Light optical and Ultrastructural Characteristics of the Maral Liver with Chronic Dicrocoeliasis

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BACKGROUND: Dicrocoeliasis is caused by trematode Dicrocelium lanceatum from the family Dicrocoeliidae, a parasite in the bile ducts of the liver of domestic and wild animals. Dicrocoeliasis mainly affects sheep, cattle, camels, zebu, deer, fallow deer, argali, less often – horses, donkeys, dogs, rabbits, hares and bears, as well as humans. Dicrocoeliasis of ruminants is widespread across the whole Kazakhstan. Invasive diseases represent a significant obstacle in the development of domestic maral breeding, among which trematodoses, and particularly dicrocoeliasis of maral play a major role.

AIM: The aim of the research was to study the influence of dicrocoelia on the ultrastructural organization of the liver of maral.

MATERIALS AND METHODS: For examination under electron microscope, biopsy pieces of liver tissue of maral were fixed in 2.5% solution of glutaraldehyde with post-fixation in 1% solution of osmium tetroxide, conducted according to a conventional method, and enclosed in epon. Semi-thin and ultra-thin sections were prepared on the ultra-microtome Leica. Semi-thin sections were stained with methylene blue, azure 2, and studied at a high-resolution light optical level. The ultra-thin sections were contrasted with uranyl acetate and lead citrate according to Reynolds method and examined under electron microscope Libra 120 (C. Zeiss).

RESULTS: The light optical examination of half-thin sections revealed that the morphological pattern of pathological changes developed in all the cellular structures of the hepatic lobules: In the form of plethora and vast enlargement of sinusoids, vacuolar and lipodegeneration of hepatocytes, destruction of the hepatic tissue with edema, hemorrhages, in the appearance of cells associated with inflammation, and the deposition of hematin crystals.

CONCLUSIONS: In the liver of maral infected with chronic dicrocoeliasis, dystrophic and destructive pathological changes developed in all the cellular structures of the hepatic lobules: In the form of plethora and vast enlargement of sinusoids, vacuolar and lipodegeneration of hepatocytes, destruction of the hepatic tissue with edema, hemorrhages, in the appearance of cells associated with inflammation, and the deposition of hematin crystals.

Introduction

Maral farming is a new, fast developing and high-income field of animal husbandry. It represents a high value for the economy of Kazakhstan. The production of velvet antlers has a strategic importance for the agro-industrial sector, socioeconomic status of the significant part of population in rural areas depends on export of the antlers products.

East Kazakhstan region is the main place of production and export of the products of velvet antlers deer farming in Kazakhstan. At present, there are 24 farms of different forms of ownership, which specialize in the maral farming. The climate and natural conditions of the region are favorable for breeding and farming of maral. The objective at this moment is to improve the current system of velvet antlers deer farming and to increase the production. To solve this problem, it is necessary for this field to reach an active development, that is, to improve the quality and the quantity of products while keeping a good control of the overall antler deer population. Part of this solution is to improve the conditions of keeping the animals and conditions of feeding but also it is important to provide an epizootic well-being of maral farms by protecting them from infectious and invasive diseases.

According to domestic researchers, outbreaks of invasive dicrocoeliasis are still present in different climatic zones of the country. Dicrocoeliasis is prevalent in the steppe, semi-desert and forest-steppe zone of the country, where it becomes a great burden to the livestock economy. Incidents of the infected livestock in southern regions of Kazakhstan reach 80%. Beginning of the pasture season and mid-summer is the season with the highest rates of infection. The disease begins to clinically manifest itself at an intensity of invasion (II) of more than 300 specimens in animals older than 1.5 years. As the animals age, the intensity of invasion increases, reaching more than 25 thousand specimens, with the extent of
invasion in sheep 80–100% and in cattle 40–98%. In the meantime, economic damage is caused by a decrease in the volume and quality of dairy and meat productivity, and also by the costs of medical and preventive measures. This is due to lack of efficiency and untimely therapeutic and preventive measures, which leads to an increase in the formation of trematode biotopes of intermediate and additional hosts [1], [2], [3].

Usenbaev et al., found a significant infestation with dicrocoeliidae of sheep – up to 78.6% and cattle – 45.0%. In this case, dicroela formed associations with other parasites inside the animals' body, consisting of two, three, four, or five subparts. Most of the times, there was a four-membered association with the following component composition: Fasciola + dicrocelia + stronygylata + Eimeria (33.3%) [4]. Kaspakbaev et al. reported that the invasion of farm animals with helminthiases in Kazakhstan is quite high, while official veterinary statistics does not correspond to the reality due to lack of control over anthelmintic activity, due to sharp decline in the production of livestock products and a reduction of the animal population [5]. Erbolat and Suleimenov, based on the results of research in seven oblasts of Kazakhstan, noted various mixed invasions in animals, including those that are formed from various ratios of Fasciola and dicrocelia. The authors also report that the diversity of helminths in farm animals and the predominance of individual helminth groups in associations with certain climatic and geographical zones of Kazakhstan make it extremely difficult to control them. The solution of the problem of helminthiases is complicated by the presence of the variety of climatic zones and subzones on the territory of Kazakhstan, by the diversity of groups and types of helminthic pathogens, by the lack of an arsenal of antihelminthic products, by the disaggregation of farms, and by the predominance of private property in the agricultural sector [6].

An important role in the evaluation of the epizootic situation of dicroceliasis and in the helminthological assessment of pastures belongs to data on the survival of eggs and larvae of dicrocelia in the external environment. Invasive elements of dicrocelia have a great survival rate in the external environment. Hence, when the moistened areas dry, the eggs of the dicrocelia retain viability for 10–12 months. They remain vial for a long time even at the temperature of 43–45°C. Dicrocoelium larvae retain the invasiveness in the body of intermediate and additional hosts for up to 3 years or more (Tverdokhlebov and Ayupov, 1980) [7]. In each climatic zone, the extent and intensity of the dicroceliasis invasion for each individual animal species varies significantly. The situation with dicroceliasis is poor in many areas of East Kazakhstan, where the infection of ruminants with dicrocelia sometimes reaches 100% with an invasive intensity of 30 thousand copies. Foothill regions, mountains, and steppe regions are especially at risk of dicroceliasis.

It was found that maral dicroceliasis invasions during the past 10 years were recorded more often – in 68.1 (2006) and 82.0% (2016) of animals [7], [8], [9].

Thus, the intensive spread of the disease is facilitated by the presence of a wide range of definitive, intermediate, and additional hosts of dicrocelia, and also by the stability of invasive elements in the external environment.

The main pathogenic effect of dicrocelia is concentrated on the liver: Clogged and damaged bile ducts, poor digestion, and bile secretion. In addition, a big part of the clinical picture has a toxic and allergic effect. The pathogenesis is distinguished by the absence of a period of hematogenous migration. Cercariae of the parasite escape the intestine through the outlets of bile ducts and undergo maturation first in the small, and then in the large bile ducts. Pathomorphology bears resemblance of parasitic cholangitis, expressed in the appearance of thick whitish crimped cords of the affected ducts under the capsule and on the cut of the liver. When invasion is intensive, the disease progresses similarly to biliary cirrhosis: The liver is enlarged, the surface is shagreen or tuberous form, brown, dense, and is penetrated by a network of connective tissue strands of the affected bile ducts, from which a mucous yellow-brown liquid containing parasites is secreted. The gallbladder is enlarged and contains viscous bile with parasites [10].

Publications on dicroceliasis in Kazakhstan are limited to the studies about the distribution of parasites among large and small cattle, their effect on animal productivity, hematologic indices, pathoanatomical changes in organs, and various economic damage. Methods of treatment and prophylactic measures of the disease are also described. However, there are no available research data from a detailed study on the effects of dicrocelia on the maral liver at the histological and ultrastructural level. Due to lack of data concerning the effect of dicrocelia on the maral organism and the demand to develop a research-based system of anti-dicroceliasis measures, our research was dedicated toward this field.

Materials and Methods

For examination under electron microscope, biopsy pieces of liver tissue of maral were fixed in a 2.5% solution of glutaraldehyde with post-fixation in 1% solution of osmium tetroxide, conducted according to a conventional method, and enclosed in epon. Semi-thin and ultra-thin sections were prepared on the ultramicrotome Leica. Semi-thin sections were stained with methylene blue, azure 2, and studied at a high-resolution light optical level. The ultra-thin sections were contrasted with uranyl acetate and lead citrate.
Research Results and Discussion

The light optical examination of half-thin sections revealed that the morphological pattern of pathological changes in liver tissue was polymorphic, even within a single hepatic lobe. The liver tissue located closer to the center of the lobule was characterized by plethora, capillarization of sinusoids, a sharp expansion and edema of perinosinoid and sinusoidal space, the appearance of lymphocytes and macrophages, and vacuolar dystrophy of hepatocytes (Figure 1).

When observed under electron microscope, the hepatocyte nuclei were characterized by a high euchromatin content, a primary location of the condensed chromatin, or contained coarse accumulations of condensed chromatin (Figure 2). Perinuclear space is narrow. Numerous large mitochondria had a round or oval shape. Some of them tool giant forms. The cristae are few, some portion of the crista is finely fragmented, a matrix of low electron density, finely grained. The tubules of the granular endoplasmic reticulum were located without strict orientation, less often in an orderly parallel series and were characterized by the degranulation of fixed ribosomes. The agranular endoplasmic reticulum was represented by small vesicles and long thin tubules. The granules of glycogen were absent. Lipid inclusions were present with signs of recycling. Numerous autophagosomes were present in the cytoplasm (Figure 3).

Elements of the Golgi complex occupied a small area and were vacuolized. The biliary surface contained rare short microvilli. A complete reduction of microvilli was also noted (Figure 4).

The destruction of the end plates was observed (Figure 5). At the same time, the intercellular spaces were significantly enlarged and connected the lumen of the bile capillaries with sinusoids (Figure 6).

The sinusoidal surface of hepatocytes was smooth, indistinct, and partially destroyed (Figure 7).

The space of Disse was expanded and almost merged with the space of sinusoids, caused by a destruction of the endothelium. Occasionally, closer to the periphery of the lobules, there were regions of hepatocytes with pronounced lipodystrophy, complete destruction of the liver structure, hemorrhages, and swelling of the interstitial tissue (Figure 8).
Lipid inclusions with signs of recycling had round shape and were distinctly of large sizes, up to a complete replacement of the hepatocytes’ cytoplasm. Closer to triads the liver structure was destroyed; the separately located hepatocytes were located in the edematous and fibrous connective tissue. In addition, there could be seen clusters of lymphocytes, freely floating red blood cells, regions of hemorrhage, and numerous long crystals in the form of rhombic plates and needles, belonging to the structures of the disbalanced process of hemoglobin pigmentation. Blood vessels of triads were significantly enlarged. Epithelium of cholangiol was high and light with signs of vacuolar...
dystrophy. Intermittent bile ducts proliferated in the form of narrow elongated structures, the epithelium nuclei looked dark pyknotic. Epithelium of cholangiol was elevated and light, with signs of vacuolar dystrophy. Intermittent bile ducts proliferated in the form of narrow elongated structures, the epithelium nuclei looked dark and pyknotic. Occasionally completely necrotic hepatic lobules were also observed.

The membranes of the granular endoplasmic reticulum were homogenized. The regions of the enlarged bile capillaries were connected with the space of sinusoids. Sinusoidal surface of hepatocytes was destroyed. The space of Disse and sinusoidal space were filled with fragments of destroyed hepatocytes, light-colored apoptotic “balls,” Kaunsilmen bodies, erythrocytes, unstructured flaky mass, and bundles of collagen fibrils (Figures 10 and 11).

During the examination under electron microscope, the signs of chromatin condensation in the nuclei of hepatocytes were becoming more apparent, up to the karyopyknosis. Part of the mitochondria was in a state of destruction (Figure 9).

Morphologically altered hepatocytes by necrosis were surrounded by structureless masses. In the region of necrosis of hepatocytes and hemorrhages, there were present accumulations of rhomboid crystals of hematin (Figure 12).
Conclusions

In the liver of maral infected with chronic dicrocoeliasis, dystrophic and destructive pathological changes developed in all the cellular structures of the hepatic lobules: In the form of plethora and vast enlargement of sinusoids, vacuolar and lipodegeneration of hepatocytes, destruction of the hepatic tissue with edema, hemorrhages, in the appearance of cells associated with inflammation, and the deposition of hematin crystals.

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