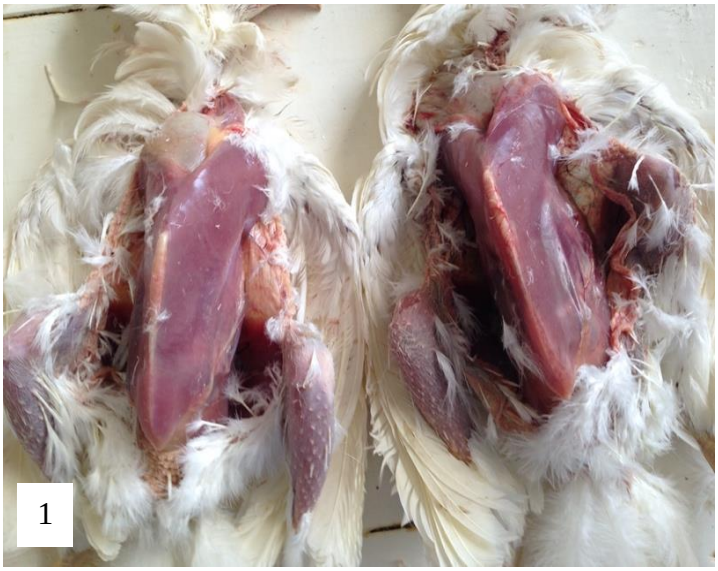

Fig. 1. The exhausted fowl with the pronounced keel bone and atrophied muscles. **Fig. 2.** The liver with miliary foci.

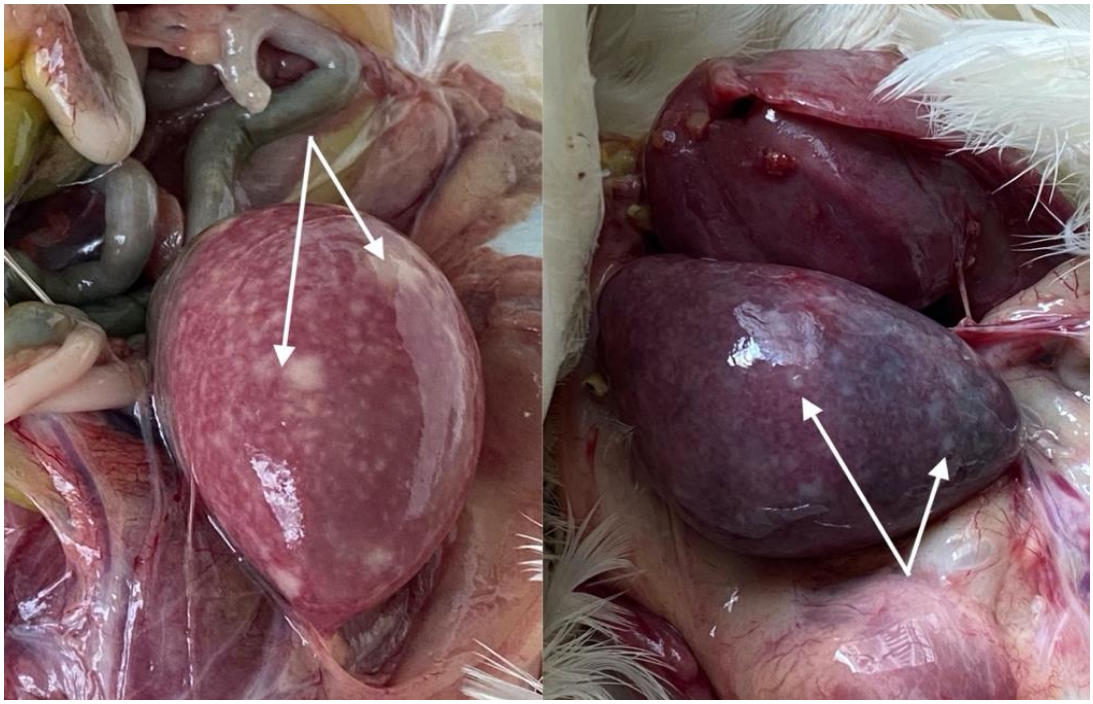

Fig. 3. Splenomegaly with proliferative foci (arrows).

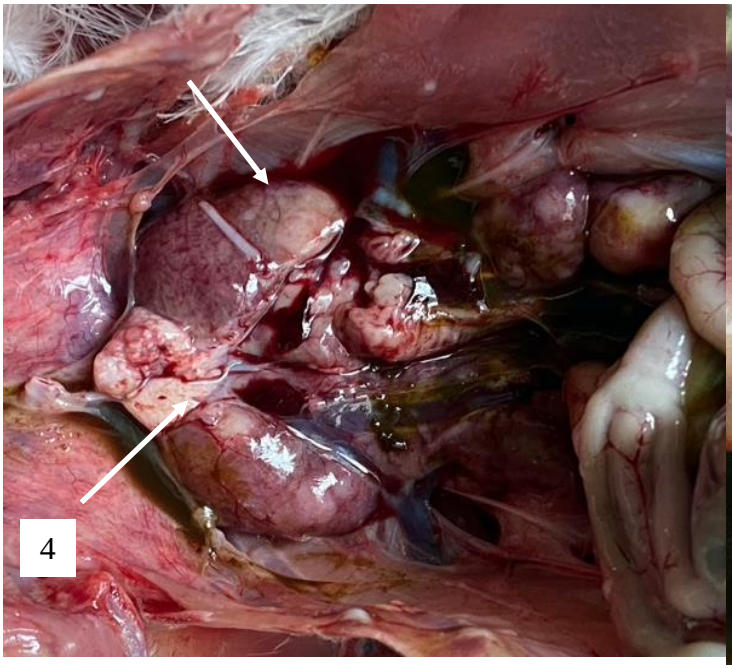

Fig. 4. The kidneys. Tumor-like over-growth (arrows). (arrows).

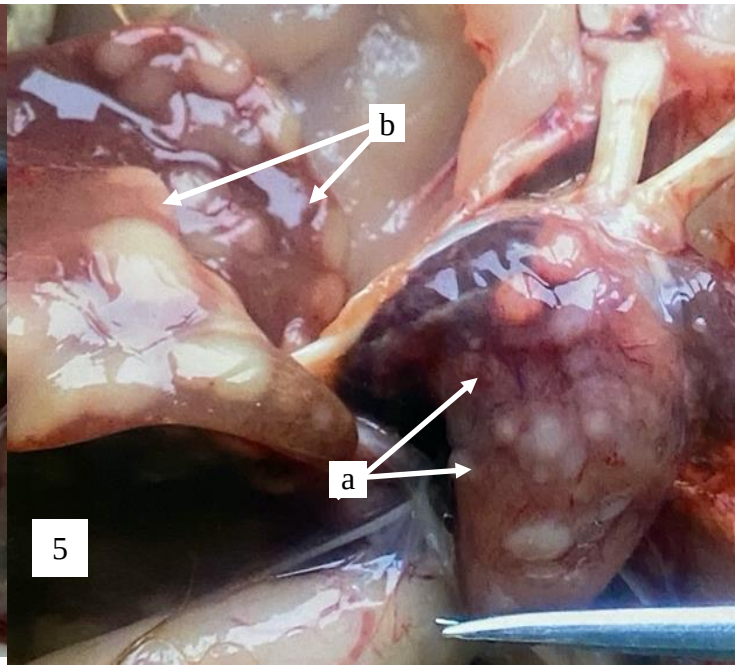

Fig. 5. The heart (a) and the liver (b). Tumor-like over-growth

The histopathological examination of the laying hens' parenchymal organs revealed the diffuse lymphocytic infiltration. Microscopically, in the affected liver, the liver lobe's typical architecture is disturbed and replaced by pleomorphic lymphoblasts, which merged into large foci, while the parenchyma and stroma were not identified (Fig. 6a, 6b). Lymphoblast proliferation was accompanied by dilation of central veins and intraparticle capillaries. Hemorrhages and stasis also emerged.

Diffuse lymphoblastic infiltration was also found in the cortical and cerebral layers of the kidneys. The combined foci of infiltration were localized mainly in the cortex (Fig. 7a). The vessels between the renal tubules were dilated and filled with erythrocytes. Cellular infiltrations merged and squeezed the tubules. The lumen of the proximal tubules was narrowed, filled with homogeneous protein masses, and desquamated epithelium (Fig. 7b). Necrosis of tubular epitheliocytes was detected in some places. The nuclei of cells were in the rhexis stage; some of them were lysed. The Shumlyansky-Bowman capsule of the renal glomeruli was compacted, and the vessels were filled with blood. The plate of the ureters mucous membrane was swollen, fibrous, and diffusely infiltrated by lymphoblastic cells (Fig. 8a).

Diffuse infiltration of lymphoblasts between the acini of exocrinocytes was detected optically in the pancreas (Fig. 8b). The glandular epithelium had a slight zymogenic layer; the cells themselves were with a homogeneous cytoplasm and slightly reduced.

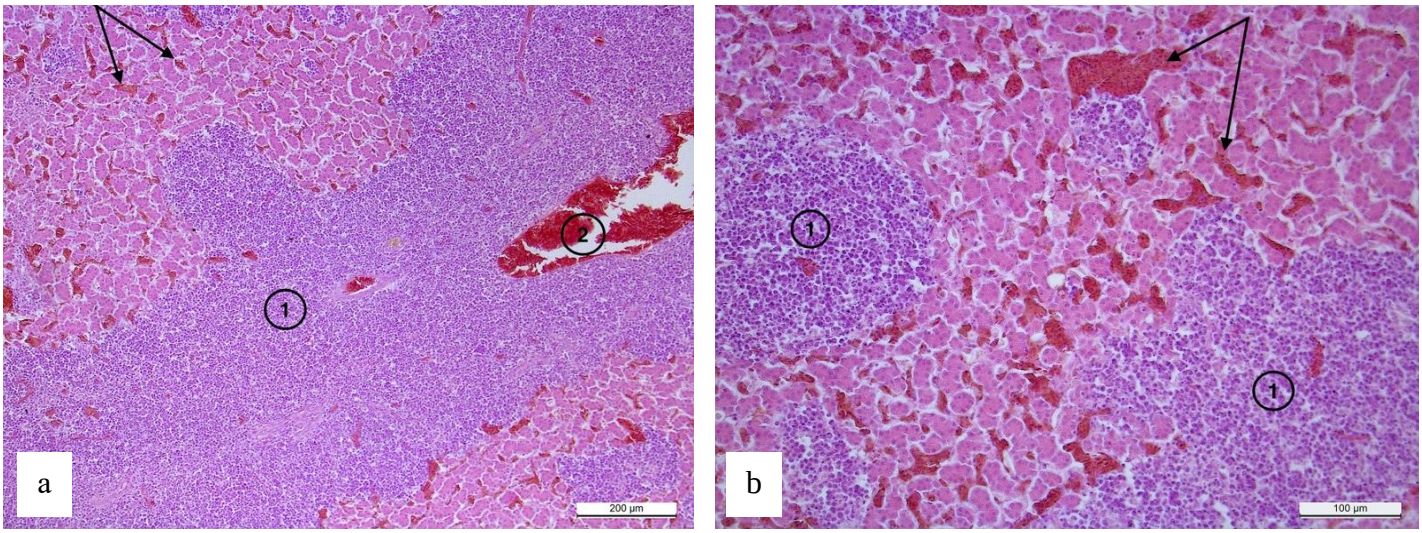

Fig. 6. A. The liver. Massive cellular infiltrations of lymphoblastic cells, prolymphocytes, and lymphocytes (1). Venous stasis (2). Expansion of intraparticle capillaries (arrows). Hematoxylin and eosin, x100. B. The liver. Diffuse proliferation by pleomorphic lymphocytes (1), dilation of intraparticle capillaries (arrows). Hematoxylin and eosin, x200.

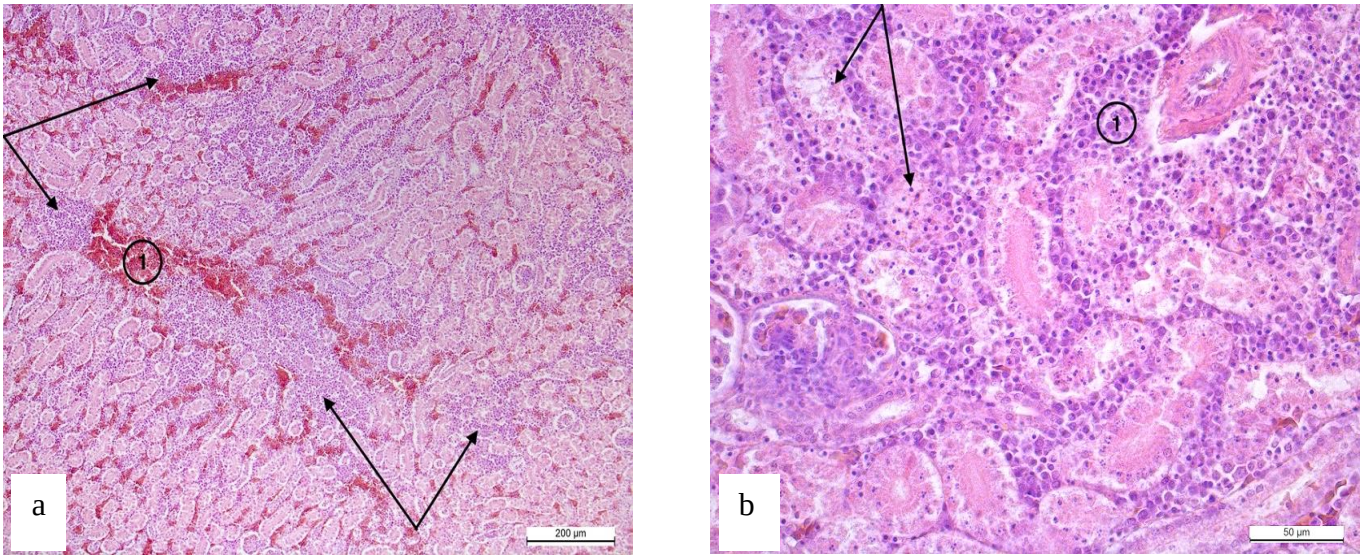

Fig. 7. A. The kidneys. Diffuse infiltration by pleomorphic lymphoid cells (arrows). Hemorrhage (1). Hematoxylin and eosin, x100. B. The kidneys. Dystrophy and necrosis of the nephrothelium of the proximal and distal tubules of the kidneys (arrows). Foci of lymphoblastic cells (1). Hematoxylin and eosin, x400.

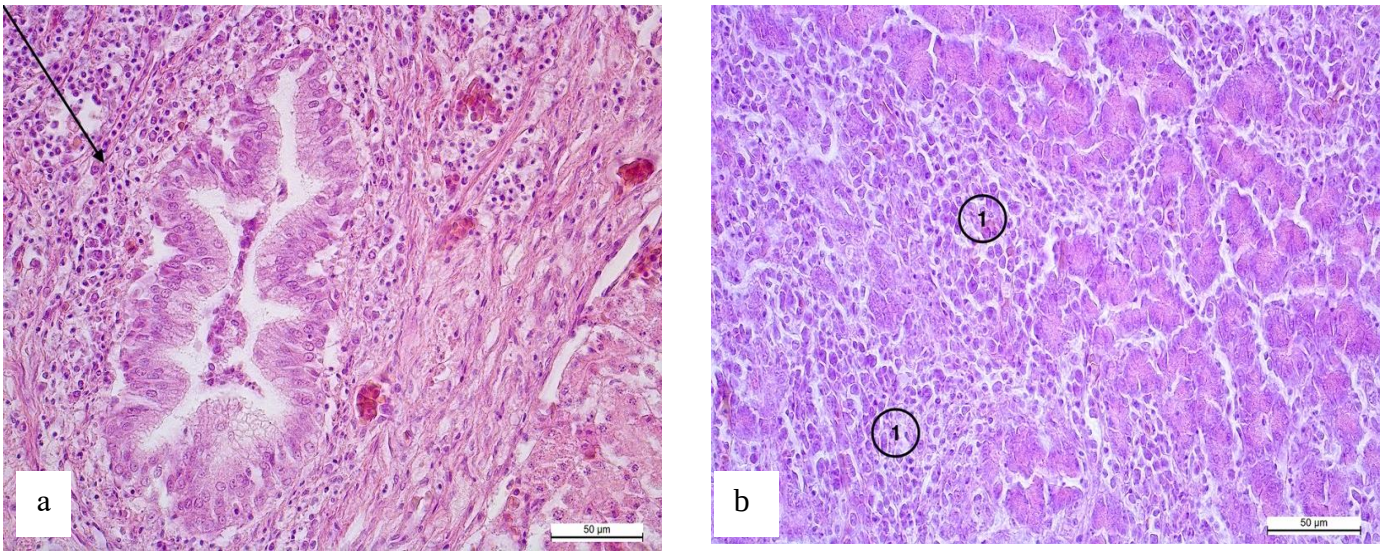

Fig. 8A. The ureters. Diffuse infiltration by lymphoid cells of the actual plate of the mucous membrane (arrow). Hematoxylin and eosin, x400. B. The pancreas. Diffuse infiltration oflymphoblasts between acinuses (1). Hematoxylin and eosin, x400.

Myocardial damage was featured by cardiomyocyte dystrophy, loss of transverse striation, and diffuse proliferation and infiltration of lymphoblasts between heart muscle fibers (Fig. 9a, 9b). Cardiomyocyte nuclei were poorly visible. The capillaries were full of blood.

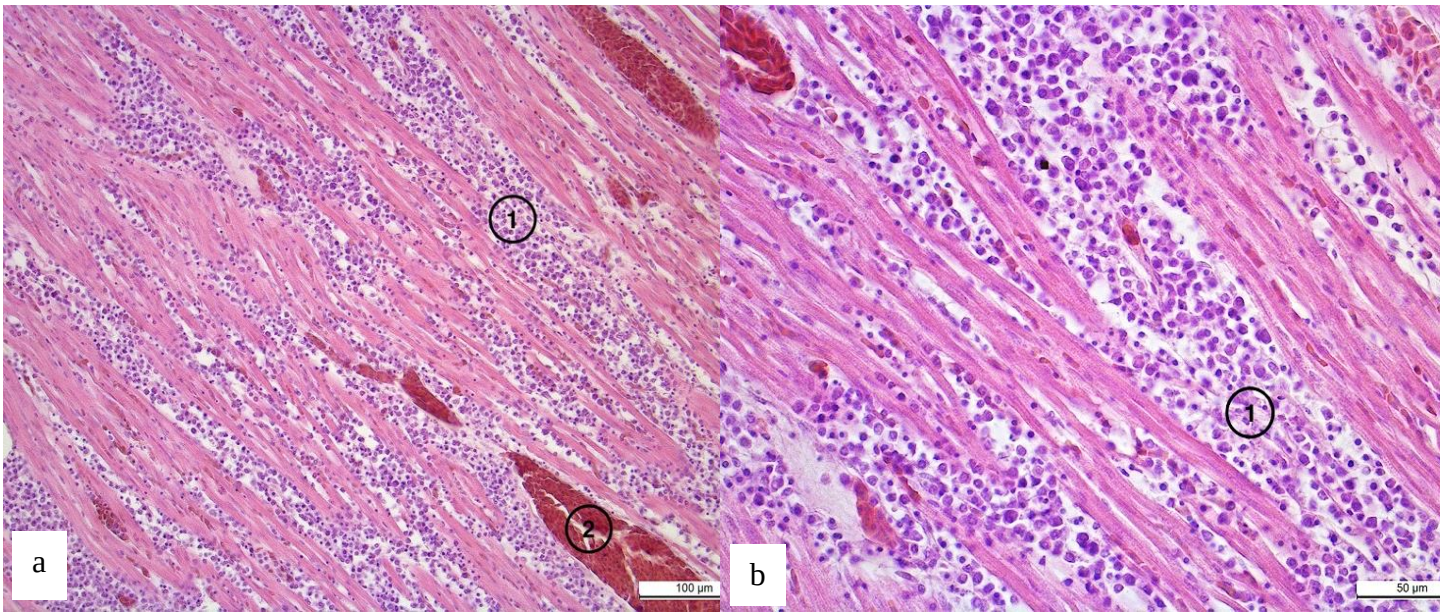

Fig. 9A. The heart. Diffuse proliferation of lymphoblasts between cardiomyocytes (1). Hemorrhage (2). Hematoxylin and eosin, x200. B. The heart. Cardiomyocyte dystrophy, infiltration by polymorphic lymphoblastic cells (1). Hematoxylin and eosin, x400.

Replication of the virus in the laying hens' bursal sac and thymus caused dystrophic changes in these organs. Lymphoid follicles of the bursal sac were reduced in size, atrophied, and somewhere replaced by cystic cavities of various sizes (Fig. 10a). In most lymphoid follicles, exposure of the reticular skeleton and necrosis of lymphocytes of the cerebral layer were detected (Fig. 10b), accompanied by the expansion of interfollicular connective tissue with its focal infiltration by lymphoblasts.

Histological examination of the thymus showed different morphofunctional conditions of the organ. Some lobes were represented by reticuloepithelium and adipose tissue with the local placement of thymocytes. The others had pronounced cortical and cerebral substances, with a part of lymphocytes being necrotized, and the rest diffusely located between the lymph nodes. It should also be noted that the number of Hassall's corpuscles was unsubstantial.

Swelling and disintegration of Schwann cells, demyelination and focal infiltration of endoneurium by lymphoid cells were pronounced in the sciatic nerve. Cellular infiltrates were mostly represented by the large lymphoblasts (Fig. 11b).

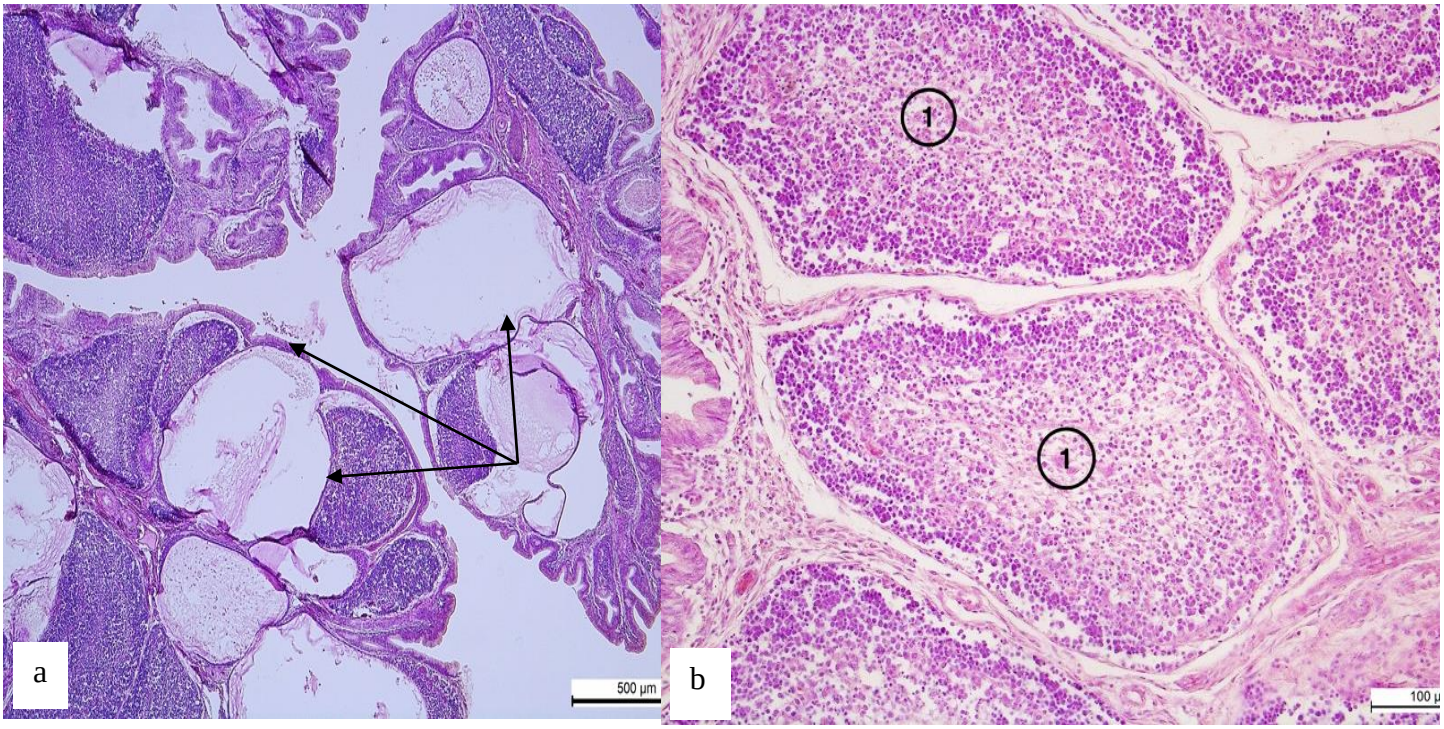

Fig. 10A. The bursal sac. Multiple cystic formations (arrows). Hematoxylin and eosin, x50. B. The bursal sac. Delimphatization of the cerebral layer of lymphoid follicles (1). Hematoxylin and eosin, x200.

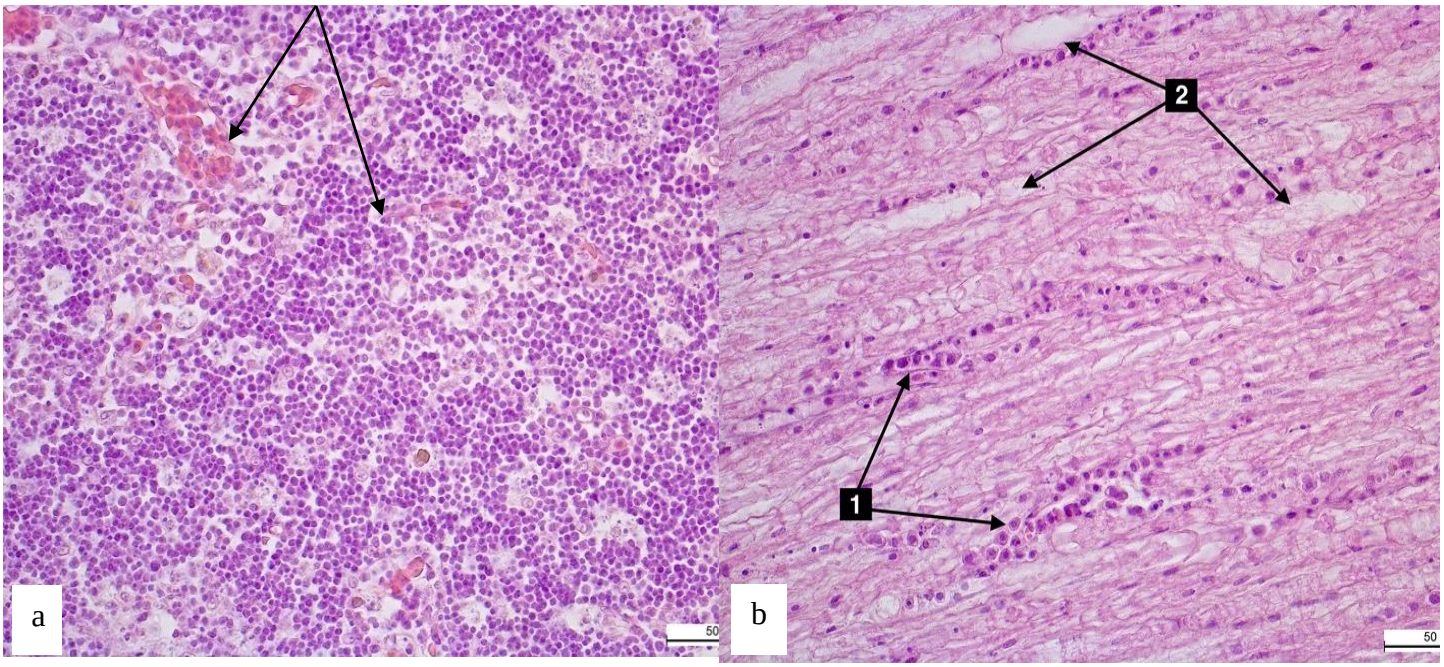

Fig. 11A. The thymus. Delymphatization of the cerebral layer. Hematoxylin and eosin, x400. B. The lumbar sacral nerve. Infiltration by polymorphic lymphoblastic cells into the nerve tissue (1), defibering and edema of the endoneurium (2). Hematoxylin and eosin, x400.

Lymphocytic infiltration in the red pulp was detected on histopreparations of the spleen (Fig. 12a). The germinal centers of the lymphoid nodules of the white pulp were necrotized (Fig. 12b).

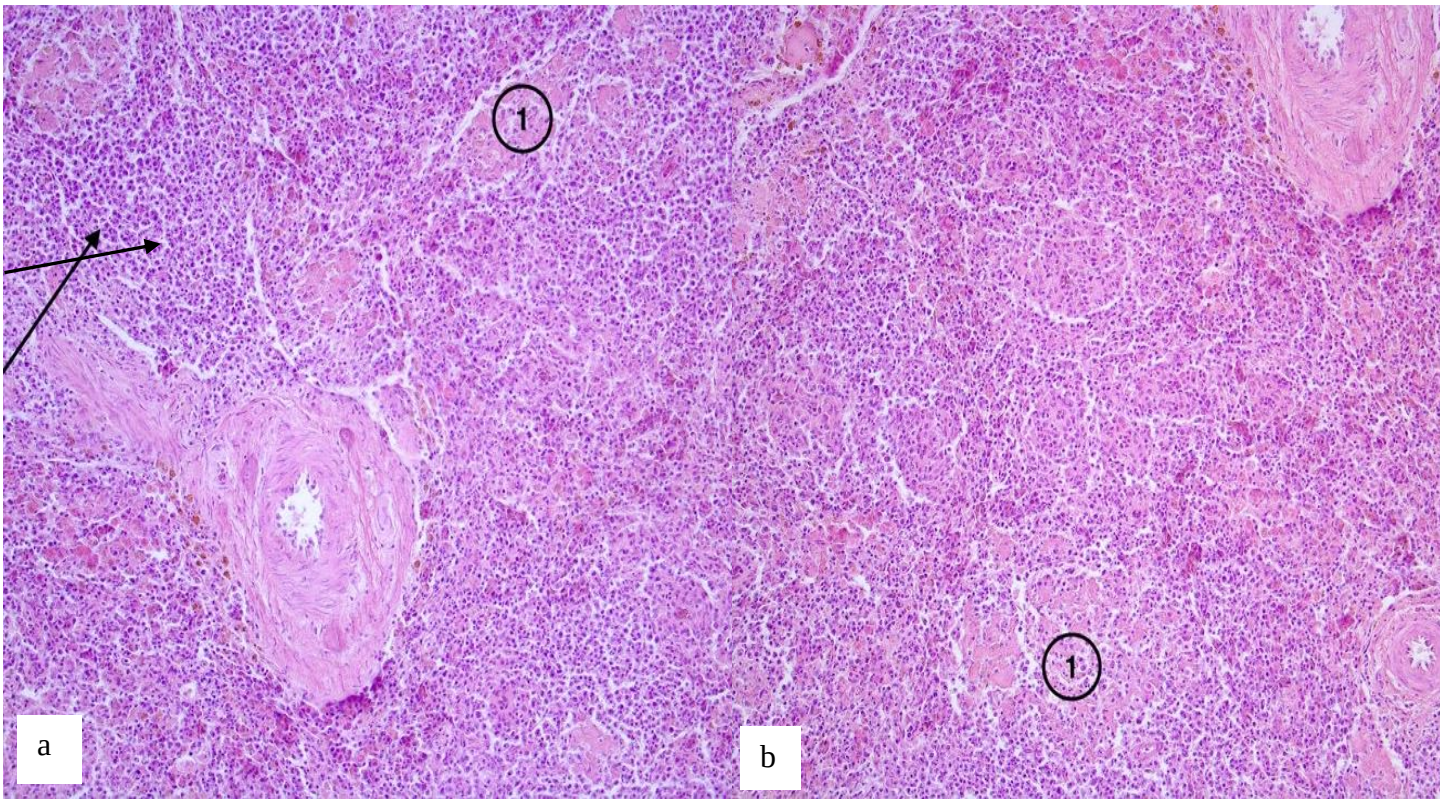

Fig. 12A. The spleen. Diffuse infiltration by lymphoblastic cells in perivascular couplings (arrow). Necrosis of the reactive centers of lymphoid nodules (1). Hematoxylin and eosin, x200. B. The spleen. Necrosis of lymphoid nodules (1). Hematoxylin and eosin, x200.

Diffuse lymphoblastic infiltration was visualized in the lungs, especially in the plate of the parabronchi, second-order bronchi, and ectobronchi (Fig. 13a). The walls of blood vessels were dilated, and the capillaries were full of blood. Severe dyscirculatory disorders were accompanied by increased vascular permeability and permeation of the stroma with fluid. Perivascular lymphoblastic infiltration was also available (Fig. 13b).

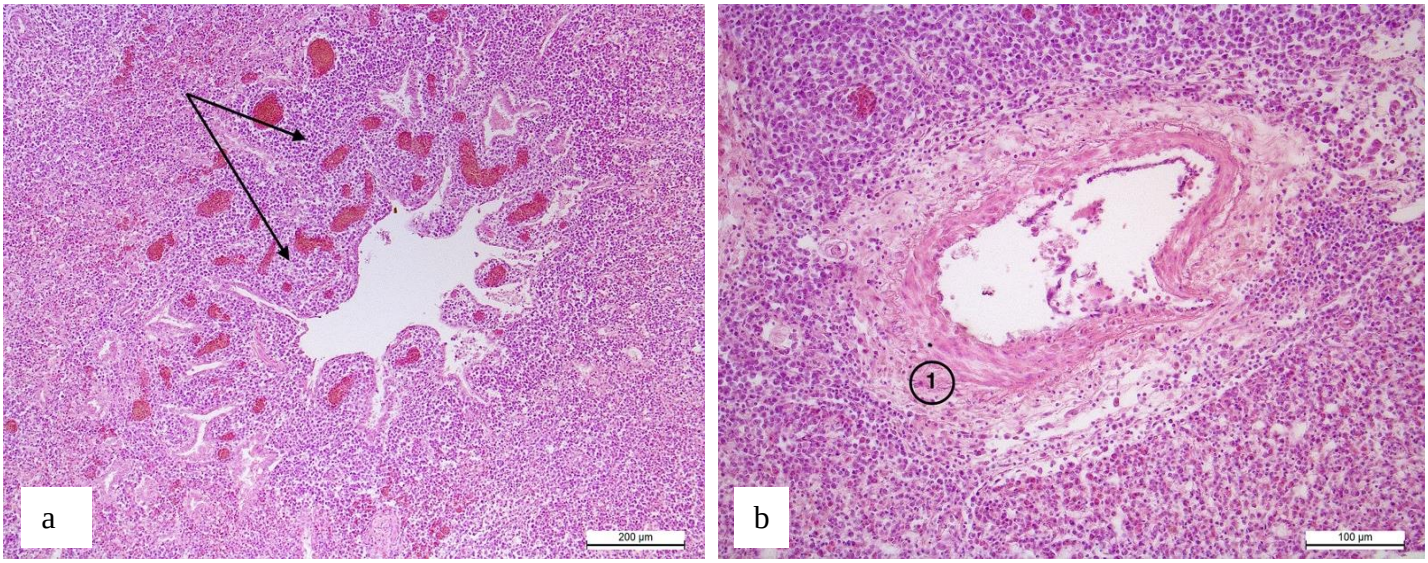

Fig. 13A. The lungs. Massive cellular infiltration into the parabronchial plate (arrows). Hemocapillaries and venous vessels are full of blood. Hematoxylin and eosin, x100. B. Perivascular edema (1), massive perivascular infiltration by lymphoblasts. Hematoxylin and eosin, x200.

Focal infiltration and proliferation by small, medium, and large lymphoblasts were detected both in the mucous membrane of the glandular stomach and between the glands of the submucosal base (Fig. 14a, 14b). There were hemorrhages, desquamation, and focal necrosis of the glandular epithelium in the mucous membrane.

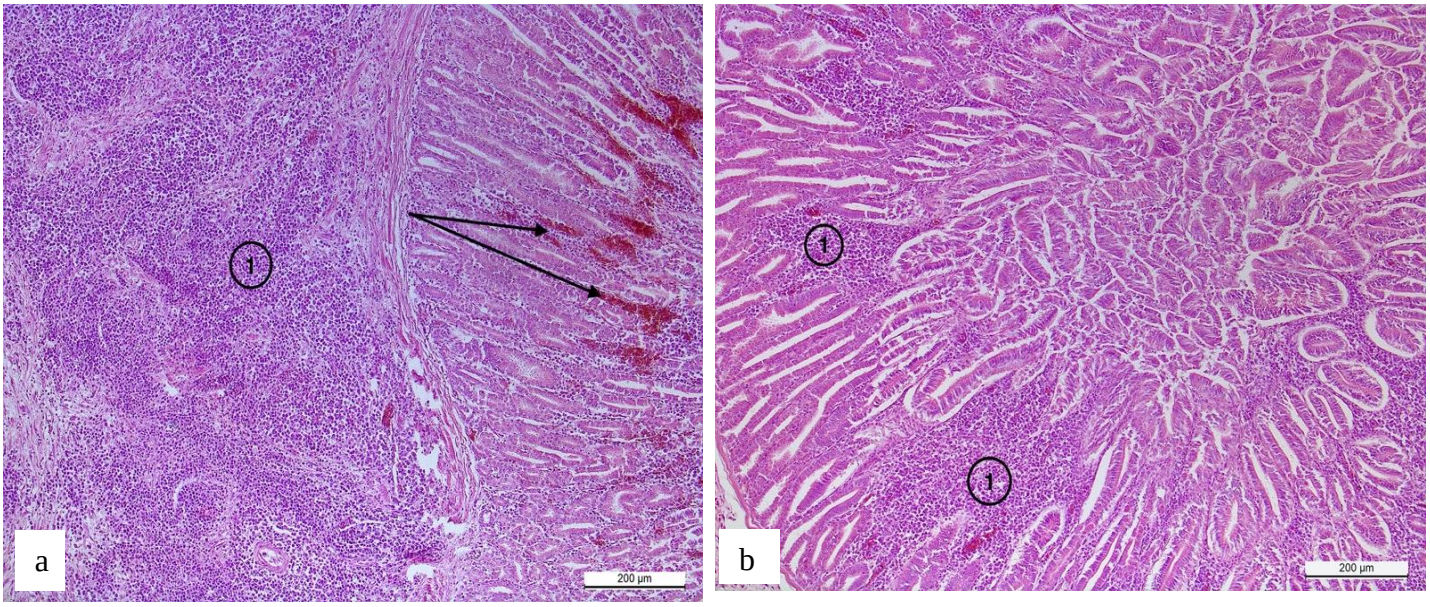

Fig. 14A. The glandular stomach. Massive diffuse infiltration by polymorphic lymphoblastic cells (1), hemorrhage into the mucous membrane (arrows). Hematoxylin and eosin, x100. B. The glandular stomach. Focal lymphoblastic infiltrationof glands under the mucous membrane (1). Hematoxylin and eosin, x100.

Conclusion

The histopathological examination of the acute form of neurolymphomatosis revealed a systemic lesion of all parenchymal organs with massive infiltration and proliferation of pleomorphic lymphoid cells. In the liver and kidneys, infiltration and proliferation of lymphoid cells with significant damage to the parenchyma of organs, as well as hemorrhage, were observed. Focal dystrophic and necrobiotic lesions with massive infiltration of the large, medium, and small lymphoblasts were detected in the heart. Changes in the spleen were characterized by necrosis of the reactive centers of lymphoid nodules, cellular pleomorphism of the red pulp, and intense perivascular proliferation of small lymphoblasts.

References

Baaten, B. J., Butter, C., Davison, T. F. (2004). Study of host-pathogen interactions to identify sustainable vaccine strategies to Marek's disease. Vet Immunol Immunopathol. 100 (3-4),165-77. Doi,10.1016/j.vetimm.2004.04.009.

Biggs, P. M. (2001). The History and Biology of Marek's Disease Virus. In, Hirai K. (eds) Marek's Disease. Current Topics in Microbiology and Immunology, Vol. 255. Springer, Berlin, Heidelberg. Doi, 10.1007/978-3-642-56863-3_1.

Biggs, P. M., Powell, D. G., Churchill, A. E. & Chubb, R. C. (1972). The epizootiology of Marek's disease, I. Incidence of antibody, Viraemia and Marek's disease in six flocks, Avian Pathology, 1,1, 5-25.
Doi, 10.1080/03079457208418048.
Calnek, B. W. (2001). Pathogenesis of Marek's Disease Virus Infection. In, Hirai K. (eds) Marek's Disease. Current Topics in Microbiology and Immunology, vol 255. Springer, Berlin, Heidelberg. Doi, 10.1007/978-3-642-56863-3_2.
Calnek, B. W. & Richard, L. Witter (1985). Marek's Disease - A Model for Herpesvirus Oncology, CRC Critical Reviews in Microbiology, 12,4, 293-320. Doi, 10.3109/10408418509104432.
Cui, X., Lee, L. F., Hunt, H. D., Reed, W. M., Lupiani, B., Reddy, S. M. (2005). A Marek's disease virus vil-8 deletion mutant has attenuated virulence and confers protection against challenge with a very virulent plus strain. Avian Dis. 49, 199–206. Doi,10.3390/vaccines9020159.
Davison, F., Nair, V. (2005). Use of Marek's disease vaccines, could they be driving the virus to increasing virulence? Expert Rev Vaccines. 4(1),77-88. doi,10.1586/14760584.4.1.77.
Frank, F. (2001). Marek's disease, History, actual and future perspectives. Lohmann Inform, 25,1-5.
Kezawa, M., Goryo, M., Sasaki, J., Haridy, M. and Okada, K. (2010). Late Marek's Disease in Adult Chickens Inoculated with Virulent Marek's Disease Virus. J. Vet. Med. Sci. 72(12),1539–1545. Doi.10.1292/jvms.10-0203.
Kiceli, D. (1962). Prakticheckaja mikpotehnika i gictohimija. Budapesht (in Russian).
Lawn, A. M., Watson, J. S. (1982). Ultrastructure of the central nervous system in Marek's disease and the effect of route of infection on lesion incidence in the central nervous system. Avian Pathol. 11(2),213-25. Doi, 10.1080/03079458208436095.
Merkulov, G. A. (1969). Kurc patologicheckoj tehniki. L. (in Russian).
Nazerian, K., Solomon, J. J., Witter, R. L., Burmester, B. R. (1968). Studies on the Etiology of Marek's Disease. II. Finding of a Herpesvirus in Cell Culture. Proceedings of the Society for Experimental Biology and Medicine. 127(1),177-182. Doi,10.3181/00379727-127-32650.
Osterrieder, N., Kamil, J., Schumacher, D. et al. (2006). Marek's disease virus, from miasma to model. Nat Rev Microbiol 4, 283–294. Doi, 10.1038/nrmicro1382.
Othman, I., Aklilu, E. (2019). Marek's disease herpesvirus serotype 1 in broiler breeder and layer chickens in Malaysia. Veterinary World, 12 (3), 472-476. Doi,10.14202/vetworld.2019.472-476.
Payne, L. N., Biggs, P. M. (1967). Studies onMarek's disease. II. Pathogenesis. J Natl Cancer Inst. 39 (2),281-302. PMID, 18623945.
Payne, L. N., Venugopal, K. (2010). Neoplastic diseases, Marek's disease, avian leukosis and reticuloendotheliosis. Rev. Sci. Tech., 19 (2), 544-564.
Pejović, N., Velhner Maja, Polaček, V., Aleksić-Kovačević Sanja, Marinković, D., Knežević Milijana. (2007). Morphological and immunohistochemical examination of tumor cells in Marek's disease. Acta veterinaria. Volume 57, Issue 1, Pages, 27-35. Doi,10.2298/AVB0701027P.
Peter, M. Biggs & Venugopal Nair (2012). The long view, 40 years of Marek's disease research and Avian Pathology, Avian Pathology, 41,1, 3-9.
Simu, G., Ciobanu, H., Ciobanu-Bîlc, A. (1980). Histological aspects of lymphoid organs in chickens with Marek's disease. Morphol Embryol (Bucur). 26 (4),345-7. PMID, 6450886.
Stamilla, A. (2020). Morphological and Immunohistochemical Examination of Lymphoproliferative Lesions Caused by Marek's Disease Virus in Breeder Chickens, Animals, 10 (8), 1280. Doi, 10.3390/ani10081280.
Suma, U. S., Rahman, M. W., Nooruzzaman, M., Chowdhury, E. H. and Islam, M. R. (2017). Pathology of Marek's disease in layer chickens in Bangladesh. The Bangladesh Veterinarian, 34 (2), 35– 41.
Witter, R. (1997). Increased Virulence of Marek's Disease Virus Field Isolates. Avian Diseases, 41(1), 149-163. Doi, 10.2307/1592455.
Witter, R. L, Calnek, B. W., Buscaglia, C., Gimeno, I. M., Schat, K. A. (2005). Classification of Marek's disease viruses according to pathotype, philosophy and methodology. Avian Pathol 34, 75-90.
Witter, R. L. (2001). Protective efficacy of Marek's disease vaccines. In, Hirai K (ed) Current topics in microbiology and immunology. Springer, Berlin, pp. 58-90.
Witter, R., Sharma, J., & Fadly, A. (1980). Pathogenicity of Variant Marek's Disease Virus Isolants in Vaccinated and Unvaccinated Chickens. Avian Diseases, 24 (1), 210-232. Doi,10.2307/1589781.

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