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MORPHOLOGY AND VETERINARY AND SANITARY EXAMINATION OF MARAL LIVER

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ABSTRACT

Velvet antler maral breeding is a profitable branch of animal husbandry in the Altai Republic. Marals provide valuable raw materials for medical purposes. Velvet antler products are in great demand in the world market. Further development of this branch of animal husbandry requires deep knowledge of the morphophysiology of maral body. Issues of veterinary and sanitary and forensic examination, abdominal surgery encourage scientists to study the features of the digestive system of these animals and, in particular, of the liver, pancreas, etc. The liver being a digestive gland is of particular interest. It is a fairly large parenchymal organ that can be edible. This article describes the study of morphology and pathology of maral liver. This topic is quite relevant and has practical value because, in addition to velvet antler products, one can use maral slaughter products what, in turn, will make this industry even more profitable. This study results demonstrated that the maral liver has morphological features similar to these of domestic ruminants. Maral liver can be affected by diseases of different etiologies. Non-infectious diseases are the least common. Tuberculosis is the most common infectious disease of marals. Parasitoses of gastrointestinal tract are absolutely the most widespread diseases. The studied results can contribute to taking therapeutic and preventive measures by veterinary specialists of maral breeding farms.

Disciplinary: Biological Science, Veterinary Medicine (Anatomy and Hystology).

1. INTRODUCTION

Velvet antler industry is a highly profitable livestock industry in the Altai Republic, piedmont

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farms of the Altai Territory, the Republic of Transcaucasia, Kazakhstan, Ukraine, Belarus, and other countries (Ershova, 2019). The profitability of maral breeding reaches 160%.

Marals (Cervus Canadensis) belong to the subfamily of Old World deer (Malofeev et al., 2019). Cervinae provides raw material which is very valuable for the medical industry. Pantocrine and pantohematogen are obtained from the blood of these animals. These medications stimulate vital processes in the human body. Velvet antler products are in great demand in the world market and are exported to the countries of the Far East and Southeast Asia (Silantyeva, 1999).

Further growth and development of this livestock industry require in-depth morphophysiological knowledge of maral organisms and improvement of veterinary service for this industry which was developed for protecting animals and humans from zoonotic diseases (Tishkov, 2007).

Maral meat and slaughter products have high eating qualities and are dietary. Issues of veterinary and sanitary and forensic examination, therapy, abdominal surgery, and rational use of feed for marals encourage scientists to closely study the morphophysiology of their digestive system.

Issues regarding pathologies of marals are no less important since there is a possibility of getting zoonotic diseases through the consumption of maral products (Lunitsyn, 1998, 1999, 2000). Besides, pathological processes reduce the quality of products obtained (Kopylov and Lunitsyn, 2000).

Marals are highly likely to have infectious and invasive diseases, since they are registered in domestic ruminants of this region (Ponamarev and Lunyova, 2018, 2019).

The liver is the largest digestive gland in the maral body of particular interest. It performs many vital functions, and an animal cannot live without it. Maral liver is a valuable food product, but at the same time, it can become a source of infecting the consumer with zoonoses (Silantieva, 1999).

The aim is to study the morphophysiological features and pathologies of maral liver in the Altai Republic.

2. MATERIALS AND RESEARCH METHODS

The object of this study was the liver obtained from apparently healthy marals aged five to ten years. Keeping and feeding of marals in farms corresponded to zootechnical standards.

The material was taken from the farms of the Ust-Koksinsky and Ust-Kansky districts of the Altai Republic during planned slaughter and shooting of animals. The material was collected for research by seasons: winter, spring, summer.

To determine the topography of the liver, a part of the abdominal wall was removed, organ location in relation to the costal wall, and other abdominal was determined.

To characterize the intraorgan vascular bed of the liver, a corrosion method was used (Bolgov and Butakov, 1975) using plastics such as AKP-7, Acronil-01, and Redont. Structures were filled with substances of different colors. Tissue maceration was carried out in a 50% hydrochloric acid solution, and then the cast was removed and washed in running water. Measurement of blood vessels and the biliary system was performed on dry casts.

For histological examination, pieces of tissue 10x10 mm in size were taken and fixed in 10% neutral formalin. Preparations were embedded in paraffin. Slices were obtained using a sliding microtome.

Histologic sections were stained with hematoxylin-eosin according to Bemer, Van Gieson, Mollory, and resorcinol-fuchsin according to Weigert (Romeys, 1953).

In order to define the characteristics of the maral liver, its morphology was correlated with

morphological data of already studied ruminants (Aleksandrovskaya et al. 1987; Bobrik et al. 1991; Volkova and Eletsky, 1996; Sotnikov, 1992).

Department of Anatomy and Histology of the Faculty of Veterinary Medicine of the Altai State Agricultural University developed a technique for making corrosion preparations of the vascular system using Makroflex foam. Before filling with mounting foam, a studied organ was kept for about one hour in warm water (37-39°C). The next step was washing vessels with a warm 3% ammonia solution to remove blood residues. Then, using Janet's syringe, we injected the solution into vessels through previously fixed in them plastic cannulas, until a colorless liquid started to flow out of large vessels. If the solution flowed out from smaller vessels, we ligated them or used hemostatic forceps, so that later there would be no leakage of mounting foam. An organ was left on the table until mounting foam hardened, and then it was examined (Malofeev et al., 2000).

Veterinary and sanitary examination of maral liver was carried out according to a special technique (Lunitsyn et al., 1999).

For the diagnosis of non-infectious diseases, methods of examination, palpation, and pathoanatomical research were used (Lunitsyn, 1990, 2002).

Infectious diseases were diagnosed using clinical and laboratory methods (Lunitsyn, 1999, 2002).

Postmortem diagnosis was carried out by the method of complete helminthological autopsy according to K.I. Skryabin (Kotelnikov, 1984).

The study results were processed statistically (Korostelyova et al., 2009).

3. RESULTS

Maral liver (hepar) is a compact flat organ, of dark red color and moderately dense consistency. Two surfaces are defined in the liver: diaphragmatic (convex and smooth) and visceral (concave and lobed); and also two edges: dull dorsal and sharp ventral.

A deep trough for caudal vena cava runs along the dorsal edge, a light esophageal indentation is also visible here. On the visceral liver surface, there is liver hilum where the portal vein and hepatic artery run through. Extraorganic common hepatic duct formed by the fusion of left and right intraorganic hepatic ducts runs out of liver hilum. Hepatic duct is 10-16 cm long, connects to a pancreatic duct, opens with a common papilla into the duodenum at the distance 14.5-18.6 cm from abomasum pylorus. Marals have no gallbladder. The caudate lobe is located at the liver hilum and has a caudate process, and there is a renal indentation for the right kidney on it.

The ventral edge of the liver has a deep notch of a round ligament lying in the plane of 8-9 intercostal space. It separates the left lobe from the square lobe of the liver. The middle part of the liver is divided by hilum into square and caudate lobes.

The liver is outside covered with a serous membrane that, in the form of an omentum, covers also duodenum and stomach.

In adult marals, the liver is located in the plane of the general body center of gravity, in the right hypochondrium, directly behind the diaphragm. The liver is oblong. The left lobe reaches the lower end of the 6^{th} rib, and the right lobe reaches the vertebral end of the 14^{th} rib. The liver extends beyond the caudal edge of the last rib only with its dorsal end. Sharp edge does not lie below the costal arch.

Absolute liver weight in maral males varies from 1,300-3,800 g, and in females 1,100-3,000 g.

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Relative liver weight in maral males is 1.15-1.52 % and in females 1-2%. Distance between its right and left edges is 31-46 cm, Width in the area of the liver hilum is 18-29 cm, and thickness in this area is 2.7-3.4 cm.

The liver surface of mature maral is covered with a fibrous connective tissue membrane (Glisson's capsule) of uneven thickness (115.2-140.9 μm). This capsule consists mainly of collagen fibers, with the presence of elastic ones (Figure 1).

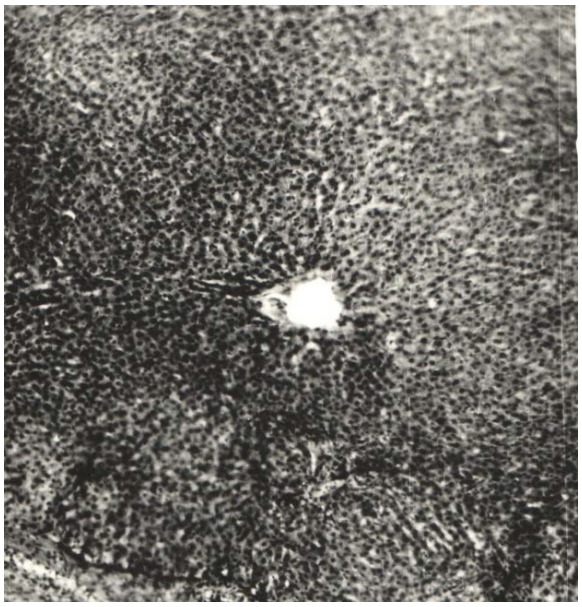

Figure 1: Microscopic photo: Liver acinus of a maral, age 5 years (magnification 140x)

The liver has parenchyma and stroma. Parenchyma is represented by hepatocyte cells and stroma, by connective tissue. Thin layers of connective tissue 3.4-11.5 μm extend from the capsule and divide the organ into separate segments of a five- or hexagonal shape.

In the center of the liver acinus, there is a central vein, round in cross-section. A large number of elastic fibers was found in its wall. Central vein wall thickness is 20-30.2 μm. Hepatic cords consisting of two rows of hepatocytes are clearly visible inside the acinus. Cells of liver parenchyma, hepatocytes, are arranged in irregular rows that branch and run radially from the periphery of the acinus and converge to its central vein. There are thin layers of connective tissue between them, with a thickness of 0.52-0.86 μm. Hepatocytes are usually round-shaped, with diameter 9.8-11.3 μm; they have one or two nuclei with one clearly visible nucleolus, less often with two (Figure 1).

Between the irregular rows of hepatocytes, there are light slit-like spaces – hepatic sinusoids. Sinusoids run through the parenchyma of acinus from the periphery to its center and flow into the central vein of the acinus. Between the rows of hepatocytes inside liver trabecules, there are capillaries that form a continuous system starting from any point in liver acinus to the portal tract and secreting into the bile duct.

The Portal triad is characterized by the presence of a hepatic artery of predominantly round shape, with the wide lumen and wall thickness 11.2-15.1 μm. The inner layer (intima) is covered with flattened endotheliocytes. The middle layer (media) is formed by a single layer of circularly located smooth muscle tissue. Nuclei of myocytes are large, rod-shaped. The outer layer (adventitia) is formed by a thin layer of loose connective tissue (Figure 2)

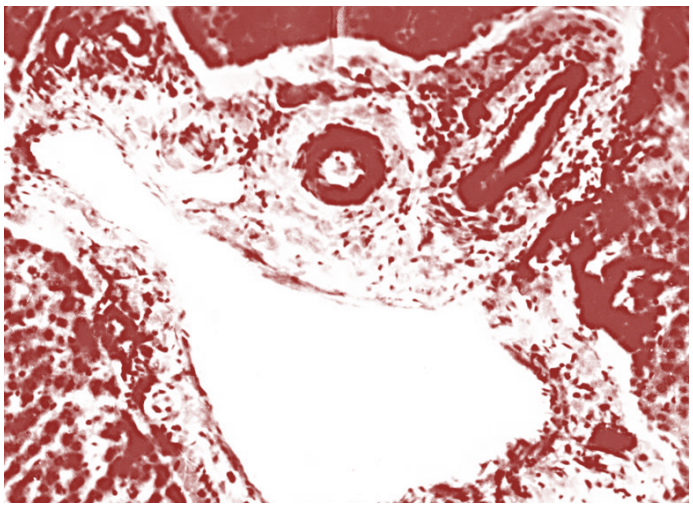

Figure 2: Microscopic photo: Portal tract of maral liver, age 7 years (magnification 70x)

Branches of the portal vein are large, cochleiform. Walls of veins have all three layers: inner, middle, and outer. Flat epithelial cells with significant nuclei are clearly visible in the intima of veins. The subendothelial layer formed by loose connective tissue is well developed. The media consists of smooth muscle tissue. The muscle layer thickness ranges 2.5-3.1 μm. Adventitia is formed by loose connective tissue.

The lumen of the venous vessel is wide, its wall is thin, 8-10 μm; it belongs to the vessels of muscle type (Figure 2). Bile ducts of the triad are of elongated, rounded or oval shape. The Wall of the bile duct is lined with a single-layer cuboidal epithelium. Lumen is narrow, its thickness is 10-13.2 μm. From the outer, the bile duct is surrounded by a layer of loose connective tissue with numerous elastic and collagen fibers (Figure 2). Maral liver has a unique blood supply, as arterial and venous blood is brought to it. Venous blood runs from the entire gastrointestinal tract and enters liver hilum, and arterial blood is delivered through the hepatic artery.

Figure 3: Macroscopic photo: Corrosion liver preparation. Injection of portal triad elements, doe maral, age 5 years (scale 1: 2.3)

Venous blood flows off through hepatic veins. Thus, the portal vein and hepatic artery enter liver hilum, and bile duct and lymphatic vessels run out of it (Figure 3). In the area of liver hilum, there

are so-called “own” hepatic lymph nodes. Also, from our studies, lymphatic vessels can flow into the pancreas, stomach, and even into the thoracic lymphatic duct.

Hepatic lymph 6-10 nodes (Figure 3) are located near the common hepatic artery in the area of the liver hilum. The size of the lymph nodes is 11-62 μm. There are also small lymph nodes of different localization. Hepatic (portal) lymph nodes are of rounded, bean-like, and oblong shape that allows studying this organ well (Figure 4).

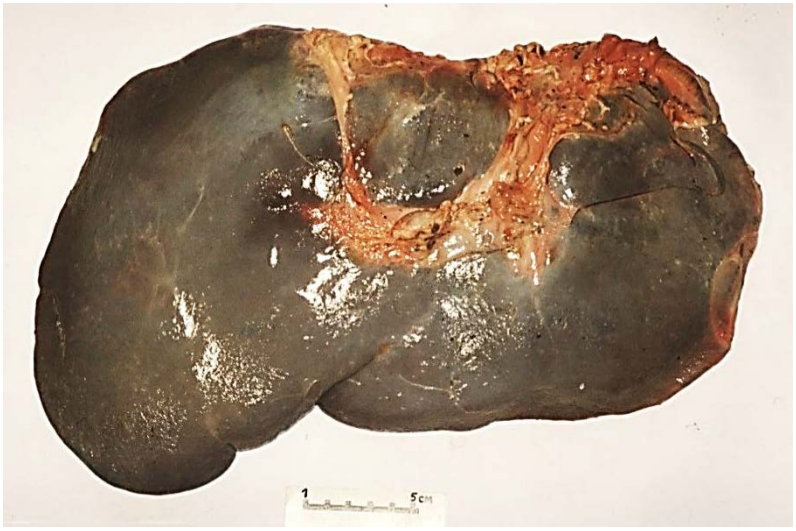

Figure 4: Macroscopic photo: Visceral surface of the liver with lymph nodes, maral, age 6 years

The tissue of these nodes is brown-gray. Portal lymph nodes collect lymph from liver, pancreas, and duodenum, abomasum lymph nodes. Excretory ducts of these nodes flow into lymphatic collecting vessels into the celiac trunk. Lymph flows through lymphatic vessels and is removed through the liver hilum. To study hepatic lymph nodes, we used a special technique. Lymph nodes of maral liver were fixed in 10% formalin solution and stained with hematoxylin-eosin according to Van Gieson. Also, we used polyurethane foam to study and define lymphatic afferent and efferent vessels (in an amount of 8-14) (Figure 5).

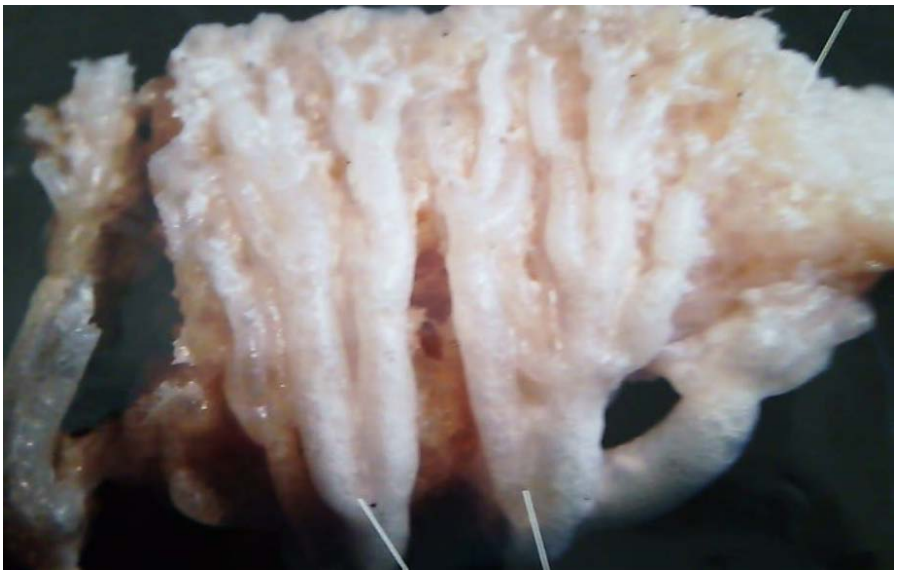

Figure 5: Macroscopic photo: Corrosion preparation. Injecting of maral liver lymph node with Makroflex mounting foam

Histological sections of a lymph node showed that it was covered with a capsule from whereof the trabeculae run which penetrate cortical substance and are a kind of skeleton for a lymph node.

Nodes are of reticular tissue, it can be either loose or dense.

Between the trabeculae, there are follicles and a network of reticulin fibers that merge into one structure-forming cord.

Lymphatic sinuses are also well visible in the form of thin spaces. Marginal sinus is located in the cortical substance of the lymph node, and central sinus is between the cords.

For studying the above morphophysiological parameters of the maral liver, organs with no pathological changes were used. However, in several cases during slaughter, it revealed that even apparently healthy animals had pathological processes of internal organs that involved the liver. Therefore, in this article, we also describe the pathologies found during our research.

To identify a pathological process, a veterinary and sanitary examination of a carcass and other maral slaughter products was first carried out. During the veterinary and sanitary examination, the liver was examined and palpated from the diaphragmatic and visceral sides. If the adhesion of diaphragm to the liver was found, then it was separated and examined for pathological changes. Then hepatic lymph nodes were cut and examined. After that, two non-through incisions were made from the visceral side along bile ducts in order to find parasites. If there were pathological signs of pathognomonic infectious diseases, then laboratory tests were performed.

As a result of research, we found that maral liver can be affected by diseases of various etiologies. Marals in the Altai Republic are least likely to have liver diseases of non-infectious etiology (cirrhosis, capillary ectasia, etc.).

With postmortem examination, we diagnose atrophic cirrhosis with the following symptoms: the liver is enlarged, solid, usually of light, or dark yellow color. With hypertrophic cirrhosis, the liver is significantly enlarged, twofold on average. It has a dense consistency, with a lot of connective tissue.

With capillary ectasia on the surface and in the parenchyma, there are bluish-red or dark-purple foci of different sizes. Under the liver capsule, there are clearly visible indentations.

Sanitary assessment of changes caused by non-infectious diseases includes evaluation of the severity of pathological changes. With non-infectious inflammation, severe cirrhosis, degeneration and other pathological changes in the parenchyma, the liver is subject to disposal. With single lesions, parts of the liver are removed. Part of the liver without lesions is used without restriction.

The most common infectious disease in marals is tuberculosis. Tuberculous lesions are most often located in hepatic lymph nodes. Pathological changes are different; they can be of productive or exudative nature. With a productive form, there are nodules with the size of millet grain which are subsequently most often calcified. With an exudative form, there is diffuse, serous-fibrinous inflammation with no calcification or formation of connective tissue capsules, although caseous degeneration areas can occur. The bacteriological test reveals tuberculosis bacilli in liver pieces. Nodules of various sizes and growth of loose connective tissue of gray-yellow color are clearly visible on serous membranes. The liver should be disposed of regardless of the form of tuberculosis.

Echinococcosis, fascioliasis, and dicroceliosis are the most common parasitoses. Echinococcosis was diagnosed by finding cyst-like formations filled with fluid. When a cyst was opened, multiple brood capsules with scolexes were found. In studies, child and grandchild cysts were often found. In marals, echinococcosis cysts were more often found in the liver than in other organs. Atrophy and growth of connective tissue were developed in the affected liver. Maral liver with echinococcosis was disposed of.

With fascioliasis, lesion of bile ducts was detected. They looked like dense white tubes, and catarrhal inflammation was clearly visible on their mucous membrane. At a high grade of invasion, the walls of bile ducts were thickened and had a tuberous shape. There were often found calcareous formations with the remains of decaying parasites. Lymph nodes of maral liver with fascioliasis are enlarged, and the brown pigment is visible in their sections. Maral liver with found fasciolas was disposed of.

Dicrocelias were also often found in the maral liver. They are quite easily palpated if you hold your hand along the incision along the bile ducts of the liver. There is a brown-black fluid in bile ducts. Organ with such lesions and a high grade of invasion during dicroceliosis were disposed of.

4. DISCUSSION

Analyzing the results of our research on the features of the anatomical structure of the maral liver, we should note that it is of a flat, oblong shape. Two surfaces are defined in the liver: diaphragmatic (convex and smooth) and visceral (concave and lobed); and also two edges: dull dorsal and sharp ventral. The liver hilum is located on the visceral surface; portal vein, hepatic artery and nerves run into it, and hepatic duct runs out of it. A distinctive feature in the structure of maral liver from domestic ruminants is the absence of gall bladder. Our data are consistent with studies performed by other authors (Chebakov, 1998; Ryadinskaya, 1999).

After liver hilum, the common hepatic duct goes to the duodenum. Its length is from 10 to 16 cm. The main pancreatic duct flows into the middle part of the common hepatic duct. When connected, they open together with a common papilla into the duodenum at a distance 14.5-18.6 cm from pylorus and from 52 to 64 cm from the jejunum. In contrast to this, according to published data, this duct in large ruminants opens into duodenum separately from the pancreatic duct.

We agree with the views of Z.S. Bruveris (1972) that the liver is divided into left, right and middle lobes; the latter is subdivided by hilum into a square and caudate lobes. But they are ill-defined in marals, furrows between lobes are absent or not clearly visible. Caudate lobe has a hardly defined mastoid process. The round ligament is sometimes absent in marals, and the coronary ligament is short.

Our data on liver topography coincide with those on reindeer (Akaevsky, 1939). The oblong-shaped liver is located in right hypochondrium, directly behind the diaphragm. The left lobe reaches the lower end of the 6^{th} rib, and the right lobe reaches the vertebral end of the 14^{th} rib. The liver extends beyond the caudal edge of the last rib only with its dorsal end. Sharp edge does not lie below the costal arch.

The microstructure of liver tissue is mainly similar to that of ruminants, especially large cattle and reindeer (Bruveris, 1972; Akaevsky and Lebedev, 1971).

The liver of a sexually mature deer is covered with a fibro-connective tissue membrane from the surface. The capsule is formed by collagen fibers with the presence of elastic fibers. Nuclei are visible between fibers. Thin layers of connective tissue running from capsules divide the organ into separate acini. In the center of the liver acinus, there is a central vein. Inside acini, liver plates are clearly visible consisting of hepatocytes inside liver trabecules followed by bile capillaries.

In interlobular connective tissue, we note a liver tetrad which includes hepatic artery, vein, bile duct, and lymphatic vessel (Figure 2). Knowing morphophysiological features and possible pathologies facilitate the veterinary and sanitary examination of maral liver. Because this organ can

become a source of human infection with zoonoses, it is important to study and monitor the epizootic situation in the region, as well as to perform a thorough veterinary and sanitary examination and mandatory identification of all found pathological processes.

Marals being wild animals are very often infected with infectious and invasive diseases. Moreover, it is important to note that parasites could be found in both individual species and their associations. For example, during our research, echinococcosis, dicroceliosis, and fascioliasis were found both separately, in the form of mono-invasion, and all together, in the form of mixed invasion.

A fairly large part of marals in the Altai Republic is affected by tuberculosis; therefore, it is important to carry out mass health and preventive measures in maral breeding farms of the region.

A small percentage of non-infectious diseases was, in our opinion, due to optimal conditions for feeding and keeping animals, in comparison with industrial complexes where farm animals are bred.

5. CONCLUSION

Lymph nodes of maral liver are extra-organic and include afferent and efferent lymphatic vessels. The number of regional lymph nodes of the liver decreases with age, this, in our opinion, is associated with merging of lymph nodes with each other. Distinctive morphological features of maral liver are the absence of gall bladder, more flattened form organ with ill-defined grooves and the joint flow of hepatic and pancreatic ducts into the duodenum.

The difference in the weight of the maral liver directly depends on its function and the intensity of using energy resources, what, in turn, depends on the sex and live weight of an animal. The liver of marals of the Republic of Altai can be affected by diseases of various etiologies; the largest extensiveness and intensity of infection belong to parasitoses; non-infectious liver diseases are found less often than other pathologies.

6. AVAILABILITY OF DATA AND MATERIAL

All relevant data are already included in this article.

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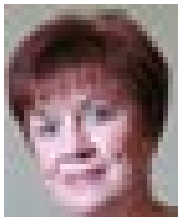

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