Pathomorphological changes in the testicles of goats in acute anaplasmosis

V Mihaylenko¹, O Dilekova¹, S Kartashov², V Meshcheryakov¹, V Shpygova¹, N Agarkov¹

¹Stavropol state agrarian University Stavropol, Zootechnical lane 12, 355017, Russia, ²Don state technical University, Gagarin Square 1, Rostov-on-don, 344000, Russia,

E-mail: viktor.mihaylenKO@yandex.ru

Abstract. The aim of the research was to study pathomorphological changes in the testicles of goats in acute anaplasmosis. Diagnosis of anaplasmosis in sick goats was carried out taking into account the characteristic clinical signs and studies of peripheral blood smears. Blood smears after fixation in alcohol ether were stained according to Romanovsky-Giemsa by hematoxilin and eosin. For histological examination, pieces of testicles and appendages of testicles of 0.5 cm thick were selected, which were fixed in an 8% neutral aqueous solution of formalin. The resulting material was poured into paraffin according to the generally accepted method. Histological sections of 5-8 microns thick were made from the obtained paraffin blocks and stained with hematoxilin and eosin using the method of Van Gieson and Mallory. Micro photos were made with a series of rendered images on the basis of Olimpus 2000. The study revealed the following changes: the spermatogenic epithelium has desquamation, in the lumen of the convoluted seminiferous, there were tubules protein detritus, exfoliated cells of seminiferous epithelium and macrophages. Sertolli cells were in a state of vacuole dystrophy and necrosis. In the interstitial tissue around arterial vessels and convoluted seminiferous tubules, focal accumulation of lymphoid-macrophage infiltration was found. Endocrine cells located in the interstitial were in a state of vacuole dystrophy and necrosis. Thus, in goats older than 6 months with acute anaplasmosis in the testicles, pathomorphological changes are characteristic of acute parenchymal orchitis.

1. Introduction
The development of goat breeding is one of the priorities in agriculture of the Stavropol region. There are pastures and climatic conditions for the development of dairy goats in the region. Currently, an important task of the industry is to reduce losses from death and reduce the productivity of animals. Anaplasmosis is one of the reasons for the death of goat-heads and the decrease in their reproductive capacity, especially newly imported from prosperous regions.

Anaplasmosis is a natural focal disease, because in the wild fauna there is a large number of susceptible animals, long-term parasitosis and the presence of ticks-carriers. Carriers of the causative agent of anaplasmosis are relapsing-fever ticks and ixodic ticks, mosquitoes, gnats, horseflies, flies [1].

According to many studies, ticks-carriers of anaplasmosis are widely distributed in many countries [2, 3, 4]. Stavropol region permanently affected with anaplasmosis of animals coincide with the areal of the tick Dermacentor marginatus.
With autoimmune orchitis, the quantity and quality of sperm decreases, sexual activity deteriorates [5, 6, 7]. According to our observations anaplasmosis disease adversely affects the sexual function of animals. Decrease in sexual activity in breeding rams in the autumn is one of the characteristic clinical signs of anaplasmosis.

In the literature available to us, there was no data on the study of pathomorphological changes in the testes of goats with anaplasmosis, which served as the purpose of our research.

2. Materials and methods of the research
Experimental studies were carried out in the period from 2010 to 2018 in farms Shpakovsky, Grachevsky, the Novoaleksandrovsky district of Stavropol region, at the Department of Parasitology and veterinary-sanitary expertise, anatomy and of pathological anatomy named after Professor S.N. Nikolskiy of the Federal State Budget Education Institution of Higher Education “Stavropol State Agrarian University”. Material for histological study were testicles pieces from the goats died during the acute period of anaplasmosis. Three dead goats of 6-7 months of age and six dead goats 1-3 years of age died from acute anaplasmosis flow were investigated. The material was taken not later than 3 hours after the death of the animal. The animals were mostly newly imported from better anaplasmosis regions. There is a massive parasite on sheep by ticks D.marginatus in Shpakovsky, Novoaleksandrovsky and Grachevsky districts of Stavropol region and as a result, these areas are affected by anaplasmosis. Diagnosis of anaplasmosis in sick goats was carried out taking into account the characteristic clinical signs and studies of peripheral blood smears taken from sick animals. Blood smears after fixation in alcohol ether were stained by Romanovsky-Giemsa method, by hematoxilin and eosin [8]. In each smear, 100 fields of vision were viewed under the immersion system of a biological microscope at a magnification of x1000.

To analyze the pathogenic impact of anaplasma on tissue of the testicles was performed postmortem of animals died from anaplasmosis. For histological examination pieces of testes and appendages of testes 0.5 cm thick from 5 places from each were selected. The obtained material was fixed in the fixer “Aldofix” for 3 days, then the resulting material was washed in running water for 24 hours. After washing - dehydrated in ethyl alcohol of increasing concentration (70%, 80%, 90%, 100%). After dehydration in alcohols, the pieces were poured into paraffin according to the generally accepted method. Impregnated with paraffin blocks were pasted on the wooden block and sledge microtome produced histological sections with a thickness of 5-8 microns. For the review study, the histological sections obtained were stained with hematoxilin and eosin [8]. Hematoxylin produced by the method of Ehrlich. To determine the connective tissue elements, the sections were colored by Van Gieson and Mallory methods. Micro photos were made with a series of rendered images on the basis of Olimpus 2000.

3. Results
Clinical signs of the disease in goats before death were characteristic of acute anaplasmosis. There was an increase in body temperature to 40.5-41.5 °C, severe oppression, refusal to feed, anemia of external mucous membranes, tachycardia. In the first day after the detection of the disease, the urine was transparent light yellow or yellow. 24 hours after the onset of the disease, the urine acquired a red-brown color. When blood was taken from a vein, it was watery and light red, and some animals had orange blood. There was a decrease in the number of erythrocytes and hemoglobin. Microscopy of stained peripheral blood smears, taken during life, observed quantitative and qualitative changes. Observed basophilic granularity of erythrocytes, anisocytosis, poikilocytosis. Erythrocytes appear with Cabo rings (figure 1), Jolly bodies and basophilic granularity.
An autopsy of goats died from acute anaplasmosis showed pathological changes similar to those for anaplasmosis of rams. Fatness of all animals was average or above average. In the study of external mucous membranes, anemia was noted (conjunctiva, oral mucosa, nasal cavities of white color with a barely noticeable pink tinge, the surface is smooth, moist). One of the eight studied dead bodies found cardiac dropsy, located in the region of the dewlap, limbs, and also in the mediastinum. In the area of the underbelly, lower abdomen, thoracic and pelvic limbs, subcutaneous tissue was impregnated with a gelatinous, transparent yellowish mass, from the surface of the incision a transparent liquid flowed. Blood in vessels was not coagulated, watery, light red, and at 4 goats the orange color. In the superficial and deep lymph nodes, there were changes characteristic to serous-hemorrhagic lymphadenitis. Atrophy of the red bone marrow, septic spleen, hemorrhagic diathesis were observed in all examined dead bodies. Dystrophic changes in the lungs and brain stagnant edema were noted in parenchymal organs. Pathological changes were characteristic of acute mucous-catarrhal gastroenteritis in the gastrointestinal tract. In the study of the testicles, no pronounced macroscopic changes were found. They had an oval shape, the parenchyma was an elastic texture of pink color with a grayish tinge, lobed structure was clearly expressed. Its weight in adult goats was about 180.0-200.0 g, which corresponds to the norm.

In the study of hysteresis of the testicles in all goats older than one year, the pathomorphological changes were similar and were detected in all of the convoluted seminiferous tubules. In arterial blood vessels, changes were characteristic of alternative arteritis. The endothelium of the arteries is partially desquamated. Connective tissue fibers of the artery wall are sometimes broken and homogenized. In the tissue surrounding the arterial vessels focal cell infiltrates consisting of macrophages and lymphocytes and fluid accumulation were seen (figure 2).
In addition, fluid accumulation and lymphoid-macrophage infiltrates were also detected between the convoluted seminal tubules, especially closer to the protein envelope. The endocrine cells located between the convoluted seminal tubules were in a state of vacuole dystrophy. Part of the endocrine cells were necrotic, in place its were visible only shadows and vacuoles filled with liquid (figure 3).

The main pathomorphological changes were found in the convoluted seminal tubules (in 100.0% of cases). Its lumen was visible with proteinaceous detritus, desquamated cells of seminiferous epithelium, macrophages, lymphocytes and isolated giant cells. The number of nuclei in giant cells did not exceed 4-5 pieces. In 11.5% of the convoluted seminiferous tubules, the spermatogenic epithelium was partially preserved. It consisted of 4-5 layers of cells (spermatogonia, spermatocytes of the first and second orders). In the lumen of these tubules were visible sperm. The spermatogenic epithelium was of uneven thickness in some places it grew thin to 2 layers of cells of spermatogonia and primary spermatocytes.

In goats aged 6-7 months, pathological changes also covered 100.0% of the convoluted seminal tubules. The spermatogenic epithelium in 70.5% of the tubules was desquamated until the spermatogonia (figure 4).
Protein detritus and single exfoliated cells of spermatogenic epithelium were visible in the lumen of the convoluted seminal tubules. In addition, macrophages were visible in the lumen of the tubules (figure 5) and isolated giant cells, exfoliated the spermatogenic epithelium. Sertoli cells in most part of convoluted seminal tubules were necrotic. Some Sertoli cells were enlarged in volume, vacuoles were visible in their cytoplasm. The spermatogenic epithelium of most convoluted seminiferous tubules was represented only by the basal cell layer and spermatogonia, while the spermatocytes of the first and second order were exfoliated. In 2.0% of the convoluted seminal tubules, the thickness of the spermatogenic epithelium was 4-5 layers of sperm cells, spermatocytes of the first and second order. In the lumen of these tubules, a single sperm was found.

Microscopy of histological sections of the appendages of the testicles in all studied goats in interstitial tissue revealed stagnant hyperemia of venous blood vessels. Arteries were poorly filled by blood. The wall of most arteries is thickened, connective tissue fibers are broken, homogenized in some places. The endothelium of arteries partially desquamated, in some areas showed focal proliferation of endothelial cells. In the interstitial tissue surrounding the arterial vessels focal cell infiltrates consisting of macrophages and lymphocytes and fluid accumulation were visible. Focal clusters of macrophages and lymphocytes were found between the tubules. The epithelium of the tubules was partially drained down to the basal membrane. In some areas its thickness was 3-4 layers of cells. In the lumen of the tubules were visible exfoliated epithelial cells (figure 6) and protein dendrite (figure...
7) and separate mature sperms.

Figure 6. Exfoliated epithelial of the epididymis of the testicle of a Zaanensky breed goat of 1.5 years old, when acute course of anaplasmosis. Stained with hematoxilin and eosin scale ×250

Figure 7. Protein detritus in the lumen of the tubules of the epididymis of the testicles of a Zaanensky breed goat of 1.5 years old, in case of acute course of anaplasmosis. Stained with hematoxilin and eosin, scale ×100.

4. Conclusion
Thus, in goats older than 6 months with acute anaplasmosis in the testicles, the pathomorphological changes were characteristic of acute parenchymal orchitis. Which was characterized by: desquamation of spermatogenic epithelium in the convoluted seminiferous tubules, phagocytosis by macrophages and giant cells of desquamated cells of spermatogenic epithelium.

Many authors [9, 10, 11] state that in violation of the hemato-testicular barrier, there is a possibility of developing autoimmune processes. Pathohistological changes in convoluted seminiferous tubules, autoimmune orchitis due to the peculiarities of the morphological structure of the testicles and the presence of hemato-testicular barrier. For autoimmune orchitis, regardless of pathogenic factors, it is characterized by a decrease in not only sexual activity but also the volume of ejaculate and the number of mature sperm in it. In addition, an increased number of pathological forms of sperm is found in the ejaculate. Histological examination is characterized exfoliated epithelial cells of convoluted seminal tubules. The presence in the lumen of the affected seminiferous tubules of macrophages and giant cells phaging the affected cells of spermatogenic epithelium was found. In interstitial tissue, there is focal cell infiltration by macrophages, lymphoid, plasma cells. Based on the results of our research, we can
conclude that in mature goats in acute anaplasmosis, the pathological changes are characteristic for autoimmune orchitis that developed as a result of damage of the hemato-testicular barrier.

References
[1] A. Torina, S. Caracappa, Clinical and diagnostic aspect Small ruminant research, 106. S6-S11 (2012) doi:10.1016/j.smallrumres.2012.04.026
[2] S.V. Bugmyrin, L.A. Bespyatova, Y.S. Korotkov, L.A. Burenkova, O.A. Belova, L.Iu Romanova, L.I. Kozlovskaya, G.G. Karganova, E.P. Ieshko, Ticks and Tick-borne Diseases 2013, 4, 57–62. (2013.) doi:10.1016/j.tibdis.2012.07.004
[3] XY. Liu, XY. Gong, C. Zheng, QY. Song, T. Chen, J. Wang, J. Zheng, HK. Deng, KY. Zheng, Acta Tropica, 167, 26-30 (2016) doi:10.1016/j.actatropica.2016.12.010
[4] H. Belkahia, M. Ben Said, N. El Mabrouk, C. Cherni, M. Ben Hassen, A. Bouattour, L. Messadi, Veterinary Microbiology, 208, 223-230 (2017) doi: 10.1016/j.vetmic.2017.08.004
[5] Bousmaha, F.; Khoudja, F. Benchaih, Bucks and Bulls of Algeria Asian Journal Of Animal And Veterinary Advances, 7, 10, 950-959 (2012) doi 10/3923|/ajava/2012/950/959
[6] HC. Schuppe, A. Pilatz, H. Hossain, A. Meinhardt, M. Bergmann, G. Haidl, W. Weidner, ResearcherID и ORCID UROLOGE, 49, 629-635 (2010) doi: 10.1007/s00120-010-2256-1
[7] CA. Silva, M. Cocuzza, JF. Carvalho, E. Bonfa, Autoimmunity Reviews, 13, 4-5, 431-434 (2014) doi: 10.1016/j.autrev.2014.01.024
[8] Staining of blood smears by using the erlich's hematoxylin and sodium eosin. russ. ru 2304776, 2007 Trukhachev V.I., Rodin V.V., Mikhailenko V.V., Dergunov A.A. Chemical Abstracts. 2007. Т. 147. С. 253438.
[9] M. Naito, Terayama, H. Hayato, , S Himai et al., Medical Molecular Morphology 45, 4, 185-189 (2012) DOI: 10.1007/s00795-012-0587-1
[10] C. Matschurat, K. Rode, J. Hollenbach, K. Wolf, C. Urhausen, A. Beineke, AR. Gunzel-Apel, R. Brehm, Histology And Histopathology, 34, 5, 525-535 (2019) doi: 10.14670/HH-18-058
[11] H. -C. Schuppe, A. Pilatz, H. Hossain, Meinhardt, A., M. Bergmann, G.Haidl, W. Weidner, Urologe, Vol. 49, is. 5 P. 629-635 (2010), doi: 10.1007/s00120-010-2256-1