



## Pathomorphological Characteristics of Listeriosis in Calves Suckling Period

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### Annotation

In this article listed results of pathological studies of listeriosis in calves suckling period. It was found that the changes are mainly characterized by hyperemia and edema of the meninges, parenchymal liver disease and infarction, acute catarrhal enteritis, acute catarrhal abomazitom, acute venous hyperemia of the kidneys, the presence of hemorrhages in various organs, inflammation of the mesenteric lymph nodes.

**Keywords:** Listeriosis; Meningoencephalitis; Redness; Granuloma; Desquamation; Hemorrhage; Degeneration; Necrosis.

### Introduction.

Listeriosis - acute natural focal zoonotic infectious disease of many species of animals and birds. History of the study listeriosis infection has more than a hundred years. It is common in many countries almost in all continents. Medical and socio-economic importance of this disease is increasing in recent years.

Annual registration of morbidity in humans and animals, food listeriosis outbreaks, accompanied by a high fatality rate, make listeriosis urgent medical and veterinary problem. [1, 2, 3].

**Goal.** Explore the pathological changes in the body listeriosiscalves in suckling period.

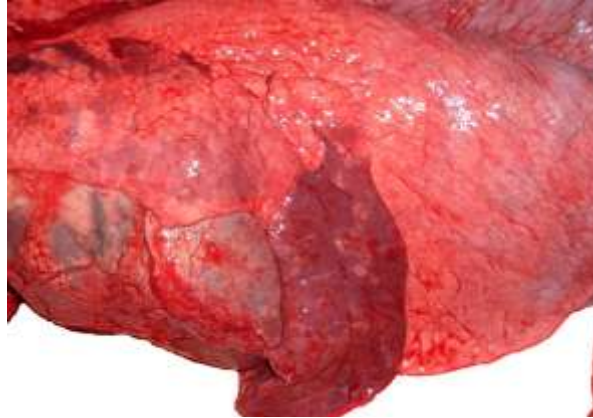
### Materials and methods.

The studies were conducted in 2015-2016 at the Department of Biosafety of the Kazakh National Agrarian University. The material for our research served as the pathological material, taken from 27 calves which died from listeriosis in farms Talgar, Ili, Balkhash Enbekshikazakh and districts of Almaty region of Kazakhstan. All calves were subjected to a detailed postmortem examination with detailed logging of each case. For histological examination of the pieces were taken from the spinal cord, and various parts of the brain, liver, kidney, heart, lung, lymph nodes, spleen, thymus, gastro-intestinal tract. Fixing of pathological material was carried out in 10% neutral formalin and Carnoy's fluid. Paraffin and frozen sections with thick 5-10 $\mu$  stained with hematoxylin-eosin, Van Gieson, azure-eosin. To determine the deoxyribonucleic acid used Feulgen method ribonucleoproteins - method of Brachet, glycogen and neutral glucose and glycan- Schiff-perio

dic acid (PAS) reaction (according to McManus). To detect Listeria in histological preparations used Levatidi method. For microstructures measurement used ocular micrometer. The histological preparations were examined under a microscope KARL ZEISS and photographed with a digital camera.

### Results and discussion.

Nearly in all calves superficial lymph nodes (submandibular, front blade, inguinal) were able serous inflammation. They were increased in size, red color, lighter consistency, cut surface was wet. Lungs were swollen, enlarged, light red, doughy consistency. On the cut surface was dripping foamy pale red liquid. Foams, light liquid were in the large bronchi and trachea in the cavity. Light pieces hard floated in water (Figure 1).



**Figure 1. Pulmonary edema, in calf's 2<sup>nd</sup> to 15<sup>th</sup> day..**

**In all cases, in epicardium calves showed dotted hemorrhages (Figure 2).**



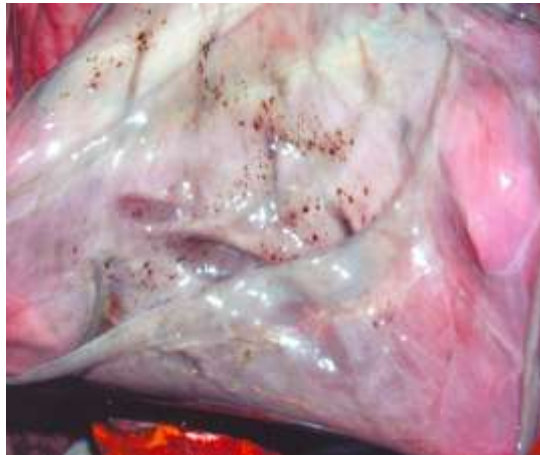
**Figure 2. Point hemorrhage on epicardium, in calf's 5<sup>th</sup> day..**

Spotty hemorrhage marked in endocarditis, exactly in the field of clamshell and tricuspid valves. The myocardium is grayish-brown, loose consistency (**Figure 3**).



**Figure 3. Spotted hemorrhage in 3 flap valve, in calf's 5<sup>th</sup> day..**

In the lumen of the abomasum was turbid whitish-gray mass with mucus, the mucous membrane was swollen, grayish. In the mucosa of the abomasum and intestines revealed hemorrhage, ulcers and erosions (**Figure 4**).



**Figure 4. Bluetongue ulcerative abomazit, in calf s 5<sup>th</sup> day.**

The mesenteric lymph nodes were swollen, reddened, drawing on the section was smoothed, the cut surface was wet (**Figure 5**).



**Figure 5. A hemorrhagic enteritis and serosanguineous lymphadenitis mesenteric lymph nodes**

Parenchymal organs (liver, kidneys, myocardium) has been increased in size, uneven color, loose texture, pattern on the cut smoothed (**Figure 6**).



**Figure 6. Parenchymal liver dystrophy, in calf s 3<sup>rd</sup> day.**

In some cases in the bodies we observed changes characteristic of venous congestion (enlarged organs, dark red color, soft texture, with cut surface liberally was dripping blood).

Under the liver capsule and the body section marked clearly defined, dense foci of necrosis. They were the size of 0.2-0.7 cm, yellow, drawing on the cut homogeneous, non-structured (**Figure 7**).



**Figure 7. Foci of necrosis in the liver, in calf's 15<sup>th</sup> day.**

The spleen was normal size or several reduced (**Figure 8**). In some cases it has been increased, softened.



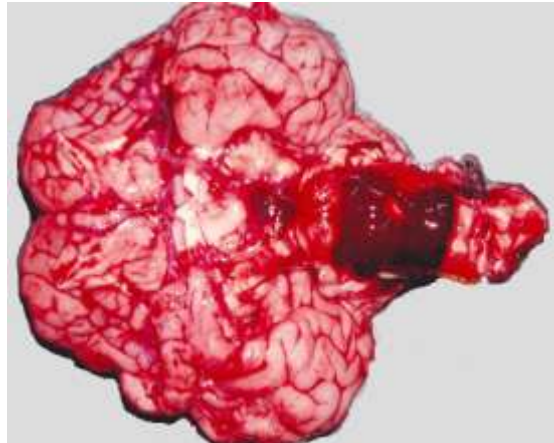
**Figure 8. Atrophy of the spleen, in calf's 5<sup>th</sup> day.**

The vessels of the pia mater were congested, marked hemorrhage, edema. Similar changes were observed in the brain: bleeding and accumulation of edema fluid, marked congestion, injection of the veins of the pia mater, redness. (**Figure 9**). Hemorrhages were observed in a large hematoma (**Figure 10**). The same changes were subjected matter of the brain. More severe changes in the soft shell and brain substance noted in the brain stem.



**Figure 9. The injection of the brain vessels, in calf's 5<sup>th</sup> day.**





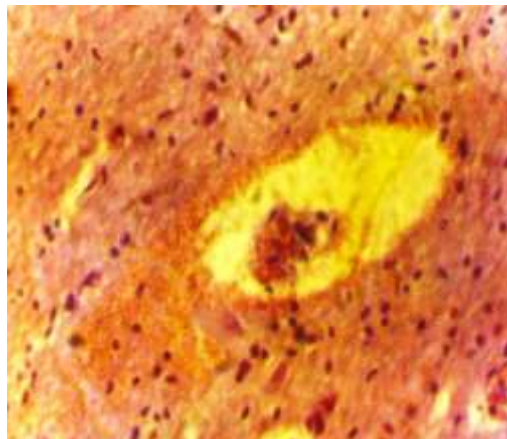
**Figure 10.**The hematoma at monthly calf, under the pia mater of the medulla oblongata.

**Histopathological changes:**

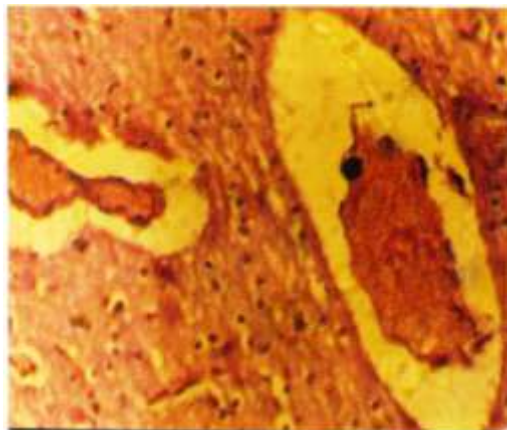
Brain. In the dead calves with nervous symptoms mentioned acute inflammation of the brain and head pia membrane irritation (encephalitis, meningitis). The severity of inflammation was different in calves and they were located primarily in the brain stem. Changes found in the medulla oblongata, pons, midbrain, thalami, cerebellum, and some calves in the cerebral hemispheres. However, we are convinced that constant inflammation exposed medulla and pons.

In the cerebral hemispheres and the horns of Ammon blood vessels were congested, noted small hemorrhages, per cellular and per vascular edema, glial cell proliferation (Figures 11, 12, 13).

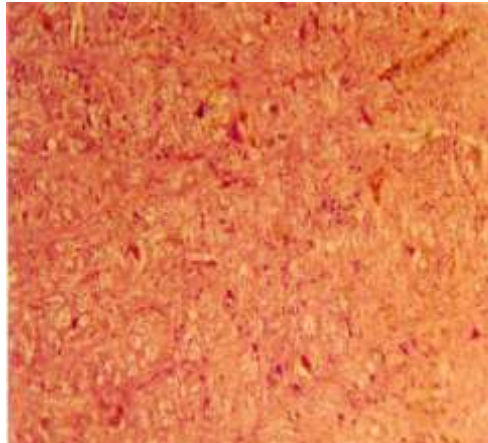
Corporaquadrigemina. There were congested of the blood vessels, perivascular proliferation of adventitial cells.



**Figure 11. Large Hemisphere: diapedetic hemorrhage. Hematoxylin-eosin X600.**



**Figure 12. Large Hemisphere: the accumulation of edema fluid around the blood vessels. Hematoxylin-eosin X600.**

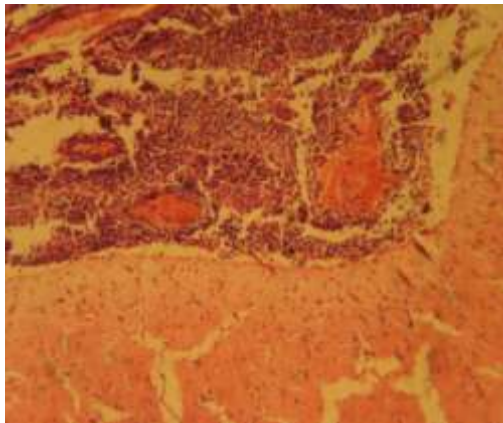


**Figure 13.**In the horns of Ammon the proliferation of glial cells.Hematoxylin and eosin x 120.

Some nerve cells were swollen, their outlines blurred, nucleus barely visible. Number of glial cells increased.

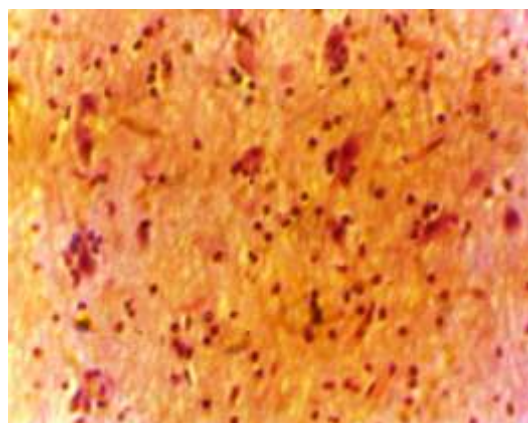
**The thalami changes were in quadrigemina.**

In the medulla and pons pia was saturated with serous fluid, lymphocytes, histiocytes and solitary segmented neutrophil leukocytes, the blood vessels were full-blooded, thickened to 5 - 319 times (16.5 - 1052.8 mm) (**Figure 14**).

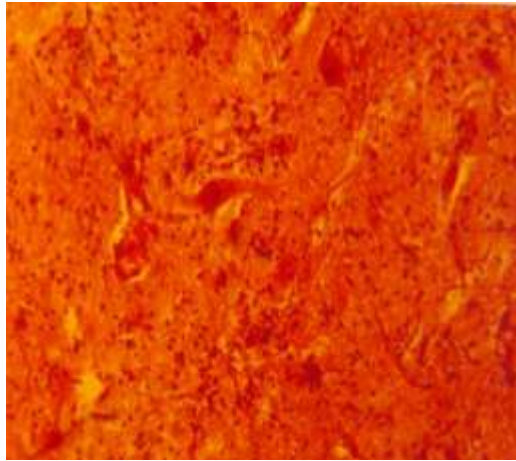


**Figure 14.** The elongated brain of calf:acute leptomeningitis. Hematoxylin-eosin x 150.

The borders with the fourth ventricle of the brain medulla pointed very strongly expressed by inflammatory processes, necrosis and leukocyte-lymphocyte cell clusters of varying size, neuronal damage (chromatolysis, acute swelling, vacuolization, pycnosis, education-shadow cells, etc.) congestion of blood vessels, diapedetic hemorrhage, cellular infiltration around the small blood vessels, glial cell proliferation, accumulation of edema fluid around the glial and neuronal cells (perivascular, pericellular edema). Damaged neurons are surrounded by glial cells, sometimes they replace neurons (true neuronophagia) (Figure 15, 16, 17).



**Figure 15.** The elongated calf brain: a phenomenon neuronophagia.Hematoxylin-eosin x300.



**Figure 16. Large Hemisphere: degeneration of neurons, glial cell proliferation. Hematoxylin-eosin x150.**

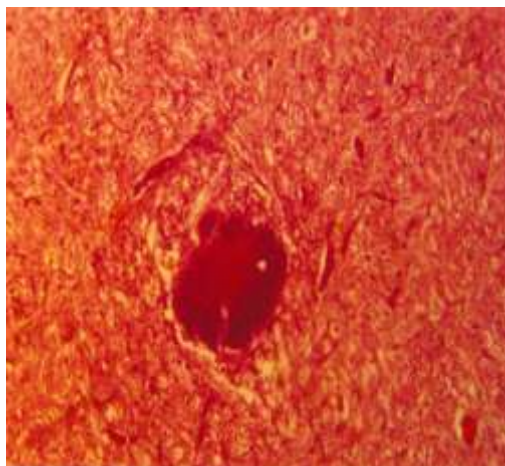


**Figure 17. Oblong brain: perivascular cell coupling. Hematoxylin-eosin x150**

In histo preparation stained by Nissl could see the collapse of tiger substance. The closer to the pia mater of the medulla oblongata of the changes we have not seen.

Directly under the ependyma of the fourth cerebral ventricle revealed edema and cellular accumulation of neutrophils and lymphocytes. It contains small-lymphocytic leukocyte accumulation and foci of necrosis about the size of 105 microns, the largest of them reach up to 1300 m (Figure 18).

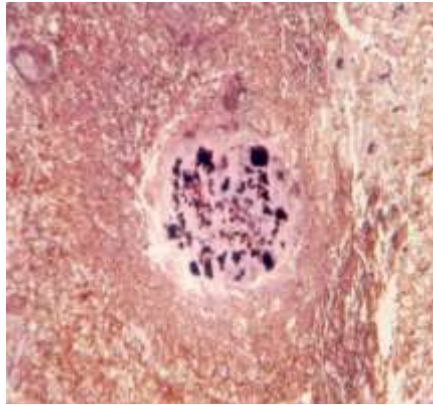
In stained with hematoxylin-eosin preparations in the past the center of the pink color seen dead weight with single nuclear debris, on the periphery - the area of nuclear debris, neutrophils and lymphocytes.



**Figure 18. Necrosis in the medulla of oblongata calf. Hematoxylin-eosin x 150.**



**The necrotic foci were found Listeria, painted black by Levatidi (Figure 19).**

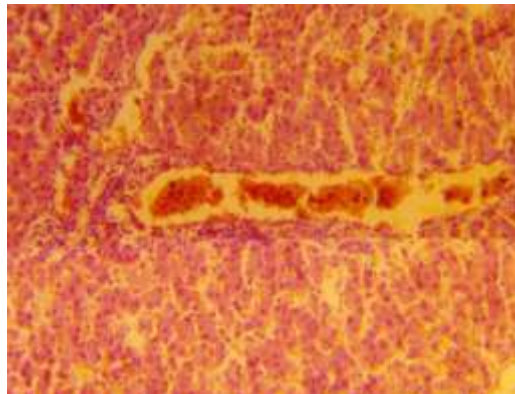


**Figure 19. Oblong brain: Listeria outbreak in necrosis. Painting by Levatidi x 150.**

In histopreparat stained with azure-eosin in necrotic foci also detected listeria.

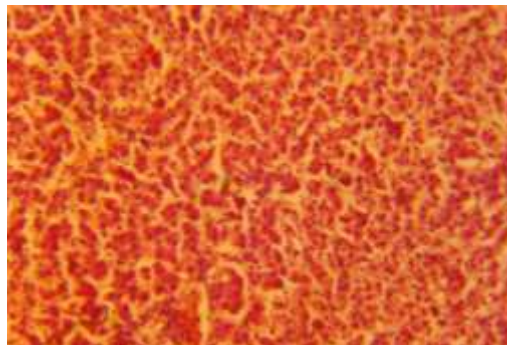
In many of the cerebellum Purkinje cells were swollen, their core wrinkled or slightly stained, congested blood vessels, marked hemorrhage, sometimes cell infiltration of histiocytes, lymphocytes and neutrophils. In the medulla, pons, cerebellum, and occasionally in other parts of the brain were observed edema and desquamation of endothelial cells of small blood vessels. So, in the brain died within 1-2 days, calves up to one month of age structural changes observed constantly, they are mainly localized in the brain stem and its soft shell. Stated in the brain changes were mainly characterized by acute lymphocytic encephalitis.

Liver. The structure of the hepatic beams broken, veins and capillaries congested, marked accumulation of edema fluid in the space of Disse (**Figure 20**).



**Figure 20. Flushing the liver blood vessels and red blood cells in the space of Disse.  
Hematoxylin and eosin x 150.**

Endothelial cells and Kupffer's cells were swollen, there was an increase in the sinusoids number of monocytes and neutrophils. Around some of the veins observed accumulation of histiocytes and lymphocytes. Many hepatocytes were able protein and fatty degeneration (**Figure 21**).



**Figure 22. Grain and fatty dystrophy of the liver. Hematoxylin and eosin x 150.**



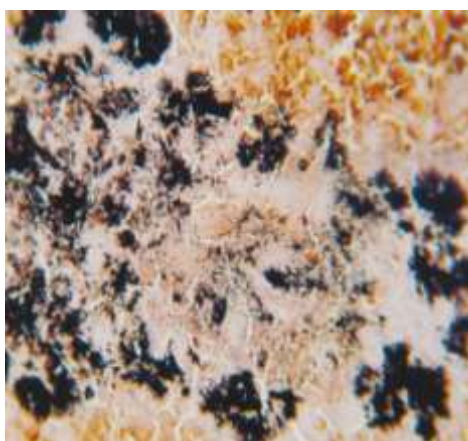
Protein hepatocyte degeneration leads to swelling of cells squeezing sinusoids. The nuclei of these cells are stained badly, becoming barely noticeable (karyolysis).

Some slices were exposed to necrosis, lost its normal structure. The boundaries were smoothed hepatocyte cytoplasm disintegrated into clumps oxyphilous colored, wrinkled kernel, broken up, dissolved, thus converted into a homogeneous structure less mass. Small dead areas cover almost half of the individual cloves - periportal their part and take a few larger lobules as a whole.

The amount of such foci ranged from 70 microns to 1200 microns. Necrosis was mostly exposed to the peripheral part of the lobes, the central part - is rare. The eosin stained preparations could be seen that the usual structure of the body in the necrotic foci smoothed. On the periphery of the pockets of the dead were a large number of nuclear fragments.

In histomorphology necrosis depending on the time of their occurrence was observed species, in the morphology of the "new" and "old" were some of the features of necrosis. Newly appeared necrotic lesions appear as a homogeneous, non-structured, eosin stained red mass of broken hepatocyte nuclei fragments, and subjected to rickettsia found there. Over time, the central necrosis deposited calcium salts (dystrophic calcification), and nuclear fragments stored only at their periphery.

If silvered thin slices of liver by Levatidi in the central part of the dead centers of detected black painted pathogen - *Listeria* (**Figure 23**).



**Figure 23. Liver 15 days calf: necrosis and disintegrated *Listeria*. Painted by Levatidi x 300.**

In histopreparat stained with azure-eosin *Listeria* can be seen in foci of necrosis and hepatic between beams.

In the liver, calves that have been clinically observed disorders of the nervous system of the state of endothelial cells of blood vessels and sinusoids, hepatocytes was largely similar to the parent description. Namely, hepatocytes were able granular dystrophy, they were increased in the cytoplasm could be seen oxyphilic colored fine grains sinusoid compressed between the beams, and interstitial central Vienna congested. Dead foci were not observed. Some hepatic lobules were awarded various sizes cell clusters are mainly composed of histiocytes and lymphocytes. Disse space has been expanded due to accumulation of edema fluid, swollen endothelial cells, been rejected, Kupffer cells are swollen, enlarged.

In histopreparat treated PAS-reaction was observed decrease in the amount of glycogen in the peripheral portion of the lobes; they were detected in the hepatocytes as small red beans. Along with this, in the painted techniques Brache and Feulgen preparations, we observed a decrease in the content in hepatocytes of RNA and DNA.

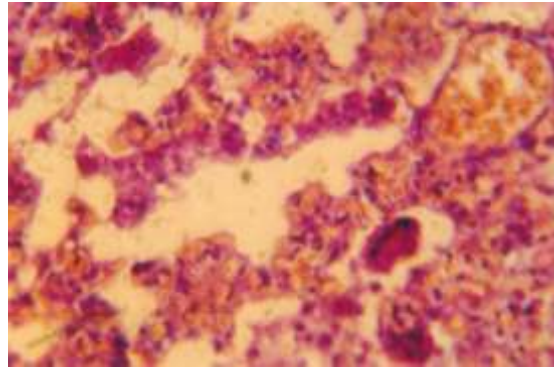
Thus, in the liver of patients develops nonspecific listeriosis calves fulminant hepatitis. Changes capture all the structural components of the liver, circulatory disorders develop and hepatocytes are exposed alterative processes. However, it should be noted that the severity of inflammation in the liver is not always the same character. Manifested in the form of septic and caused the death of calves listeriosis characterized by the severity of inflammation in the liver. *Listeria* has evolved from actions and their toxic products of protein and fatty degeneration of parenchymal cells necrosis passed, his small pockets located on the periphery of the lobules, their merger appeared more extensive, which occupied an entire slice or multiple slices. There were marked by clusters of *Listeria*. In calves, dead with obvious signs of brain damage, nonspecific reactive inflammation of the liver were observed foci of necrosis, and in different parts of the segments found accumulations of macrophages, lymphocytes, neutrophils isolated.

Small intestine. The small intestine is mainly observed changes characteristic catarrhal enteritis. Epithelial cells of the crypts and villi of the intestinal mucosa as a result of degeneration were rejected from the basement membrane. In its own layer of the mucous membrane was observed accumulation of serous fluid, congestion of the blood vessels. On the

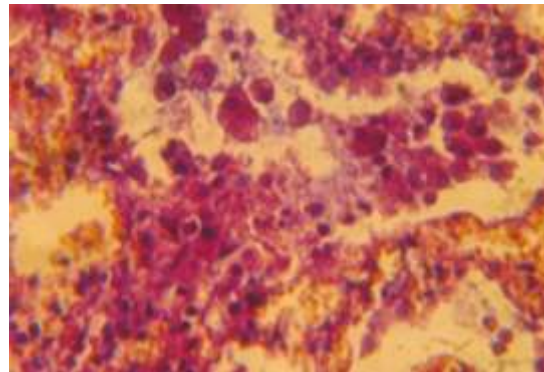
surface of the mucous membrane and preserved between the fibers by the sword exudates from serous fluid, mucus and desquamated epithelial cells, segmented leukocytes and lymphocytes. Adventitial cells of blood vessels and endothelial cells of capillaries were swollen, their number increased.

The colon is also noted changes characteristic catarrhal. The lamina propria was detected clusters of lymphocytes and histiocytes.

Lungs. The agency noted the productivity and exudative reaction changes characteristic of acute venous congestion are clear. Respiratory capillaries and small blood vessels were filled with blood in the alveoli contained edematous fluid around the blood vessels marked accumulation of neutrophils, histiocytes and lymphocytes (**Figure 24**). The walls of the alveoli were swollen, their lumen were lymphocytes, histiocytes and exfoliated ACs. The bronchi catarrhal exudate (**Figure 25**) was visible.



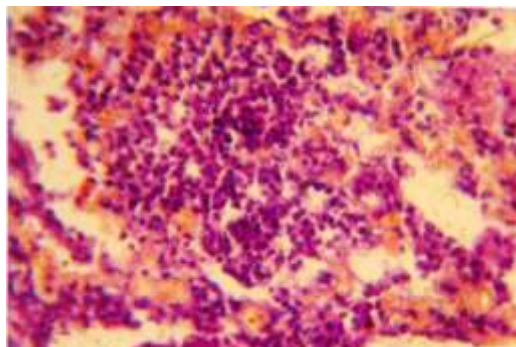
**Figure 24. Congestion pulmonary vessels. Hematoxylin-eosin  $\times$  400.**



**Figure 25. Catarrhal pneumonia. Hematoxylin-eosin  $\times$  400.**

Acute venous congestion in the lungs characterized by the following changes: due to the plethora of capillaries interalveolar walls were thickened, there has been accumulation of edema fluid in the cavity of some alveoli. The capillary lumina erythrocytes were arranged in 3 - 4 rows.

In addition, except for changes in the lung characteristic catarrhal pneumonia in the bronchioles and bronchi walls in the interalveolar septum in the blood vessel wall is small nodules consisting of macrophages, lymphocytes, leukocytes and single segmented (**Figure 26**).



**Figure 26. Histiocytic-lymphocytic granuloma in the lung of a calf.**

**Hematoxylin-eosin × 150**

In the central part of their cells are able to collapse and necrosis. The size of these foci was different. If the small ones occupied one alveoli and bronchioles, the larger covering several of the alveoli and bronchioles. Where there are some pockets of the body structure is not preserved. The fabric around the granulomas was saturated with edema fluid, and blood vessels are filled with blood.

Analyzing it may be noted that in the lungs to the blood circulation disorders joins secondarily evolved catarrhal, sometimes it took place, and purulent inflammation.

Spleen. As noted in calves that died from listeria in some cases the spleen increases in size. Microscopic examination found that the body increase is due to the plethora body. Endothelial cells plethoric venous sinuses swell.

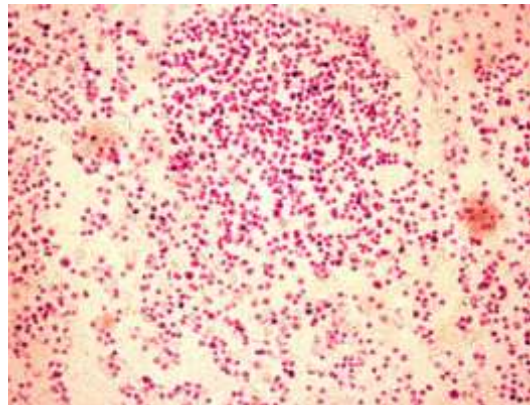
Lymphoid follicles were reduced their breeding centers have not been determined, endothelial wrinkled around the central artery of the number of cells has been reduced.

Some calves drawn the attention of the sharp decrease in the number of cells in the lymphoid follicles and red pulp.

Dilution of cells in lymphoid follicles observed not only in the central part, but in the marginal zone (Figure 27).

The lymph node changes were of two types. The follicles of the cortex and brain strands observed decrease in the number of lymphocytes. Sinuses expanded, there was observed the accumulation of serous fluid. Sinus macrophages were in varying degrees of degenerative changes.

**In some cases characteristic hyperplasia in the lymph nodes changes.**

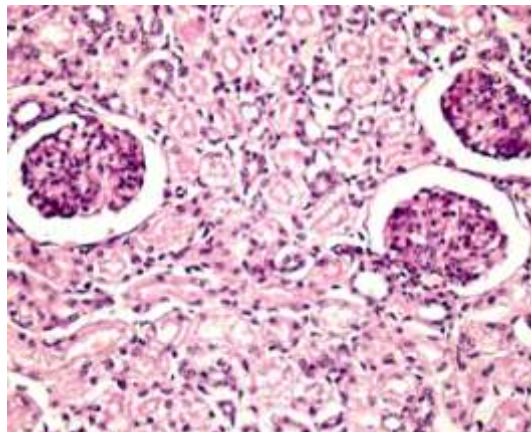


**Figure 27. Spleen: depression lymphocytes lymphohaliculitis,**

**Did not see breeding centers. Hematoxylin and eosin x 150.**

Thus, in the peripheral organs of the immune system suppressing changes indicate reaction of the immune system.

Kidneys. Epithelial cells of the convoluted tubules of the cortical layer were swollen, thickened wall of the tubular lumen of narrowed border between cells smoothed wrinkled nucleus in the cytoplasm were many small grains, eosin stained in red (Figure 28).



**Figure 28. Kidneys: granular dystrophy. Hematoxylin and eosin x 150.**

Interstitial capillaries and glomerular capillaries were filled with blood, interstitial tissue impregnated with edematous fluid. Edema fluid was in the lumen of the convoluted tubules.

Thus, the changes observed in the kidney that are typical granular dystrophy and acute venous congestion.

### **Conclusion.**

Some farms Talgar, Ili, Balkhash Enbekshikazakh and Almaty region of Kazakhstan regions ("Almaty", "Asyl" them. Konaeva "Zhanashar" and others.) in the cold season, observed disease of suckling calves period of listeriosis and their waste. The structural changes that have developed in the body of patients with listeriosis calves comply with clinical symptoms of a nervous or septic character.

Acute listeriosis encephalomyelitis manifested nervous symptoms, mainly develops in the medulla and pons calves: leptomeninges due to heavy congestion there lymphocytes, histiocytes, single segmented white blood cells thickens; in gray and white matter of the brain appearing necrotic foci, small clusters of lymphocytes, neutrophils and glial cells, perivascular cellular Clutch, neuronal damage, sometimes neuronophagia phenomenon. Macroscopic changes were limited congestion of the blood vessels of the pia mater, the advent of large-sized hematoma.

The calves livers, patients with listeriosis stated nonspecific reactive hepatitis. Manifested by external examination of parenchymal degeneration, this process is complicated by the emergence of pockets of various sizes dead in septic form.

The spleen and lymph nodes of the calves diseased listeriosis, marked atrophic changes, manifested vacuum lymphocytes in the follicles, smoothing breeding centers, decreased immune system function.

Picture autopsy died from listeriosis calves characterized by punctate and spotted bleeding on the endocardium and the epicardium under, on the mucous membrane of the digestive tract, acute catarrhal enteritis and abomasitis, signs of parenchymal liver disease, kidney and myocardium, lung congestion.

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