Pathomorphological changes in the cerebellum in rat chlamydial infection

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Abstract. Chlamydiosis of farm animals is a large group of diseases united etiologically. They differ in the nature of the course of the infectious process and the forms of its clinical manifestations. Often, chlamydiosis of pets shows neurological symptoms. Thus, the study of hemato-encephalic barrier passability by experimental chlamydiosis is of some interest. The final brain hemispheric tissues from rats experimentally infected with the chlamydiosis agent of the Laurie strain were chosen as the subject of the studies. The results of studies have shown that chlamydia disrupt the barrier functions of endothelium. Endothelial cells within blood vessels form a semipermeable barrier between the contents of the vessels and the tissues surrounding them. When examining the vascular channel in the control group of rats, it was clearly observed that endothelial cells fit closely together and interact poorly with cells circulating in the blood. The presence of chlamydia in capillary wall in rats infected with the agent results in endothelial cell hypertrophy. As a result, some of the endotheliocytes flow into the lumen of the vessels and break down and contribute to the generalization of infection. As a consequence of this process, changes in dyscirculatory character were observed primarily in gray and white matter and the soft cerebral membrane of the cerebellum with vascular channel damage. Consequently, the pathomorphological changes that occur in the brain substance in experimental chlamydia infection can be regarded as secondary ones that arise in response to circulatory disorders.

1. Introduction
Chlamydiosis infection affecting mammals and birds is caused by antigen-related microorganisms from Chlamydiaceae family [1]. A whole range of diseases are considered under chlamydiosis of animals. These diseases due to their polymorphism, cannot be combined by a specific symptom complex, and sometimes affect all systems and organs. Due to the lack of certain organotropy and host specificity among the various chlamydia members, the chlamydiosis clinic is extremely diverse. The pathological process in chlamydiial infection can be localized in many organs, thereby causing pathomorphological changes in various body structures [2]. Chlamydia refers to such diseases, in
which the passability of the hemato-encephalic barrier leads to degenerative changes of brain cells and accordingly to the development of neurological symptoms in animals [3]. Chlamydia disrupt the barrier functions of the endothelium, which within blood vessels forms a semipermeable barrier between the contents of the vessels and the tissues surrounding them. As a result of this process, some of the endothelial cells flow into the lumen of the vessels and break down, contribute to the generalization of infection in the body.

2. Equipment and devices used in studies
Research work was carried out on laboratory animals. Chl Psittaci, a strain of "Laurie" isolated from parrot in 1957, was used to infect rats; A description of the microorganism is given in the "Catalogue of Strains" 1962. As a subject of research to describe morphological signs we present cerebellum.

After the animals were sacrificed, the test material was fixed in 10% formalin. The next day the pieces were cut, followed by wiring on alcohols of increasing strength. For histological studies, the material was fixed in 4% formaldehyde solution and poured into paraffin. Histological sections were stained with hematoxylin and eosin, hematoxylin stains in blue-violet tones the shell of cell nuclei, chromatin. Eosin dyes in pink-red-orange tones of the cytoplasm and some structures (fibers), (review technique), and by Van Gison to determine the degree of expression of the scleroplastic processes. Sections up to 5 microns thick were made from the finished blocks on the slant microtome. The obtained preparations were studied with Zeiss microscope (Axioskop 40) with ocular magnification x10, with lenses x 4, x10.

3. The results of the study and their discussion
In microscopic examination of gray and white matter, the cerebellum observed sharp swelling and swelling of tissues [4]. The division of the cerebellum into grey and white matter persisted. In the grey material, the cerebellum clearly showed all three cell layers - molecular, gangliosis and grainy (figure 1). The most striking changes were noted in Purkinje cells. Individual cells that retained shape increased in size (figure 2). Cytoplasm of them stained weakly eosinophilic, inhomogeneously, nuclei had indistinguishable contours, they were often absent [5,6]. Dendric cell processes were poorly traced. Purkinje cell death processes were also recorded when the morphological structure of them could not be differentiated. Molecular layer cells have also undergone significant changes, with pericellular edema, cariopycnosis and destructive changes in the cytoplasm. In the cells of the grain layer there was observed colliquation of the cytoplasm, cariolyis and an increase in intercellular spaces. The neurocytes of all layers of grey cerebellum matter were further reduced in size, their nuclei were weakly stained, or absent. As a result of these changes, shadow cells surrounded by astroglial macrophages were formed. Astroglia elements performed both drainage and macrophagal function. On the one hand, their activities were aimed at resorption of excess fluid in the brain substance, on the other hand at licking dead neurocytes.

Perineural edema, as well as glial cell elements in dystrophy, were well observed in the grey cerebellum. In the soft sheath of the cerebellum there was also swelling, full blood of vessels, small centers of hemorrhages were detected [7]. Fibroblast clusters and excess fibrous structures were observed around blood vessels, and single large cells of macrophage species were also encountered. In the capillaries of the soft membrane of the cerebellum, such phenomena as full blood, stases, dissociation of blood into plasma and form elements were observed [8].

The vascular channel of gray and white brain matter, including capillaries, small arteries and veins, underwent morphological changes. The venous vessels had extended lumen. The wall of the veins was thinned, the gaps between the endothelial cells were increased, as a result of which it was not clearly traced (figure 3). Veins lumen were characterized by uneven blood filling, in them blood dissociation into plasma and form elements took place. As a result of the increase in interendothelial spaces, perivenous edema was rapidly formed. However, some veins were dormant.

Capillary channel was characterized by excessive blood filling, with stasis phenomena, without any clear morphological changes of vascular wall (figure 4).
Figure 1. Maintaining the structural organization of the cerebellum cortex against the background of pronounced edema. Color hematoxylin and eosinom. x 400.

Figure 2. Dystrophic changes in Purkinje cells and pericellular edema in the cerebral cortex. Color hematoxylin and eosinom. x 400.

Figure 3. Perivenous edema in the white-brain substance of the cerebellum. Colour with hematoxylin and eosin. x 400.

Figure 4. Increase the lumen of the vessel with separation of endotheliocytes in the white cerebellum. Color hematoxylin and eosin x 400.
The most distinct changes were also observed in the walls of small arteries. The endothelial layer was not expressed uniformly as a result of focal desquamation of endothelial cells, an increase in the volume of the nuclear-containing part, which strongly protruded into the lumen of the vessel. Phenomena of myocyte hypertrophy, edema with separation of cell elements were observed in the muscle shell. Edema extended to perivascular zones and was extensively traced in surrounding tissues. In the lumen of the arteries there was excessive blood filling, stases, formation of near small erythrocytic clots, dissociation of blood into plasma and form elements were observed. Some arteries had weak blood filling.

4. Conclusion
Thus, in gray and white matter and soft brain membrane of the cerebellum, changes of dyscirculatory character with damage of vascular channel were observed primarily. Pathomorphological changes arising in a brain substance in experimental chlamydia infection can be considered secondary, arising in response to circulatory disorders.

References
[1] Yu H, Lin H, Xi L et al. 2019 Chlamydia muridarum induces pathology in the female upper genital tract via distinct mechanisms Infection and Immunity 87(8) 00145
[2] Sereda T G, Tatarnikova N A 2019 Development of an automated system histology security of food production IOP Conference Series: Earth and Environmental Science 315 032003
[3] Zhang J, Wang H, Zhang L et al. 2014 Chlamydia pneumoniae infection induces vascular smooth muscle cell migration via Rac1 activation Journal of Medical Microbiology 63(2) 155-61
[4] Petyaev I M, Zigangirova N A, Chalyk R Y 2012 Chlamydial antigen and nucleic acid detection in liver biopsies from patients with chronic cholelithiasis Experimental and Clinical Hepatology 8(1-2) 45-52
[5] Cingolani G, McCauley M, Lobley A, Perilla J R, Paumet F 2019 Structural basis for the homotypic fusion of chlamydial inclusions by the SNARE-like protein IncA Nature Communications 10(1) 2747
[6] Motrich R D, Sanchez L, MacCioni M 2012 Male rat genital tract infection with Chlamydia muridarum has no significant consequence on male fertility Journal of Urology 187(5) 1911-17
[7] Markelova E V et al. 2016 Study of the system of cytokines in case of herpetic and chlamydia-herpetic infection Journal of Global Pharma Technology 8(9) 10–14
[8] Henning K, Reinhold P, Hotzel H, Moser I 2008 Outbreak of a Chlamydophila psittaci infection in laboratory rats Bulletin of the Veterinary Institute in Pulawy 52(3) 347–9