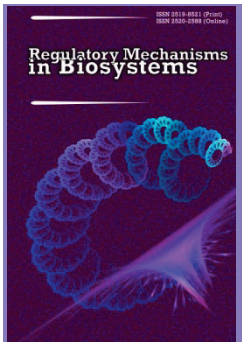

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Morphofunctional features of the esophageal tonsil in some wild and domestic bird species

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It is well known that the esophageal tonsil belongs to the peripheral immune system of birds. Its functional basis is formed by lymphoid tissue, in which, under the influence of antigens, lymphocytes differentiate into effector cells, causing specific immunity. Material for histological research was selected from adults of 18 species of wild and domestic birds, belonging to 6 orders. When performing the experiment, the classical methods of staining histopreparations with hematoxylin and eosin, according to Mallory, Van Gieson, were used and impregnated with argentic nitrate according to Kelemen. The object for cytological, electron microscopic and immunohistochemical studies was the esophageal tonsil of *G. gallus*. In the course of this work it was demonstrated that the esophageal tonsil is located in the mucous membrane and submucosa of the junction between the caudal part of the esophagus and the glandular stomach (proventriculus). Most of the examined bird species (Galliformes – *G. gallus*, *N. meleagris*, *C. coturnix*, *M. gallopavo*, *P. colchicus*, *B. bonasia*, *P. crictatus*; Anseriformes – *A. platyrhynchos*, *A. anser*, *B. canadensis*; Passeriformes – *P. pica*, *C. cornix*; Ciconiiformes – *C. ciconia*) have an esophageal tonsil which was detected macroscopically. It has an annular shape, a plicated and uneven surface with holes of crypts and light pink colour. Its size is different in the birds of the studied species. It was recorded that the greatest length is in *B. canadensis* and *C. ciconia*, and the smallest is in *C. coturnix* and *P. pica*, the maximum width is in *A. platyrhynchos*, and the minimum width is in *P. pica* and *C. cornix*. In *L. lagopus* (Galliformes), *G. glandarius* (Passeriformes), *G. chloropus*, *F. atra* (Gruiformes) and *C. livia* (Columbiformes) the esophageal tonsil is not macroscopically visible. Most birds of the examined species have tonsils apposing directly with the glandular part of the stomach. Only *A. anser* and *B. canadensis* have a transition zone between them, the morphology of which differs from that of the tonsils and the glandular part of the stomach. Lymphoid tissue in birds of most species is represented by all levels of structural organization. It occupies the largest area in the tonsil of *A. platyrhynchos* and the smallest in *L. lagopus*. The area of lymphoid tissue and its location are different in the birds of the examined species. According to this criterion it is proposed to classify the tonsils into compact and diffuse ones. Reticular cells, lymphocytes, immunoblasts, plasma cells, monocytes and macrophages have been identified in lymphoid tissue of esophageal tonsil of *G. gallus*. Among the lymphocytes their subpopulations were found that respond to monoclonal antibodies with markers CD4+, CD8+ and CD20+. CD34+cells were not found in the tonsil.

Keywords: esophageal tonsil; lymphoid tissue; lymphoid nodules; lymphoid cells; CD-markers; birds.

Introduction

It is known that the esophageal tonsil belongs to the peripheral immune system of birds. Its functional basis is formed by lymphoid tissue, in which, under the influence of antigens, lymphocytes differentiate into effector cells, causing specific immunity (Korver, 2006; Davison, 2014; Koutsos & Klasing, 2014; Kaspers & Göbel, 2016). In addition, there are data in scientific articles that the development of B-lymphocytes occurs in mammalian intestinal lymphoid tissue, whereas in birds, B-lymphocytes are formed in the cloacal bursa (Fellah et al., 2014). The cloacal bursa is absent in mammals.

One of the main features of peripheral organs of the immune system is their location. They are always localized in areas of possible penetration of antigens into an animal's organism (Nasrin et al., 2012; Doneley, 2016; Junior et al., 2018; Nochi, et al., 2018). It is well known, that most antigens penetrate into an animal's body through the digestive system. According to this, there is about 70% of the lymphoid tissue in their walls, this tissue forms the functional part of peripheral organs of the immune system. In its development, it has four levels of structural organization: a diffuse form, prenodules, primary and secondary lymphoid nodules, which are developed in the same sequence and provide for the needs of an animal's organism at certain stages of its growth. Presence of all levels of the structural organization of lymphoid tissue in the peripheral organs of the immune system indicates its complete morphofunctional maturity and, consequently, the maturity of these organs, that is, their ability to give a full response to the action of the antigens (Nochi et al., 2018).

In mammals, the first barrier to the penetration of antigens through the digestive system is the pharyngeal lymphoid ring or Waldeyer's lymphatic ring (Cesta, 2006; Ruddle et al., 2009), which is absent in birds. In birds, this function is performed by the esophageal tonsil, which is located in the mucous membrane and submucosal part of the caudal part of the esophagus before its penetration into the glandular part of the stomach (proventriculus) (Casteleyn, 2010; Samour, 2015; Al-Juboury et al., 2016).

Despite the significant role of the esophageal tonsil in maintaining the immune homeostasis, its structure and, consequently, functional features in birds are insufficiently studied. The most complete morphofunctional features of the esophageal tonsil are studied in the ontogenesis of *G. gallus* (Dyshliuk, 2010, 2018) and *A. platyrhynchos* (Khomich & Usenko, 2013) and fragmentarily in other species of poultry (Dyshliuk & Orlova, 2017; Lyashchinskiy & Usenko, 2019). Survey studies

of topography and the esophageal tonsil structure in certain species of wild birds were conducted by Kovtun & Kharchenko (2005) and Kharchenko & Lykova (2013). In their opinion, the development of the esophageal tonsil depends on the trophic specialization of birds. However, literature data of the morphology of the esophageal tonsil of wild and domestic species of birds require significant rectification. This is connected to the topography, linear macroscopic measurements, the features of the location and content of lymphoid tissue and individual levels of its structural organization. Cellular composition of the lymphoid tissue of the esophageal tonsil of birds is insufficiently studied. There is also no information about the placement of subpopulations of lymphocytes in it.

Materials and methods

The histological studies were conducted in 2016–2020 at the immunomorphology scientific laboratory of the Department of Anatomy, Histology and Pathomorphology of Animals named after academician G. Kasianenko, National University of Life and Environmental Sciences of Ukraine (Kyiv). Electron microscopic studies were conducted at the laboratory of electron microscopy at Bogomolets National Medical University and immunohistochemical studies – in the pathomorphological laboratory of "CSD HEALTH CARE" (Kyiv). All manipulations with birds in the experiment were performed in accordance with the ethical norms of international and Ukrainian law. The birds were decapitated under light ether anesthesia.

Material for research was selected from 18 species of wild and domestic adult birds, which belong to 6 orders (Table 1). Domestic birds were purchased from farms in Kyiv, Zhytomyr and Cherkasy regions. Material from wild birds was taken from collections of the I. I. Schmalhausen Institute of Zoology of the National Academy of Sciences of Ukraine. The birds were clinically healthy and had no signs of diseases.

Table 1
Characteristics of the birds from which the material was taken

Order	Species	Poultry/ wild	Number	Body weight, kg (x ± SE)
Galliformes	*Gallus gallus* (Linnaeus, 1758)	poultry	5	1.42 ± 0.02
	Numida meleagris (Linnaeus, 1758)	poultry	5	1.58 ± 0.02
	Coturnix coturnix (Linnaeus, 1758)	poultry	5	0.17 ± 0.01
	Meleagris gallopavo (Linnaeus, 1758)	poultry	5	4.78 ± 0.07
	Phasianus colchicus Linnaeus, 1758	wild	5	1.05 ± 0.01
	Bonasa bonasia (Linnaeus, 1758)	wild	3	1.05 ± 0.01
	Lagopus lagopus (Linnaeus, 1758)	wild	3	0.63 ± 0.08
	Pavo crictatus Linnaeus, 1758	wild	3	4.19 ± 0.08
Anseriformes	*Anas platyrhynchos* (Linnaeus, 1758)	poultry	5	2.68 ± 0.04
	Anser anser (Linnaeus, 1758)	poultry	5	3.10 ± 0.03
	Branta canadensis (Linnaeus, 1758)	wild	3	4.19 ± 0.25
Passeriformes	*Pica pica* (Linnaeus, 1758)	wild	3	0.17 ± 0.08
	Garrulus glandarius (Linnaeus, 1758)	wild	3	0.15 ± 0.04
	Corvus cornix Linnaeus, 1758	wild	3	0.51 ± 0.02
Gruiformes	*Gallinula chloropus* (Linnaeus, 1758)	wild	3	0.24 ± 0.05
	Fulica atra Linnaeus, 1758	wild	3	0.85 ± 0.02
Ciconiiformes	*Ciconia ciconia* (Linnaeus, 1758)	wild	3	3.43 ± 0.07
Columbiformes	*Columba livia* Gmelin, 1789	wild	3	0.28 ± 0.01

The studies began with the weighing of the birds' carcasses, after that the prosection was made and the caudal part of the esophagus was prepared in the chest-abdominal cavity. On the mucous membrane of this part, we made the topography of the esophageal tonsil, its surface, colour, linear measurements (length and width) and the number of folds of the mucosa membrane. After this, the material for histological studies was selected, it was labeled and fixed in a 10% solution of neutral formalin. After fixation in formalin, the selected material was washed in running water, dehydrated in alcohols of increasing concentration, sealed and poured into paraffin according to the conventional method. The paraffin-embedded material was placed on wooden blocks, of which histological sections 5–10 μm thick were made on the MPS-2 microtome. The histological slides of esophageal tonsil were stained by hematoxylin and eosin, Mallory and Van Gieson (Goralsky et al., 2011). Levels of structural organization of lymphoid tissue of the esophageal tonsil were determined on preparations impregnated with argentic nitrate by Kelemen. Morphometric methods of examination were used to compare the quantitative characteristics of the structures of the esophageal tonsil of the birds. The object for cytological, electron microscopic and immunohistochemical studies was the esophageal tonsil of *G. gallus*. Cytological studies were carried out on imprints that were stained with the commercial paints LeikoDiff 200 (Erba Lachema, Czech Republic). The cells of the esophageal tonsil were identified by microscope "Olympus".

Electron microscopy studies were performed according to the method of Vlasov et al. (2011). For these studies, the material was taken no later than 5 minutes after the birds were decapitated. The examined structures were cut into pieces of 1.5 mm^3, fixed in 2.5% glutaraldehyde for 1 hour at +4 °C, washed with 0.1 M Na-cocadylate buffer and again fixed in 2% solution of osmic acid. The slices were then dewatered in ethanol of increasing concentration and in acetone and poured into a mixture of epon araldit according to the generally accepted method. The specimens were placed in a capsule and filled with a mixture of epoxy resins (epon and araldit), which were polymerized for 24 h at +37 ºC and 24 h at +60 ºC. Ultra-thin sections 50–90 nm in thickness were obtained on ultramicrotome LKB-III B with the use of glass disposable knives. The slices were applied with the support (collodium) and transferred to grids, contrasted with solutions of uranyl acetate and lead citrate, and examined under a SELMI transmission electron microscope PEM-125K. Morphological subjects were photographed with a camera built-in an electron microscope on a black-and-white film and analyzed.

Immunohistochemical studies of the main reactions of cellular and humoral immunity were performed on histological sections using monoclonal antibodies (Danish firm DAKO) and visualization system (DAKO EnVision FLEX+ detection system). The preparations were additionally stained with Mayer's hematoxylin for 1–3 minutes, after that they were placed in an Eukitt. On histopreparations, we detected the subpopulations of lymphocytes expressing antigenic markers of CD4+ (T helper cells), CD8+ (T-cytotoxic / T-suppressors), CD20+ (mature B-lymphocytes), and cells that react to monoclonal antigens CD34+. They were examined by using the "Olympus" microscope and the characteristics of the lymphocytes' placement were determined due to the different types of markers.

Results

Topography and macrostructure of the esophageal tonsil of birds. It is confirmed that the esophageal tonsil of birds is located in the mucous membrane and submucosa of the junction between the caudal part of the esophagus and the proventriculus. In *A. anser* and *B. canadensis* (Anseriformes), according to our observations, there is the area of the esophagus between the esophageal tonsil and proventriculus which is different in macro- and microstructure. We called this area a transition zone (Fig. 1). There are no esophageal glands and lobules of the deep glands in this area, and the slight local accumulations of diffuse lymphoid tissue are found in the mucous membrane and submucosal basis.

In most examined bird species (Galliformes – *G. gallus*, *N. meleagris*, *C. coturnix*, *M. gallopavo*, *P. colchicus*, *B. bonasia*, *P. crictatus*; Anseriformes – *A. platyrhynchos*, *A. anser*, *B. canadensis*; Passeriformes – *P. pica*, *C. cornix*; Ciconiiformes – *C. ciconia*), the esophageal tonsil is detected macroscopically (Fig. 1, 2). It has an annular shape, a plicated and uneven surface with holes of crypts and light pink colour.

Length and width of the esophageal tonsil of birds could be different (Fig. 3). The largest length is registered in the *B. canadensis* and *C. ciconia*, and the smallest – in *C. coturnix* and *P. pica*. The maximum values of the width are registered in the *A. platyrhynchos*, and the minimum are in the *P. pica* and *C. cornix*. In *L. lagopus* (Galliformes), *G. glandarius* (Passeriformes), *G. chloropus*, *F. atra* (Gruiformes) and *C. livia* (Columbiformes) the esophageal tonsil is not macroscopically visible. The number of longitudinal folds which the mucous membrane form of the area of esophageal tonsil in birds is significantly different. *C. ciconia* has the largest number of these folds (11–13), and the Galliformes – the smallest number (5–7). In birds of other species there could be from 6 to 12. It was found that the thickening of the wall of the esophagus is mainly due to

increasing of the height and width of folds of the mucous membrane in most of birds in which the esophageal tonsil is revealed macroscopically.

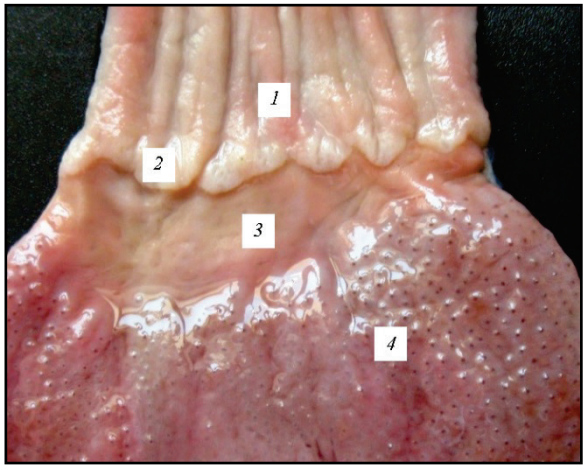

Fig. 1. Area of esophageal tonsil of *A. anser*: esophagus (*1*), esophageal tonsil (*2*), transition zone of the esophagus (*3*), proventriculus (*4*)

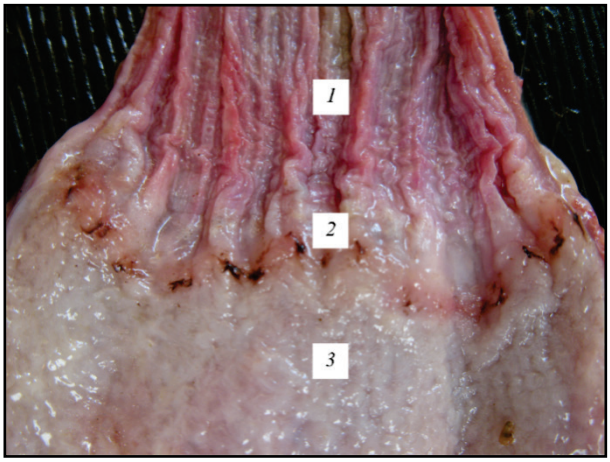

Fig. 2. Area of esophageal tonsilof *C. ciconia*: esophagus (*1*), esophagealtonsil (*2*), proventriculus (*3*)

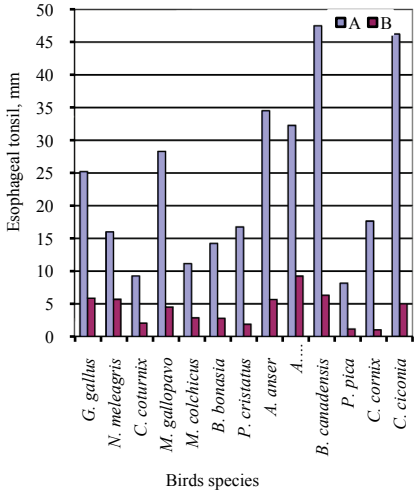

Fig. 3. Result of length (*A*) and wight (*B*) of esophageal tonsil of birds in which it is detected macroscopically

Microstructure the area of esophageal tonsil of birds. The mucous membrane in the area of esophageal tonsil is formed by the epithelium, lamina propria, lamina muscularis, which borders with the submucosa. The mucosal epithelium is stratified squamous. In some species of birds it can be keratinized (Galliformes – *M. gallopavo*, *P. colchicus*, *B. bonasia*, *L. lagopus*, *P. crictatus*; Anseriformes – *A. platyrhynchos*, *A. anser* and Ciconiiformes – *C. ciconia*), non keratinized (Galliformes – *C. coturnix*; Passeriformes – *P. pica*, *G. glandarius*, *C. cornix*; Gruiformes – *G. chloropus*, *F. atra* and Columbiformes – *C. livia*) and partly keratinized (Galliformes – *G. gallus*, *N. meleagris* and Anseriformes – *B. canadensis*). Lamina propriamucosae and submucosa are formed by a loose connective tissue, and the lamina muscularis is by the smooth muscle. The latter is well developed in *N. meleagris*, *C. coturnix*, *P. crictatus*, *A. platyrhynchos* and *B. canadensis*. The lamina muscularis is weakly developed in birds of other examined species.

The lamina muscularis of the area of the esophageal tonsil is formed by a smooth muscle tissue. The number of its layers in birds is uneven. In *N. meleagris*, *M. gallopavo*, *P. colchicus*, *L. lagopus*, *P. crictatus* and *C. ciconia* it is formed by three well-developed layers of bundles of smooth muscle cells: internal and external longitudinal and middle circular. In *G. gallus*, *C. coturnix*, *B. bonasia*, *A. platyrhynchos*, *B. canadensis*, *G. chloropus*, *F. atra* and *C. livia* the smooth external layer is weakly expressed, represented only by separate bundles of smooth muscle cells. In *A. anser*, *P. pica*, *G. glandarius* and *C. cornix* the muscular layer has a two-layer structure and is formed by internal longitudinal and external circular layers. The serous membrane is formed by a loose connective tissue that is covered with a mesothelium.

The acini of the esophageal glands and lymphoid tissue are in the lamina of the mucous membrane and the submucosal basis of the location of the esophageal tonsil of the birds of the examined species. In the area which is located closer to the proventriculus there are the deep sections of glands in the submucosa. In some species of birds the lymphoid tissue is located compactly, in others – diffusely. Therefore, it was proposed to classify esophageal tonsils into two types: compact and diffuse. Galliformes (*G. gallus*, *N. meleagris*, *C. coturnix*, *M. gallopavo*, *P. colchicus*, *B. bonasia*, *P. crictatus*), Anseriformes (*A. platyrhynchos*, *A. anser*, *B. canadensis*), Passeriformes (*P. pica*, *C. cornix*) and Ciconiiformes (*C. ciconia*) have compact esophageal tonsils (Fig. 4–6). *L. lagopus* (Galliformes), *G. glandarius* (Passeriformes), *G. chloropus*, *F. atra* (Gruiformes) and *C. livia* (Columbiformes) have diffuse esophageal tonsils (Fig. 7, 8).

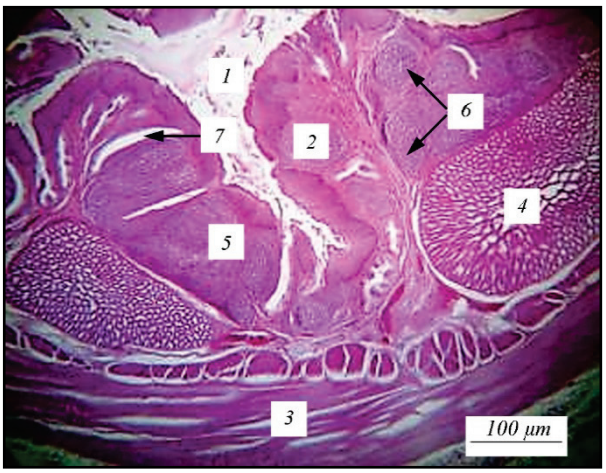

Fig. 4. Area of esophageal tonsil of *B. bonasia*: lumen of the esophagus (*1*), fold of mucous membrane (*2*), muscular layer (*3*), lobule of deep gland (*4*), diffuse lymphoid tissue (*5*), lymphoid nodule (*6*), esophageal gland (*7*); staining with hematoxiline and eosin

The lymphoid tissue occupies an unequal area in the mucous membrane and in the submucosal base of the area of the esophageal tonsil. It is the largest in the birds with its compact location and much smaller in those with a diffuse location. Lymphoid tissue replaces part of the acini of esophageal glands and partially their excretory ducts. The replacement of acini begins with the infiltration of their epithelium by lymphoid cells from adjacent lymphoid tissue. At the same time, there is also the infiltration of the epithelium of excretory ducts (Fig. 9). The result is the formation of lymphoepithelium. Later, the lymphoid tissue deforms acini of glands and enters them. It also partially penetrates the

ducts of the glands. However, there is a formation of crypt-like formations, instead of secretory units and the ducts of the glands. The crypt-like formations are similar to esophageal crypts and they are the epithelium deepening into the thickness of the esophageal tonsil.

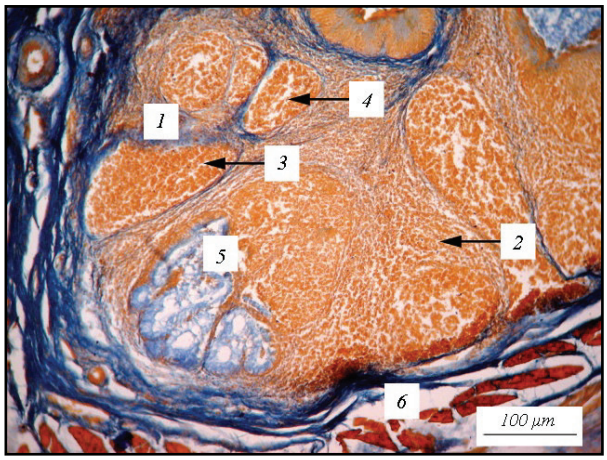

Fig. 5. Area of esophageal tonsil of *G. gallus*: mucous membrane (*1*), diffuse lymphoid tissue (*2*), primary lymphoid nodule (*3*), secondary lymphoid nodule (*4*), esophageal gland (*5*), muscular layer (*6*); staining by Mallory

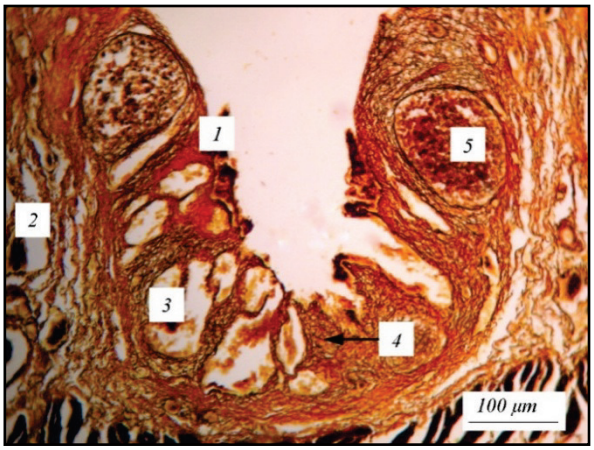

Fig. 6. Area of esophageal tonsil of *C. cornix*: epithelium (1), fold (*2*), esophageal gland (*3*), diffuse lymphoid tissue (*4*), lymphoid nodule (*5*); argentic nitrate impregnation by Kelemen

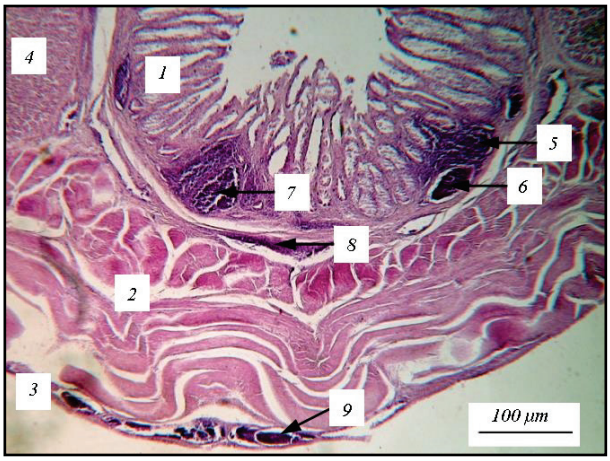

Fig. 7. Area of esophageal tonsil of *F. atra*: mucous membrane (*1*), muscular layer (*2*), serous membrane (*3*), lobules of deep glands (*4*), diffuse lymphoid tissue (*5*), primary lymphoid nodule (*6*), secondary lymphoid nodule (*7*), lymphoid tissue bordering with muscular layer (*8*), lymphoid tissue in serous membrane (*9*); staining with hematoxiline and eosin

The lymphoid tissue of the esophageal tonsil in most of examined bird species is represented by all levels of its structural organization: a diffuse form, prenodules, primary and secondary lymphoid nodules. Its area in tonsils is different (Fig. 10). The largest area of the esophageal tonsils in all birds of the examined species is the diffuse lymphoid tissue and the smallest is the prenodules. The largest area of primary lymphoid nodules is registered in *B. bonasia*, and secondary lymphoid nodules – in *M. gallopavo*. In birds of the Gruiformes and *L. lagopus* we did not detect prenodules, but in *G. glandarius* and *C. livia*, lymphoid tissue is represented only by a diffuse form.

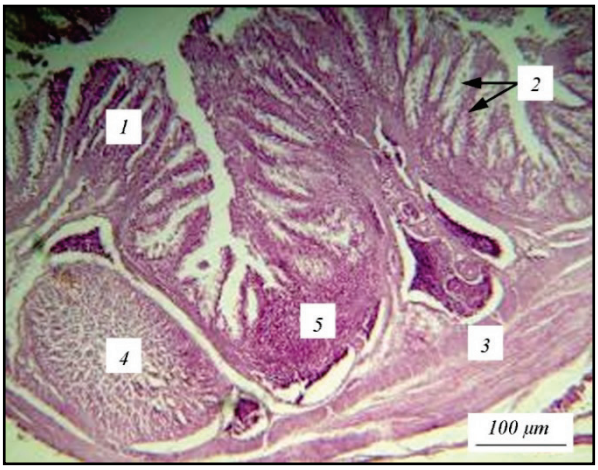

Fig. 8. Area of esophageal tonsil of *C. livia*: mucous membrane (*1*), esophageal gland (*2*), muscular layer (*3*), lobule of deep gland (*4*), diffuse lymphoid tissue (*5*); staining with hematoxiline and eosin

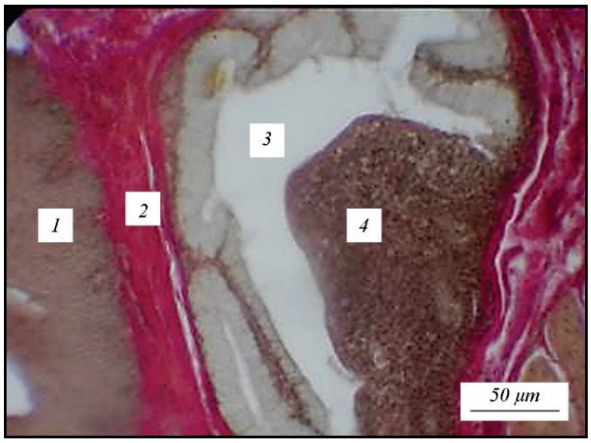

Fig. 9. Lymphoid tissue in gland of esophageal tonsil *G. gallus*: epithelium (*1*), collagen fibers (*2*), esophageal gland (*3*), lymphoid tissue (*4*); staining by Van Gieson

Cellular composition of lymphoid tissue of the esophageal tonsil of G. gallus. On imprints and on the electrongrams of the esophageal tonsil *G. gallus*, we found cells that are characteristic of the peripheral organs of immune system. They are reticulocytes, large, medium and small lymphocytes, immunoblasts, plasmocytes, monocytes and macrophages (Fig. 11, 12). Reticular cells form the basis of lymphoid tissue, its cells are in the loops of the fibers. They are masked by cells of the lymphoid series. Reticular cells form lots of ramified processes. The nucleus is located in the centre, it is large and mostly has an oval shape. It contains one nucleole and some heterochromatin. There are small, rounded and elongated-oval shapes of mitochondria, ribosomes, elements of a granular endoplasmic reticulum and Golgi apparatus in the cytoplasm.

Lymphocytes predominantly have small and medium forms. Lymphocytes have a round shape and a large nucleus, which occupies almost the whole area of the cell. There is a lot of heterochromatin in the nucleus. It is located freely, with separate lumps, throughout the plane and near the nuclear membrane. There are few mitochondria with a round shape and weakly expressed cristae, elements of the granular

endoplasmic reticulum, ribosomes and their accumulations in the cytoplasm of the lymphocytes.

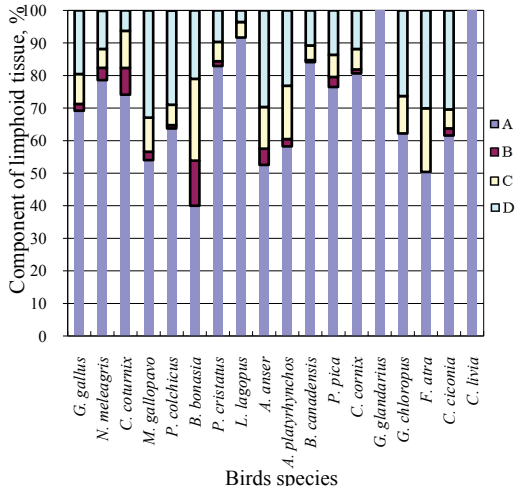

Fig. 10. Content of components (diffuse form (*A*), prenodules (*B*), primary (*C*) and secondary (*D*) limpoid nodules) lymphoid tissue in esophageal tonsil of the birds (%)

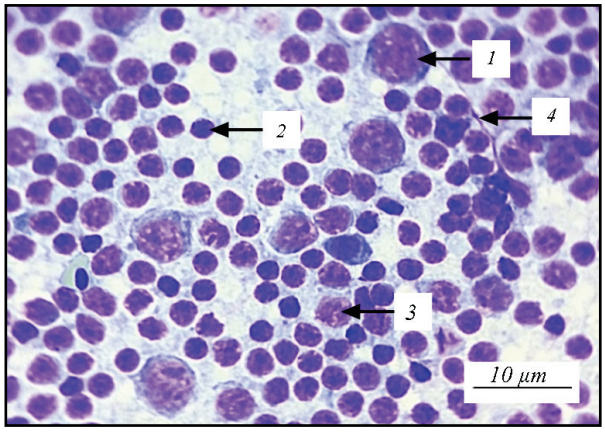

Fig. 11. The cells of esophageal tonsil of *G. gallus*: immunoblast (*1*), small (*2*) and middle (*3*) lymphocytes, reticular cell (*4*); Staining Leukodif 200

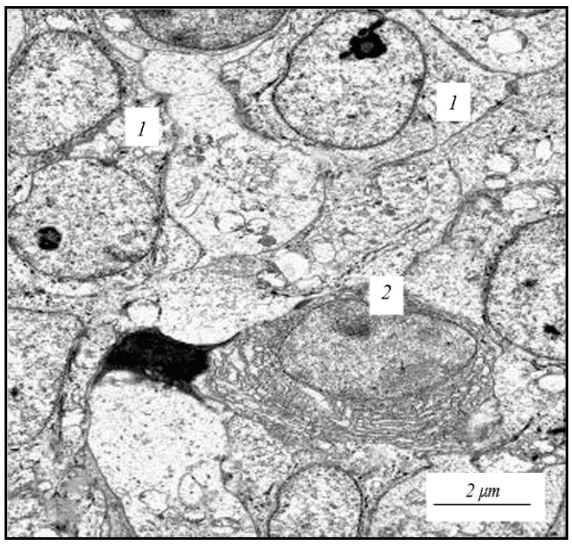

Fig. 12. Ultrastructure of cells in esophageal tonsil of *G. gallus*: lymphocytes (*1*), plasmacyte (*2*)

Immunoblasts are cells of the fifth class of lymphopoiesis (by Chertkov & Vorobyov, 1981). They are larger than lymphocytes, have a round or slightly elongated shape. The size of their cytoplasm significantly exceeds that of cytoplasm in lymphocytes. The cytoplasm of these cells is stained weakly basophilic. The nucleus is spherical, contains preferably two nucleoles, the heterochromatin is uniformly spread in the nucleoplasm. The cytoplasm is light, has a small number of organelles, such as large mitochondria of round shape with a light matrix and weakly expressed cristae, polyribosomes, elements (tubules and cisternae) of the endoplasmic reticulum, lysosomes, elements of the Golgi apparatus.

Plasmocytes are effector cells of B-lymphocytes. The nucleus is located more eccentrically there. It contains well-defined lumps of heterochromatin, which form a definitive pattern in the form of knitting needles of a wheel. There is a noticeable area of clearing near the nucleus. The size of the basophilic cytoplasm significantly exceeds the size of the nucleus. It is almost completely filled with granular endoplasmic reticulum, its tubules are considerably widened. A few large mitochondria of oval and round shapes with a light matrix, ribosomes and their accumulation are also noticeable in the cytoplasm.

Monocytes have a large size and a horseshoe nucleus. The lumps of heterochromatin are uniformly spread throughout the nucleoplasm in the nucleus. The cytoplasm has rounded mitochondria with light matrix, lysosomes and endoplasmic reticulum. Monocytes are differentiated into macrophages. Macrophages are branching, elongated shape and have got an oval nucleus, which contains a small amount of heterochromatin, which is fixed to the internal membrane of the nucleus membrane and is sputtered in the nucleoplasm. The cytoplasm has a considerable size and forms processes of various shapes and sizes. There are many large round-shaped lysosomes, small phagosomes, but there are slightly fewer mitochondria and tubules of the endoplasmic reticulum in the cytoplasm.

Placing subpopulations of lymphocytes, expressing antigenic markers in the esophageal tonsil of G. gallus. In the esophageal tonsil of *G. gallus* among the lymphocytes, there are three groups with markers CD4+, CD8+ and CD20+. The most of them are CD20+ lymphocytes (Fig. 13). They are located diffusively in lamina and mucous membrane, under epithelium, around esophageal glands and their ducts, blood vessels and in lymphoid nodules. CD4+-lymphocytes are located locally near the esophageal glands, between them, in the epithelium of their ducts, in the lower layers of the surface epithelium, near the lymphoid nodules and in them. In these nodules, they are arranged in a chain on the periphery and singly in the center (Fig. 14). CD8+ lymphocytes are recorded singly in diffuse lymphoid tissue between the nodules, and they form cell accumulations in the form of a crescent in separated lymphoid nodules (at one of the poles). Cells responsive to monoclonal antibodies CD34+ in the tonsil were not detected.

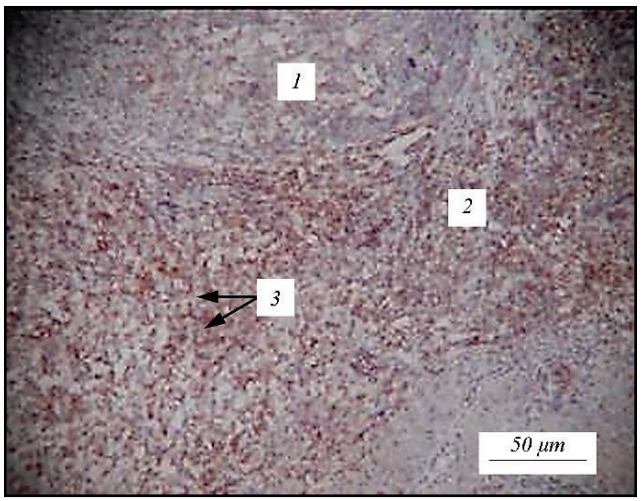

Fig. 13. The placement of lymphocytes with markers CD20+ in the esophageal tonsil of *G. gallus*: lymphoid nodule (1), diffuse lymphoid tissue (*2*), markers (*3*); histopreparation with the use of monoclonal antibodies

Discussion

It is known that the development of lymphoid tissue that is associated with the mucous membrane of the tubulose digestive organs depends on the action of the antigens on their wall and the duration (Jeurissen et al., 1989; Sharma, 1991; Friedman et al., 2003; Davison et al., 2014). In our opinion, this became the cause of the development of the esophageal tonsil in birds, which is located in the wall of the esophagus before it passes into the proventriculus. The supply of feed to the proventriculus is regulated by its sphincter (Wakhhab & Bushukina, 2017). If this part of the stomach is filled with food, the sphincter closes the entrance to it. Therefore, feed and water that contain antigens have a relatively long contact with the wall of the esophagus.

As noted above, the esophageal tonsil is not macroscopically noticeable in all birds. In birds that have a macroscopically evident tonsil, we can see that it is located along the perimeter of the esophagus and has a shape of a ring. Its length and width are uneven in the examined birds, which, in our opinion, is connected with their trophic specialization (the size of the particles of the feed which passes through the esophagus and the development of the lymphoid tissue of the tonsil).

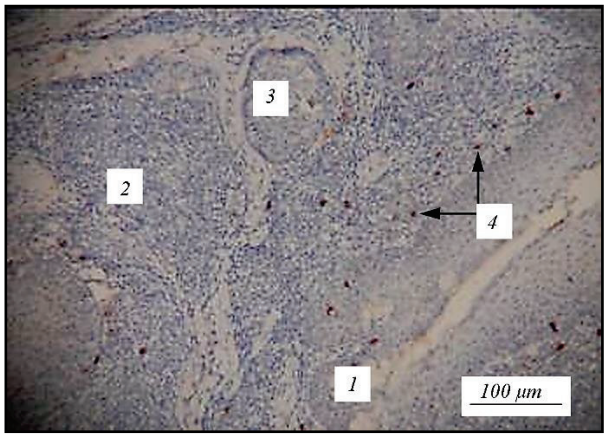

Fig. 14. The placement of lymphocytes with markers CD4+ in the esophageal tonsil of *G. gallus*: epithelium (*1*), diffuse lymphoid tissue (*2*), lymphoid nodule (*3*), markers (*4*); histopreparation with the use of monoclonal antibodies

The form of the esophageal tonsil is similar to the form of the mucous membrane from the adjacent part of the esophagus. Its folds continue on the tonsil. As a result, its lumen increases with the passage of feed. The number of folds is different in birds, this being a species feature. The folds of the tonsil mucosa are larger than the folds of the mucosa in the adjacent part of the esophagus, which is due to the development of lymphoid tissue in them.

In *A. anser* and *B. canadensis* there is an area of the esophagus between the esophageal tonsil and the proventriculus, we have called this area the transitional zone. Its presence is a special feature of species of these birds.

Histological studies confirmed that the wall of tonsil in the adjacent area to the esophagus is formed by mucous membrane, submucosa, muscular layer and serous membrane, which have a characteristic structure to them. The epithelium of mucosa is stratified squamous, but depending on the trophic specialization of birds (namely on the consistency of the feed), it can be keratinized or not keratinized. The muscular layer of the area of tonsil in certain species of birds can be two- or three-layered, which is their feature. In the lamina propria mucosae and submucosal base of the tonsil there is a lymphoid tissue, which determines its functions, and glands. The ducts of the glands are opened on the surface of the mucosa. Lymphoid tissue in the tonsil of some birds is contained continuously and compactly, and in others – in separate accumulations. On this basis, we propose to classify esophageal tonsils of birds as compact and diffuse. In compact tonsils, lymphoid tissue occupies a much larger area than in diffuse.

The lymphoid tissue of the esophageal tonsil in most of the examined birds is represented by all levels of the structural organization: a diffuse form, prenodules, primary and secondary lymphoid nodules, which indicates its full morphofunctional maturity. That is, this tissue has all structures for the formation of the cellular and humoral parts of the immune protection to the action of antigens (Sapin & Etingen, 1996). Among the separate levels of structural organization of the lymphoid tissue, diffuse form takes the largest area. In the tonsils of the *G. glandarius* and *C. livia*, only the diffuse form of the lymphoid tissue is revealed, but we cannot say that it is the only one in these birds. To solve this problem, it is necessary to conduct a study of the lymphoid tissue of the tonsil in the ontogenesis of these birds.

We agree with Gritsenko (1966) and Oláh et al. (2003), that the connection of the lymphoid tissue of the esophageal tonsil with the secretory unit of esophageal glands deserves special attention. In the studied species of birds, the lumen of the secretory unit is completely or partly replaced by a diffuse lymphoid tissue, and in some species – also by primary and secondary lymphoid nodules. The location of the lymphoid tissue around the esophageal glands and its replacement with lymphoid tissue is not accidental. We share the view of Sharma (1991) and Korver (2006) that antibodies are produced here: an immunoglobulin A is connected with a secretory component produced by these glands.

Cytological studies of the lymphoid tissue of the esophageal tonsil of *G. gallus* have confirmed the results of Oláh et al. (2003) and Nagy et al. (2005), that it contains reticular cells, lymphocytes, immunoblasts, plasmocytes, monocytes and macrophages. Their submicroscopic structure is the same as the structure of those cells of the immune system organs of birds (Stojanovskyj et al., 2016).

It is known that B-lymphocytes in birds are and T-lymphocytes are developed in the thymus. There is no analogue of this organ in mammals. In this regard, there is an assumption that these lymphocytes are formed in the immune formations of the digestive system. The cloacal bursa of birds is a non-permanent organ. After their sexual maturity, it becomes completely reduced (Ciriaco et al., 2013; Stojanovskyj et al., 2016). Birds of this age also have an age involution of the thymus, where T-lymphocytes are formed (Kendall, 1980). It is still unknown where the lymphocytes are formed in older birds. Some think that lymphocytes are formed in red bone marrow, others, that in lymphoid tissue that is associated with the mucous membranes of the tubular digestive system. In this regard, we conducted immunohistochemical studies of the esophageal tonsil of *G. gallus* to detect stem cells in it using monoclonal CD34+ antibodies and to identify individual subpopulations of T and B lymphocytes expressing monoclonal antibodies with markers CD4+, CD8+, CD20+. It was found that in the esophageal tonsil, stem cells that express monoclonal antibodies with the CD34+ marker are absent. Detected individual subpopulations of T-lymphocytes, mature B-lymphocytes and their subpopulations confirm, that here occurs antigen-dependent differentiation of lymphocytes into effector cells of the specific immunity in the esophageal tonsil.

Conclusions

The entry of antigens with feed and water through the esophagus and their delay before passing into the glandular part of the stomach, leads to the formation of esophageal tonsil, which is developed differently in adult birds. The information about the peculiarities of the esophageal tonsil structure and its function in wild and domestic birds can be used to evaluate comparison with other bird species, the state of the functioning of the immune system in the postnatal ontogeny, to understand pathological processes, to establish the mechanisms of impact of environmental factors on the body of birds, as well as in breeding work.

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