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Morphology and specifics of morphometry of lungs and myocardium of heart ventricles of cattle, sheep and horses

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Respiratory organs and cardiovascular system are interconnected and perform extremely vital functions of the organism, the main goals being performing gas exchange with the environment and emitting carbon dioxide produced in the organism into the environment. Therefore, we carried out comparative histo- and cytomorphometric evaluation of morphological structures of the heart and lungs of cattle, sheep and horses. Using complex methods of research, we determined specifics of microscopic structure and histometric parameters of parenchyma of the lungs and myocardium of the ventricles of the hearts of clinically healthy animals in species aspect – cattle, sheep and horses. The studies revealed that the histoarchitectonics of the studied animals were similar in structure, characteristic of other species of agricultural mammals, and distinct morphometric peculiarities of their histostructures. The study indicated that the respiratory zone of the lungs is most developed in horses. This parameter was lower in ruminants – cattle and sheep. Connective tissue septum of parenchyma of lungs was better expressed in cattle and sheep, and less in horses. At the same time, mean volume of lung alveoli in clinically healthy animals varied: being highest in horses – 699 ± 106 thou μm^3, then cattle – 337 ± 43 thou μm^3 and sheep – 158 ± 37 thou μm^3. Such variation in histometric parameters of parenchyma of the lungs in experimental animals indicates adaptive specifics of the organism of animals in terms of living conditions. Therefore, the respiratory zone was most developed in the lungs of horses, animals that experience significant physical and physiological load on corresponding organs and systems. As a result of histometric studies of myocardium, we determined patterns of sizes of thickness of its cardiomyocytes and volume of their nuclei. We determined that in myocardium of the heart ventricles, in the same microscope field of view, cardiomyocytes of varying thicknesses occur. At the same time, thickness of cardiomyocytes, their mean volume of nuclei in the ventricle myocardium were expressed the most in cattle, then in horses and sheep, and their histometric parameters in myocardium of the left ventricle of the heart in the experimental animals were higher than in the right one. Therefore, thickness of cardiomyocytes of the left ventricle in cattle equaled 14.06 ± 0.41 μm, and volume of nuclei of cardiomyocytes respectively 124.55 ± 7.99 μm^3. Similar changes in such parameters of histometry were found in sheep and horses. We attribute such varying histometric parameters of the thickness of cardiomyocytes and volume of their nuclei in myocardium of the left ventricle of the heart in experimental animals, compared with such parameters in the right ventricle, to the activities of the ventricles (the left one generally functions as a pump, right one – as a volumetric) and functional specifics of this myocardium tissue, which is capable of spontaneous rhythmic contractions, resulting in blood flow in the vessels: cardiomyocytes of the left ventricle carry greater load, promoting blood flow in vessels of greater (somatic) blood circulation, respectively cardiomyocytes of the right ventricle – less load, promoting blood flow in vessels of lesser (pulmonary) blood circulation.

Keywords: pulmonary acinus; bronchial tree; alveolar tree; cardiomyocytes; myofibrils; bronchi; pulmonary alveoli.

Introduction

Organisms of humans and animals are an extremely complex biological system (Havrylin & Hibert, 2018) composed of interrelated structural elements that interact, thereby forming its morphofunctional complex – organism ↔ environment (Stakhyv & Shemediuk, 2016; Yuskiv & Shyder, 2018). At the same time, response of the organism to the influence of the environment, which transforms all organs and systems into an integral whole (Parshina & Musabaeva, 2016; Borshch et al., 2020; Fedorovych et al., 2021), requires normal morphofunctional activity of all systems of the organism, including respiratory organs (Prokushenkova, 2009; Horvat & Dankovych, 2020) and the cardiovascular system (Xanthos et al., 2011).

Therefore, for successful development in the sphere of livestock farming, increase in productive qualities of animals, prevention of diseases of various genesis, along with organization-agricultural measures, it is necessary to conduct in-depth study of the organisms of agricultural animals in general and the microscopic structure of organs and systems in particular (Gutyj et al., 2017; Stravsky et al., 2020; Bashchenko et al., 2021; Mylostyvyi et al., 2021). At the same time, one of the relevant topics is study of the contents, structural-functional peculiarities and chemical architectonics of the parenchyma of the lungs and the heart in agricultural animals in species aspect (Tkachenko & Konovalov, 2010). At the same time, studies of their morphofunctional characteristics are not only of educational value, but fundamental for clinical veterinary medicine, as well as study of constitution of animals in zootechnics (Vansjackaja & Kirpaneva, 2014). Furthermore, the lungs and heart in the organisms of humans and animals perform important vital functions (Prokushenkova, 2009; Tkachenko & Konovalov, 2010).

Respiratory organs of all terrestrial animals, including agricultural, include airways (nasal cavity, larynx, trachea, bronchus), and the gas exchange organs are lungs. Pulmonary respiration is characteristic for all terrestrial vertebrates: adult amphibians, reptiles, birds and mammals, and – unlike diffusive, gill and trachea respiration – pulmonary respiration is the best way of gas exchange. It provides external respiration (gas exchange between air and blood), providing oxygen to the organism of animals and removing carbon dioxide. Furthermore, morphological specifics and functional condition of the respiratory organs are important and actually influence the vitality of the most crucial systems of the organism of animals in normal conditions, as well as during pathology associated with respiratory organs (Goralskyi et al., 2021).

At the same time, despite prophylaxis of diseases of the respiratory system, over the recent years, an increase has been observed in the morbidity from diseases of the respiratory organs of various etiologies (Koptiev, 2011). Therefore, effective treatment and prophylaxis of those pathologies are obviously impossible without knowledge of breed and species specifics of anatomy and histology, morphofunctional parameters of which should be taken into account during diagnostic and preventive measures to prevent animal diseases, as well as during medical treatment. Moreover, knowledge of natural and species peculiarities of the respiratory system of agricultural animals would help to solve issues of veterinary-sanitary and forensic expertise of products of their slaughter.

The cardiovascular system is one of the most important in the organism of animals, which plays an important role in regulation of functions of the organs and systems of organisms, it takes part in providing trophic, respiratory and excretory functions (Ciszek et al., 2007). Together with the nervous system, the cardiovascular system connects all the organs into an integral system (Zhurenko et al., 2018). The central organ of the cardiovascular system is the heart, which provides metabolism (Gushhin, 2021). The cardiovascular system, which is a component of greater (somatic) and lesser (pulmonary) circulations, delivers nutrients, hormones, oxygen to the tissues with blood, and removes metabolic products. At the same time, the heart – because of contraction of myocardium cardiomyocytes – causes blood flow in the enclosed system of blood vessels (Krešáková et al., 2015).

Thus, an important topic in the morphology is study of the lungs and heart myocardium as an integral system of greater and lesser circulations, which performs gas exchange in the organism, regulatory activity of which is carried out with involvement of the nervous system that coordinates and regulates their operations, uniting the organism as a whole (Sysyuk et al., 2017). Moreover, such parameters characterize the dynamics of the development and structure of the lungs and heart in general and are morphological criteria of physiologic and pathological changes in the organs of respiratory apparatus (Nebesna & Yeroshenko, 2015) and the cardiovascular system (Slabyi, 2017; Symivska, 2018).

Therefore, we performed histo- and cytomorphometric evaluation of morphological structures of the heart and lungs in cattle, sheep and horses in a comparative aspect.

Materials and methods

During the studies, we followed the general rules of the appropriate GLP laboratory practice (1981), positions of the General Ethical Principles of the Experiments and Animals, approved by I National Congress of Bioethics (Kyiv, 2001). All the experimental part of the study was conducted according to the requirements of the International Principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986), Rules of Studies using Experimental Animals, approved by the order of the Ministry of Healthcare No. 281 as of November, 1, 2000, "On Measures of Further Improvement of Organization Forms of Studies using Experimental Animals and the corresponding Law of Ukraine "On Protection of Animals against Abuse (No. 3447-IV as of 21.02.2006, Kyiv).

The studies were performed at the Department of Anatomy and Histology of the Faculty of Veterinary Medicine of the Zhytomyr National Agroecological University. We employed macro- and microscopic, morphometric and statistical methods of studies. The objects of the study were the heart and lungs of clinically healthy mature animals (n = 5) – cattle, sheep and horses.

Anatomic dissection was performed on fresh lungs of the experimental animals. The heart was removed from the rib cage of animals using a pericardium. Incision of the heart was made after measuring parameters, and then we conducted lengthwise dissection of the ventricles through corresponding atrioventricular orifices.

To conduct histological studies, we used generally accepted methods of fixation and preparation of histo-sections (Pishak, 2008; Horalskyi et al., 2019). At the same time, pieces of this material were fixated in 12% frozen solution of neutral formalin for 24 h and more, and then embedded in paraffin according to the schemes proposed in the guide by L. P. Horalsky, V. T. Homych, O. I. Kononsky (Horalskyi et al., 2019). Paraffin sections were prepared using MC-2 sledge microtome, their thickness did not exceed 10–12 µm.

To perform studies of morphology of cells and tissues, the histo-sections, after their deparaffinization, were stained with hematoxylin (Diapath, Italy, 2020) and eosin (LeicaGeosystems, Germany, 2020). Furthermore, for microscopic study of transversally striped muscular tissue of myocardium, determining the cytostructure of cardiomyocytes, we used staining of histo-preparations according to the Heidenhain's technique. Stained histo-sections were used to obtain preparations and conducting histometric studies.

Histometric studies of structural elements of the tissues: determining the respiratory part and connective-tissue septum of the lungs (per unit area, accounting for 5.0 mm^2), mean volume of alveoli, measurements of the thickness of transversal section of cardiomyocytes, and volume of their nuclei were carried out in light microscopy using Micros microscopes (Micros, Austria, 2012) and MBS-10 (Micromed, Russia, 1998) with fixed tube length, according to the recommendations in the guide of Horalskyi et al. (2019). Histological preparations were photographed using CAM V–200 videocamera (InterMed, PRC, 2017), installed in the microscope. Anatomical and histological terms for the structural parts of the lungs and heart are given according to the International Veterinary Histological Nomenclature (Terminology Dictionary) (2019) and the International Veterinary Anatomical Nomenclature (2012).

Mathematical analysis of the results of the studies was performed statistically using software pack Statistica 7.0 (StatSoft, Tulsa, USA). Differences between the values in the control and experimental groups were determined using ANOVA, where the differences were considered significant at $P < 0.05$ (taking into account Bonferroni's correction).

Results

The lungs in the experimental animals were rosy coloured, located in the thorax cavity and had lobular structure. The surface of the lungs of cattle and sheep had a clearly manifested clustered pattern because of the development of interlobular connective tissue. Such a pattern on the surface of the lungs of horses was smoothened. Lung lobes were ventrally separated from each other by deep fissures, and dorsally had grown together, forming blunt lung margins: in the left lung, the lobes grew together to a higher degree than in the right, which is possibly due to the lower respiratory activity of the left lung. At the same time, the volume of the left lung and its absolute weight were smaller than such of the right lung. Therefore, according to the results of our organometric studies of the lungs of animals, we saw a clearly expressed asymmetry in the structure of the right and left lung, accounting for 1:1.37 in sheep and cattle and 1:1.20 in horses.

The left lung of sheep and cattle comprises three lobes – cranial, middle and caudal, the right lung has four lobes – cranial, middle, caudal and accessory. Horses only have two lobes in the left lung – cranial and caudal, and three lobes in the right lung – cranial, caudal and accessory. Caudal lobe of lungs in horses is formed by fusion of cranial and middle lobes in one. According to our data, such lobe is caudal (diaphragm), for there are no interlobular fissures between the middle and caudal lobes in the right and left lungs, and their surface is adjacent to the diaphragm, and therefore we propose to name the heart-diaphragm lobe – diaphragm (caudal) lobe.

Relative weight of the anatomic lobes of the lungs – cranial, middle, caudal, accessory lobes in the experimental animals – compared with mean absolute weight of their lungs, was directly proportional to the weight of animals and absolute weight of the organ. At the same time, the highest percentage in the total weight of the lungs of the experimental animals accounted for caudal lobe of the right and left lungs, the parameters of which respectively equaled: 34.8 ± 3.0% in the right lung of sheep, 29.1 ± 2.5% in the right bovine lung, 42.9 ± 4.1% in the right lung of horses; and lower – 27.4 ± 2.4%; 24.4 ± 1.9% and 39.4 ± 3.6% in the left lungs respectively. At the same time, the highest percentage among the animals we studied was taken up by the caudal lobes of the lungs of horses. It is likely related to the fact that horses have no middle lung lobes due to fusion (unification) of them into a component of caudal lobes. Lower relative weight, compared with the absolute weight of both lungs, was seen for he cranial lobes: 11.9 ± 1.1% in the right lung of sheep, 10.3 ± 1.0% in cattle, 6.5 ± 0.6% in horses; lower in the left lung of the experimental animals. Those parameters were lower compared with the right lung, equaling 8.4 ± 1.0%, 8.4 ± 0.6%, 5.9 ± 0.5%. Relative weight of the middle lobes of the lungs equaled 7.4 ± 0.9 in sheep, 12.9 ± 1.3% in cattle, in the left lung respectively – 6.4 ± 0.6% and 9.4 ± 0.9%. This lung lobe is absent in horses. The lowest relative weight according to the results of our studies was observed for additional lobes of the right lungs, relative weight of which against total absolute weight of the organ was 3.8 ± 0.1% for sheep, 5.4 ± 0.6% for cattle, and 5.3 ± 0.7% for horses.

Lungs in the experimental animals were formed by ramification of the bronchi, forming a bronchial tree and ramification of the respiratory zone, formed by alveoli tree. The structural basis of the lungs of ruminants and horses comprise a pyramid or cone or cone-like lobes, which form the lung stroma. Acini are covered by thin layer of connective tissue and are constructed of alveolar pathways, alveolar sacs and alveoli. Between the lung alveoli, a significant amount of bronchi of various sizes was seen, which, on the bronchial tree, are developed non-uniformly and according to morphotopography are divided into extra-pulmonary (main and interlobular) and pulmonary bronchi, which enter the lung parenchyma, where they ramify and form the bronchial tree. The specifics of ramification of the bronchial tree in cattle and sheep are presence of trachea bronchus for the right lung. Such a bronchus, even before the bifurcation of the trachea, ramifies as a separate branch running to the right lung and almost from there divides into two branches (cranial and caudal): the cranial branch goes to the cranial lung lobe, whereas the caudal – to the middle lobe. There is no such a bronchus in horses.

The main bronchi – according to histometric studies in all species of experimental animals – were the largest in diameter: in their inner mucous membrane, there are smooth muscles, formed by bundles of soft muscular cells, which form two layers – inner circular and outer lengthwise. At the same time, rings of bronchi cartilages in the wall of large bronchi have integral structure. Similar histoarchitectonics, according to the results of our studies, are also characteristic for interlobular bronchi.

Large bronchi, among all pulmonary bronchi, are the largest in diameter. Their inner wall is formed by multi-row ciliated epithelium, under which the smooth muscles lie. The latter is formed by one layer of bundles of smooth muscular cells – myocetes, which have circular-oblique direction and border the mucous membrane. The muscular layer, as a ring, surrounds the entire lumen of bronchi. Because of the same structure of smooth muscles, the mucous membrane of large bronchi forms lengthwise folds (Fig. 1a), none of which are usually found in the wall of the main bronchi. Moreover, the lamina propria of such bronchi contains an accumulation of lymphoid tissue. In the mucous membrane of bronchi contains a large number of glands. The middle membrane (fibrous-cartilage) of large bronchi is formed of non-integral rings of bronchial cartilages, which are presented by separate cartilage plates of various sizes and forms, which form the so called cartilage "islands" (Fig. 1a).

The wall of middle bronchi is much thinner than in large bronchi. The wall of bronchi of average caliber is covered by single-layered multi-row respiratory epithelium. Smooth muscles of the mucous membrane are distinct and form well notable folds, and glands are found in the submucous membrane. The middle membrane of such bronchi usually contains separate cartilage islands, formed by hyaline cartilage tissue (Fig. 1b). However, there are plates of elastic cartilage in some places.

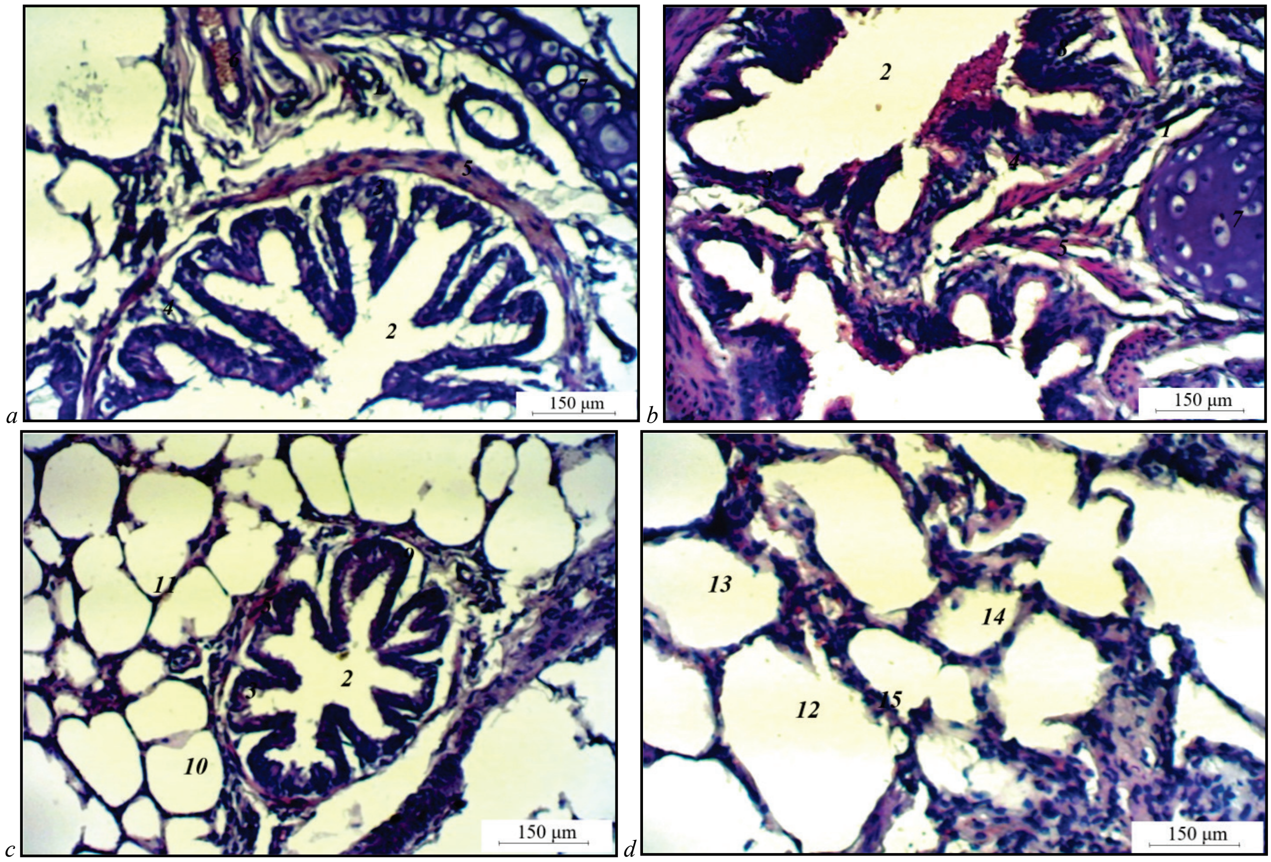

Fig. 1. Fragment of microscopic structure of lungs: *a* – structure of large bronchus of horse lung; *b* – structure of middle bronchi of horse lung s; *c* – structure of lungs of sheep; *d* – structure of cattle lungs: *1* – wall of bronchus; *2* – lumen of bronchus; *3* – epithelial plate of mucous membrane; *4* – lamina propria of mucous membrane; *5* – smooth muscles of mucous membrane; *6* – vessel; *7* – cartilage island; *8* – folds of mucous membrane; *9* – small bronchus; *10* – alveoli; *11* – membranes between alveoli; *12* – large alveolus; *13* – middle alveolus; *14* – small alveolus; *15* – membranes between alveoli; hematoxylin and eosin

The wall of small bronchi is not only formed by mucous membrane and adventitia. Epithelial lamina is formed by two-row ciliated epithelium. In the content of epitheliocytes, there are Clara cells that produce enzymes that ruin surfactant. Smooth muscles of small bronchi are distinctly formed, and in the submucous layer, compared with such in large and middle bronchi, glands are absent. Furthermore, there are no cartilage plates in the wall, only their traces were occasionally found (Fig. 1c).

Terminal bronchioles (ramification of small bronchi) are formed by extremely thin wall, similar to such in small bronchi. Epithelial lamina is formed by simple cubic ciliated epithelium. Smooth muscles are formed by smooth muscular cells, arranged as a wall, and therefore their mucous membrane forms no folds. Bronchial arteries of varying form and size are located around the bronchi of various sizes.

The histoarchitectonics of the respiratory zone of lungs are formed by the histostructure (alveolar tree), the wall of which bears alveoli. Therefore, they form a structural-functional unit – pulmonary acinus, which is a place of ramification of one terminal bronchiole and is composed of respiratory bronchioles, alveolar ducts and sacs. Microscopically, the wall of respiratory bronchioles is structurally similar to the wall of terminal bronchioles. However, in epitheliocytes of epithelial lamina, ciliated cells are absent, and their walls contain alveoli. Alveolar ducts are usually formed as a result of ramification of bronchioles of the second and third rows. At the same time, their diameter is almost two or three-fold larger compared with the respiratory bronchioles. The histostructure of alveolar ducts contains a large amount of pulmonary alveoli, between which there are intervals of the wall of those pathways, which is structurally similar to the wall of respiratory bronchioles. Alveolar sacs are terminal ends of the alveolar ducts. The wall of such sacs is formed by several alveoli, situated near each other. Alveoli of the lung which are parts of alveolar tree are open clusters combined by interalveolar membranes, which contain blood capillaries. Interalveolar alveoli are formed by lower layers of swollen connective tissues containing numerous elastic fibers. The inner wall of lung alveoli is formed by alveocytes (respiratory epithelium) situated on the basal membrane, forming a continuous layer below them. Lung alveoli vary in forms and sizes – small, average, large (Fig. 1d).

According to the results of our study, the alveolar tree in horses was shortened, large and swollen. Alveolar bronchioles were poorly differentiated. Because of expansion, alveolar sacs are large and have smoothened alveoli. Alveolar sphincters (muscular elements of the alveolar tree) are developed very poorly. The alveolar tree in lungs of ruminants, compared with horses, was shorter and expanded. Such morphofunctional characteristics of the alveolar tree in the experimental animals, in our opinion, cause increase in excessive reserve air in the organism, which is used much slower, because of slowing of the respiratory rhythm in such animals. According to the results of histometric studies, mean volume of lung alveoli in clinically healthy animals varied: it was the highest in horses (699 ± 106 thou μm^3), second highest in cattle (337 ± 43 thou μm^3) and lowest in sheep (158 ± 37 thou μm^3). At the same time, compared with horses, this parameter was 2.1 times lower in cattle and 4.43 in sheep (Table 1).

According to the histometric studies of lung parenchyma in horses, cattle and sheep, the respiratory zone of lungs was to the highest degree developed in horses – 54.8 ± 7.4% of the total area of lung parenchyma. In ruminants, this parameter was somewhat lower, equaling 44.8 ± 3.4% in cattle, 47.0 ± 7.6% in sheep. By contrast, connective tissue was more developed in cattle and sheep and less in horses, occupying respectively 55.2 ± 3.4%, 53.0 ± 7.6% and 45.2 ± 7.4% (Fig. 2; Table 1).

Therefore, the histometric studies we carried out indicate that the respiratory zone of lungs was developed the best in horses – 54.8 ± 7.4% of the total area of lung parenchyma. In ruminants, this parameter was lower.

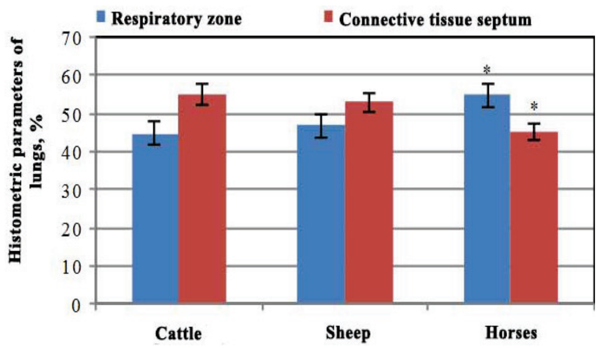

Fig. 2. Histometric parameters of lungs of the experimental animals (x ± SE, n = 5): * – P < 0.05 compared with cattle

Table 1
Histometric parameters of lungs of cattle, sheep and horses (x ± SE, n = 5)

Parameter	Measurement unit	Animals		
		Cattle	Sheep	Horses
Respiratory zone of lungs per unit area (5 mm^2)	mm^2	2.24 ± 0.24	2.35 ± 0.32	2.74 ± 0.47
	%	44.8 ± 3.4	47.0 ± 7.6	54.8 ± 7.4
Connective tissue septum of lungs per unit area (5 mm^2)	mm^2	2.76 ± 0.24	2.65 ± 0.32	2.26 ± 0.47
	%	55.2 ± 3.4	53.0 ± 7.6	45.2 ± 7.4
Mean volume of alveoli	thou μm^3	336.9 ± 42.5	158.1 ± 7.4**	699.8 ± 106.4**

Note: statistically significant differences were taken into account compared with cattle: * – P < 0.05; ** – P < 0.01; *** – P < 0.001.

In the atria, two layers of the muscular membrane were found: first the outer and second the deep layers. Moreover, the outer layer of myocardium is shared by both atria. At the same time, muscular fibers are oriented transversally from one atrial appendage to another. The deep layer of myocardium in the left atrium has lengthwise direction. However, in the area of venous orifices, ring bundles of fibers are formed. Walls of ventricles are much thicker than the walls of the atria, which is related to their functional activity. Myocardium of ventricles is formed by five layers: outer and inner (muscular fibers have diagonal-lengthwise direction); then outer and inner by deeper layers and deepest layers, fibers of which are oriented in a figure resembling number eight.

Themicroscopic structure of myocardium is formed of cardiac muscular tissue and interlayers of swollen fibrous connective tissue, which contain blood and lymphatic vessels and nerves. Cardiac muscular tissue is constructed of cardiomyocytes (cardiac myocetes). Myocardium cardiomyocytes are arranged as a chain (one near the other) and, by binding together at their ends, form histostructures, similar to muscular fibers of somatic muscular tissue (Fig. 3a, b). Cardiomyocytes are bound together into muscular fibers by intercalated discs (Fig. 3c), which perform support function for contractile elements of cells (miofilaments) and provide integral contraction of myocardium, thereby creating functional syncytium. Cardiomyocytes, in microscopic structure, are cylindrical and are transversally and lengthwise striped (Fig. 3c). They form a network of thin and thicker transversally striped muscular fibers. Under light microscope, cardiomyocytes look like dark transversal stripes. In them, there are distinguished sarcolemma, myofibrils and nuclei, situated in the central part of cell. Between cardiomyocytes, there are spaces filled with dense vascularized connective tissue (Fig. 3b, c). The specifics of the structure of myocardium, compared with the structure of somatic muscular tissue, are presence of anastomoses between muscular fibers, by which the latter are combined in an integrity, forming a net-like structure: parallel-located cardiomyocytes, connected between one another by anastomosis, form a contractile system (Fig. 3b). Between muscular tissues, there are interlayers of swollen connective tissue containing blood and lymphatic vessels. In myocardium muscular fibers of experimental animals, during staining of histopreparations with hematoxylin and eosin, there is a clearly manifested lengthwise (as a result of myofibrils) and transversal (as a result of proteins actin and myosin) striped pattern (Fig. 3c). At the same time, densely located myofibrils are situated closer to the periphery of fibers, connected by anastomosis. Having a relatively small amount of myofibrils, the lengthwise striped pattern of muscular tissue is clearly distinct, while the transversal – relatively poorly. Moreover, very thick muscular tissues poorly react to staining, and their striped pattern is poorly visible, while myofibrils look thin. Very thin muscular fibers in the transversal section are oval. Myofibrils in them are located densely. The central part of cardiomyocytes contains one, more rarely two nuclei, oval or elongated, located non-uniformly. Nuclear chromatin is seen as small or larger grains throughout the perimeter of karyoplasms. At the same time, the central position of the nuclei is clear on the transversal sections of muscular fibers (Fig. 3b).

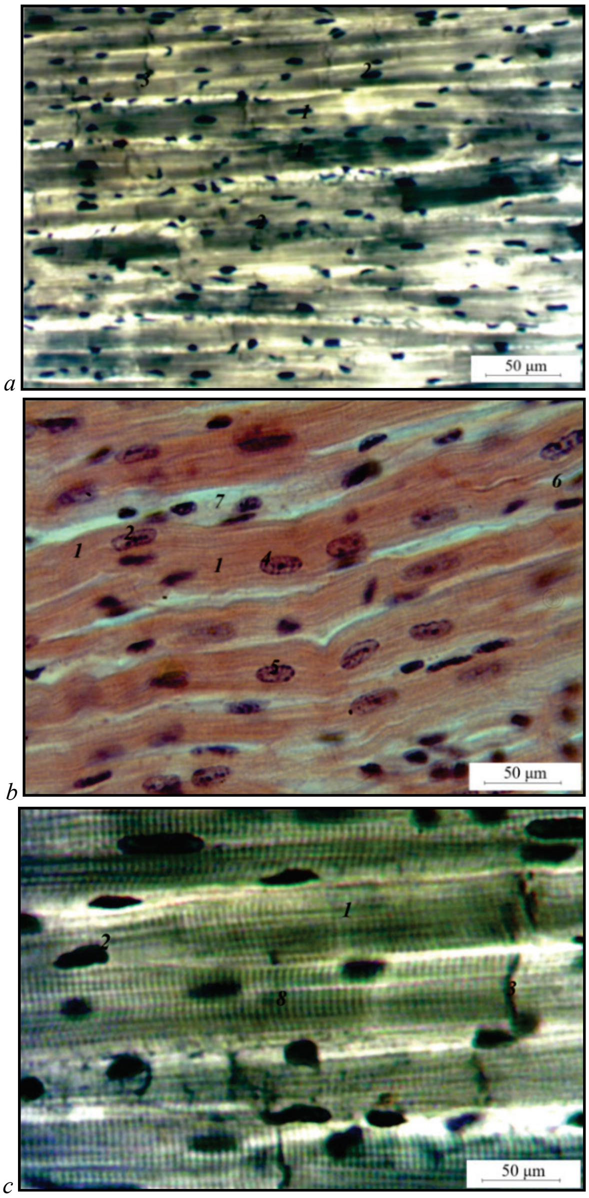

Fig. 3. Microscopic structure of myocardium: *a* – right ventricle of horse; *b* – left ventricle of cattle; *c* – left ventricle of horse: *1* – cardiomyocytes; *2* – nucleus of cardiomyocyte; *3* – intercalated disc; *4* – nucleolus; *5* – nuclear chromatin; *6* – anastomoses; *7* – connective tissue between muscles; *8* – transversal stripes; heidenhain's staining

According to the results of the studies, cardiomyocytes that form muscular tissues vary in length and thickness depending on the species of animal and their morphotopography (myocardium of left and right ventricles). At the same time, thickness of cardiomyocetes in the left ventricle was almost the same for sheep and horses, equaling 8.98 ± 0.64 µm and 9.32 ± 0.72 µm respectively. This parameter was higher in cattle, measuring 14.06 ± 0.41 µm (Fig. 4, Table 2). According to cariometric studies, mean volume of nuclei in experimental animals varied. The highest parameter was found in cattle (124.55 ± 7.99 µm^3), then in horses (102.96 ± 7.54 µm^3) and lowest in sheep (53.42 ± 5.18 µm^3), which was likely related with species specifics of the organism of animals in general (Fig. 5, Table 2).

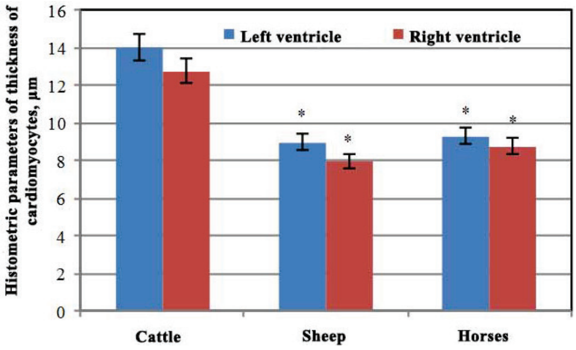

Fig. 4. Histometric parameters of thickness of cardiomyocytes of the experimental animals (x ± SE, n = 5): * – P < 0.05 – compared with cattle

The detailed analysis we conducted in morphometric studies of myocardium microstructures indicates that the layer of cardiomyocytes and volume of their nuclei in the right ventricle of the heart of experimental animals were slightly lower than in the left. Therefore, in cattle, thickness of cardiomyocytes of the right ventricle was 1.10 times less than the left, equaling – 12.8 ± 0.4 µm, the volume of nuclei of cardiomyocytes – 121.7 ± 7.0 µm^3. Similar changes in those histometric parameters were found in sheep and horses (Fig. 4, 5, Table 2). Increase in thickness of cardiomyocetes of myocardium of the left ventricle of experimental animals, their nuclei, compared with the right ventricle, are related to the functional peculiarities of this myocardium tissues, which are capable of spontaneous rhythmic contractions, promoting blood flow in the vessels: cardiomyocytes of the left ventricle carry heavier load, promoting blood flow in the vessels of greater circulation, and therefore, cardiomyocytes of the right ventricle have a lower load, promoting blood flow in the vessels of lesser circulation.

Table 2
Histometric parameters of myocardium of cattle, sheep and horses (x ± SE, n = 5)

Groups of animals	Thickness of cardiomyocytes of heart ventricles, µm		Volume of nuclei of cardiomyocytes of heart ventricles, µm^3	
	left	right	left	right
Cattle	14.06 ± 0.41	12.79 ± 0.38	124.55 ± 7.99	121.67 ± 7.02
Sheep	8.98 ± 0.64*	7.96 ± 0.56*	53.42 ± 5.18**	52.85 ± 4.33**
Horses	9.32 ± 0.72*	8.77 ± 0.74*	102.96 ± 7.54	101.44 ± 6.71*

Note: statistically significant differences were taking into account compared with cattle: * – P < 0.05; ** – P < 0.01; *** – P < 0.001.

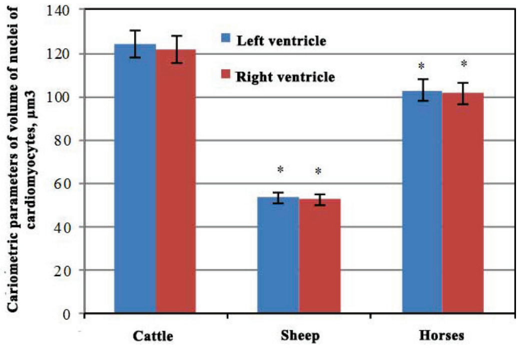

Fig. 5. Cariometric parameters of volume of nuclei of cardiomyocytes of experimental animals (x ± SE, n = 5):* – P < 0.05 – compared with cattle

Discussion

Currently, important parameters are histo- and cytometric studies of organs and systems in clinically healthy people and animals (Hnatiuk et al., 2010; Autifi et al., 2015; Blagojević et al., 2018; Bilash et al., 2019), which are criteria for diagnosing diseases of infectious (Johnson-Delaney

& Orosz, 2011; Zhang et al., 2011) and non-infectious pathologies (Dzau et al., 2006; Stakhurska & Pryshliak, 2014). The effectiveness of morphometric evaluation of the structural-functional condition of the organism of animals was confirmed at organic, tissue and cellular levels (Ramchandi et al., 2001; Mits et al., 2016). Mathematical analysis of structures of morphometric objects became recognized as a method characterized by objectiveness and accuracy. This direction is broadly used in modern pulmonology and cardiology (Hyde et al., 2009).

Thus, respiratory organs and cardiovascular system are interrelated and perform vitally important functions in the organism, the main being gas exchange by inhaling from environment and emitting carbon dioxide from organism into environment (Hyde et al., 2009; Koptyev et al., 2014). Gas exchange is performed directly in the lungs, between air and blood by diffusion of air and carbon dioxide through the walls of the pulmonary alveoli into blood capillaries (Kargopol'ceva et al., 2020).

The research we conducted revealed that the histological structure of myocardium and lungs in the studied clinically healthy farm animals – in comparative aspect – has distinct histoarchitectonics, characteristic for other species of mammals (Bahar et al., 2007; Misfeld & Sievers, 2007), though it varies by morphometric parameters. Such studies are not only educational, but also fundamental for clinical veterinary medicine, and also important for studying the constitution of animalsin zootechnics (Vansjackaja & Kirpaneva, 2014).

Therefore, according to the results of histometric studies, the respiratory zone of the lungs is most developed in horses. In cattle and sheep, such parameters were much lower. Connective tissue septum of lung parenchyma, by contrast, was most notable in cattle, then in sheep and even less in horses. At the same time, the mean parameter of volume of lung alveoli varied: the highest parameter was seen in horses, the lowest in sheep, and average in cattle. In our opinion, such contradictory morphometric results for lung parenchyma in studied farm animals indicate adaptive specifics of the organism of animals to living conditions, and therefore the most developed respiratory zone of lungs was found in horses, the animals that experience notable physical and physiological load on corresponding organs and systems.

As a result of morphometric studies of myocardium, we determined some characteristics of sizes of thickness of its cardiomyocytes and volume of their nuclei, depending on species of animals and morphofunctional activity of muscular tissue of myocardium of heart ventricles, which is to a certain degree coherent with results obtained by other scientists (Zagorujko & Zagorujko, 2017). Therefore, according to the results of M. S. Hnatiuk, cardiomyocytes of the atria had similar diameter, compared with cardiomyocytes of ventricles, measuring 5 to 30 µm in left and right atria, while equaling 10 to 45 µm in left and right ventricles. According to our histological studies, in myocardium of heart ventricles, there are cardiomyocytes of various thicknesses. At the same time, morphometric parameters (thickness of cardiomyocytes, mean volume of their nuclei) in ventricle myocardium were expressed the most in cattle, then in horses and sheep, and their histometric parameters in the left ventricle were higher than in the right ventricle.

We attribute such contradictory histometric parameters (thickness of cardiomyocytes, volume of their nuclei) of myocardium of the left ventricle in experimental animals – compared with the right ventricle – to the activities of the ventricles: the left mainly functions as a pump, and the right – as a volumetric, and also to the morphofunctional specifics of this myocardium tissue, capable of spontaneous rhythmic contractions. As a result of such contractions, there is blood flow in vessels, and at the same time, cardiomyocytes of the left ventricle carry a heavier load, promoting blood flow in the vessels of the greater circulation, respectively cardiomyocytes of the right ventricle carry much less load, promoting blood flow in the vessels of the lesser circulation.

Therefore, analysis of the results of histological studies indicates that microscopic structure of myocardium of the right and left ventricles of the heart of the experimental animals was similar. However, taking into account the expediency of using histometric methods of study (Hnatiuk et al., 2010; Slabyi, 2017), which make it possible to determine insignificant changes which it would be impossible to notice in regular histological studies, we carried out histometry of thickness of contractile cardiomyocytes, volume of their nuclei of left and right ventricles in experimental animals.

Conclusion

The left lungs of sheep and cattle consist of three lobes – cranial, middle and caudal, the right lung comprise four – cranial, middle, caudal and accessory lobes. The left lung of horses comprises two lobes – cranial and caudal, the right has three lobes – cranial, caudal and accessory.

Relative weight of lung lobes in experimental animals varied: the highest percentage was seen for caudal lobes of right and left lungs, then for cranial, middle and accessory. At the same time, morphometric parameters of the lobes of the right lungs of experimental animals were higher than in the left lung, resulting into clearly expressed asymmetry between right and left lungs, equaling 1:1.37 in sheep and cattle, and 1:1.20 in horses. The respiratory zone of lung parenchyma was better expressed in horses – 54.8 ± 7.4% of the general area of the organ. In ruminants, this parameter was lower: 44.8 ± 3.4% in cattle and 47.0 ± 7.6% in sheep. Connective tissue septum, by contrast, was developed better in cattle and sheep and less in horses. Mean volume of lung alveoli in clinically healthy animals varied: the highest was in horses – 699 ±106 thou μm^3, then in cattle – 337 ± 43 thou μm^3 and sheep – 158 ± 37 thou μm^3.

The microscopic structure of the heart myocardium in cattle, sheep and horses was similar, but varied by certain cytometric parameters, depending on morphofunctional activity of ventricles (the left functions mainly as a pump, the right – as volumetric) and species specifics of organisms of experimental animals:

– thickness of contractile cardiomyocytes in ventricle myocardium was greatest in cattle (right – 12.8 ± 0.4 µm, left 14.1 ± 0.4 µm), then in horses (8.8 ± 0.7 and 9.3 ± 0.7 µm) and sheep (7.9 ± 0.6 and 8.9 ± 0.6 µm) respectively;

– mean volume of nuclei of cardiomyocytes was highest in cattle (121.7 ± 7.0 μm^3 in the right ventricle, 124.6 ± 7.9 μm^3 in the left ventricle, then in horses (101.4 ± 6.7 and 102.9 ± 7.5 μm^3) and sheep (52.9 ± 4.3 and 53.4 ± 5.2 μm^3, respectively). At the same time, their histometric parameters of myocardium of left ventricle of the heart in experimental animals were higher than in the right.

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