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Macro- and microstructure of the oesophageal tonsil in turkeys during the post-vaccination period

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Abstract. The successful development of poultry farming depends on the state of the immune system of birds, which from the first days of life come into contact with a significant amount of antigens that enter the body mainly through the digestive tract and provoke the development of diseases. Food retention in the transition zone between the oesophagus and stomach leads to the development of the oesophageal tonsil, as one of the most developed immune formations. The purpose of this study was to find out the development of the oesophageal tonsil in turkeys and determine the timing of its full morphofunctional maturity by vaccine prevention. Material for macro- and microscopic studies was selected from 66 individuals of Big-6 turkeys in the early stages of the postnatal period of ontogenesis, which were divided into experimental and control groups. When performing the study, classic morphological research methods were used. It was shown that in both groups of turkeys, the levels of structural organisation of the lymphoid tissue that forms the base of the oesophageal tonsil arise in a certain sequence, but with different intensity. In day-old birds, the first level was revealed – diffuse lymphoid tissue, represented by local clusters of diffusely located lymphocytes, some of which migrate into the surface epithelium and have close contact with epithelial cells. On day 10, the experimental group of turkeys developed pre-nodes with dense arrangement of lymphocytes without a capsule (second level) and primary lymphoid nodules with

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compact arrangement of lymphoid cells, limited by a capsule (third level), and on day 20, secondary lymphoid nodules with light centres and a well-defined mantle zone (fourth level) appeared, while in the control group, pre-nodes were recorded at 10 days of age, and primary and secondary lymphoid nodules at 20 days of age. This may indicate that on day 20, the oesophageal tonsil, as an immune formation, acquires morphofunctional maturity, and its cells are able to recognise and destroy specific antigens. The results obtained contribute to elucidating the natural mechanisms of development of immunological processes in poultry in ontogenesis, which should be considered by veterinarians when developing new vaccine prevention strategies

Keywords: junction of the oesophagus and stomach; lymphoid tissue; lymphoid nodules; morphological studies; lymphocytes; poultry

Introduction

The digestive organs of poultry are constantly exposed to various negative environmental factors that come with food and water. On their penetration, specific cellular reactions of the immune system occur, which neutralise foreign genetic information and ensure the development of cellular and humoural immunity. The development of an immune response largely depends on immunoprophylaxis against infectious diseases of poultry.

Along with obtaining highly productive poultry, according to T.S. Budnik & S.V. Guralska (2023), there is a need to ensure its sustainable well-being, a more in-depth knowledge of the mechanisms of natural immunological processes, and the impact of vaccination on them. Unlike mammals, as found by J.-C. Weil *et al.* (2023) and R. Capotă *et al.* (2025), the organs of the immune system of birds are characterised by weak development of lymph nodes (ducks, geese, swans) or their absence (chickens, turkeys, quails), the absence of a pharyngeal lymphoid ring and the presence of a cloacal sac, Gardner's gland, and well-developed lymphoid tissue that is associated with mucous membranes (mucosa associated lymphoid tissue – MALT) and as a morphological substrate forms the basis of specialised structures – immune or lymphoid formations. The latter are peripheral organs of the immune system and are always located on the path of possible penetration of genetically foreign substances into the body and participate in the mechanisms of immune defence. One of the first powerful protective barriers of the digestive canal, as reported by G. Garagulya *et al.* (2022), is the oesophageal tonsil, which is located in the thoracic-abdominal cavity at the junction of the oesophagus with the glandular part of the stomach. Its development in birds of different species depends on age, sex, conditions of keeping and feeding, physiological characteristics of the body, and the activity and duration of action of feed and water antigens on the junction. In addition, within the same age group, there are always slight fluctuations between the maximum and minimum numbers, content, size, and other parameters of the structural elements of the oesophageal tonsil, which serve as a manifestation of the body's immune defence.

As noted by S. Ceccopieri & J.P. Madej (2024), the lymphoid tissue of the peripheral organs of the immune system, including the oesophageal tonsil, is quite early exposed to various antigenic substances, under the influence of which T- and B-lymphocytes acquire specific immunoreactivity and provide a full-fledged immune response. In this regard, T. Etekhari *et al.* (2022)

highlighted four stages of differentiation of lymphoid tissue. The first stage can be considered the appearance of diffuse ("associated") lymphoid tissue in the mucous membrane of the digestive canal organs, which is represented by lymphocytes forming several rows of cells, plasmocytes and macrophages. The presence of lymphoid cells in the mucous membrane is considered as the body's readiness to meet, recognise, and neutralise foreign substances (antigens) that are located in the external environment (digestive canal). The second stage is the development of separate clusters of lymphoid cells near blood vessels and in the thickness of exocrine glands, etc. Such structures are the pre-nodular stage of development of peripheral organs of the immune system. The next, third stage in the development of lymphoid tissue is the appearance of primary lymphoid nodules, which are represented by denser clusters of cells of the lymphoid series of rounded and oval shapes and clear contours. Their presence can be considered as a state of high morphological maturity of the immune system organs, that is, their readiness to form reproduction centres for local production of lymphoid cells. The last, fourth stage of development of lymphoid tissue, which is the highest degree of differentiation of the immune system organs, is considered to be the appearance of reproduction centres (germinal, light) in the nodules. Such centres, as noted by M. Bemark *et al.* (2024) and N.J. Monisha *et al.* (2024), occur due to long-term active strong antigenic effects, when immunological activity appears and, accordingly, the need to increase the population of lymphoid elements. For the peripheral organs of the immune system, as found by G. Garagulya *et al.* (2022), is characterised by early involution, which begins with the onset of sexual maturity and is manifested by a gradual decrease in the area of lymphoid tissue, which significantly reduces the number of lymphoid nodules with the growth (replacement) in its place of loose fibrous connective and adipose tissues.

The features of the microstructure and cellular composition of the lymphoid tissue of the digestive canal are relatively well studied in chickens, ducks, and individual wild birds with different types of nutrition and digestive activity, as noted by N.D. Rahman & S.K. Waad (2025). Information on the morphological aspects of the development of the oesophageal tonsil in turkeys is scarce and insufficiently clarified, and there is no information on the effect of vaccination on its development and the onset of its morphofunctional maturity. In this regard, the purpose of the current study was to investigate the development and morphofunctional structure of the oesophageal tonsil in vaccinated and unvaccinated turkeys, which is important for establishing their immunological status in different age periods and evaluating the effectiveness of veterinary and preventive measures in poultry farms.

Literature Review

T.S. Budnik & S.V. Guralska (2023) noted that the epizootic well-being of poultry complexes largely depends on the state of the bird's immune system and provides for immunoprophylactic measures against infectious and bacterial diseases. Specialised lymphocytes, plasma cells, and macrophages, which are part of the lymphoid tissue associated with the mucous membrane of the digestive canal, participate in immune responses. V. Khomich *et al.* (2020) based on morphological features, classified the lymphoid tissue of the digestive canal of birds into structured (tonsils, single and aggregated lymphoid nodules) and diffuse, including cellular elements (intraepithelial lymphocytes, macrophages, plasma cells, lamina propria lymphocytes).

L.O. Bugay (2008) noted that macroscopically, the oesophageal tonsil is visible in musk ducks at 5 and 10 days in the form of indistinct spots, and from 15 days it acquired a rectangular shape with multiple elevations of a rounded shape, which, according to the researcher, indicates a significant localisation in these areas of lymphoid tissue. Its most intensive growth was noted during the periods from 15 to 30 days and from 90 to 180 days, simultaneously, the indicator of maximum length was recorded in ducks on day 150, and width – in 120-day-old birds. V.R. Indu *et al.* (2020) conducted histomorphological studies of the oesophageal tonsil of broiler ducks aged 6 to 8 weeks. They found six to eight isolated tonsils near the base of mucosal folds that did not form a continuous ring and consisted of many large lymphoid nodules separated by internode sections. According to H.H. Dönmez *et al*. (2012), a well-developed oesophageal tonsil compensates for the absence of palatine tonsils in birds. The secretory sacs of the ducks' oesophageal glands were in cooperation with lymphoid tissue, and the simple cylindrical epithelium turned into lymphoepithelium, as reported by V.T. Khomych & S. Usenko (2013). The researchers noted that the composition of lymphoid tissue includes small, medium, and large lymphocytes, plasma cells and macrophages, and the presence of venules with high endothelium, in their opinion, indicates a close immunological connection of the oesophageal tonsil with other organs of the lymphatic system.

According to N. Nagy *et al.* (2005), the oesophageal tonsil of chickens is anatomically located proximal to the stomach, which allows it to be exposed to antigenic environmental influences. It contains about eight isolated units (accumulations of lymphoid tissue) at the base of each fold of the oesophagus, and therefore, their number corresponds to the number of oesophageal folds. Each isolated unit contained crypts bounded by the lymphoepithelium and adjacent lymphoid tissue. A similar number of isolated units in the oesophageal tonsil of 12-week-old White Leghorn chickens was observed by V.R. Indu & K.M. Lucy (2021). The researchers noted a close relationship between the epithelium of the oesophageal glands and lymphoid tissue.

V.T. Khomich *et al.* (2020) conducted morphological studies of the oesophageal tonsil of sexually mature birds of various species in a comparative aspect. They noted that chicken, duck, goose, quail, turkey, guinea fowl, pheasant, grouse, peacock, pheasants, black goose, stork, magpie, and crow, it was visible as a thickened whitish-pink stripe that covers the perimeter (compact tonsils), and in pigeon, waterhen, partridge, jay, and coot, immune formation was not visible (diffuse tonsils). The maximum values of oesophageal tonsil length were recorded in the black goose (47.5 ± 0.44 mm) and stork (46.23 ± 0.37 mm), and the smallest – in quail (9.26 ± 0.09 mm) and magpie (8.19 ± 0.13 mm). Its largest width was inherent in the duck (9.25 ± 0.08 mm), and the smallest – magpie (1.16 ± 0.06 mm) and crow (1.04 ± 0.06 mm). The largest area of the mucous membrane with lymphoid tissue was occupied in the duck (68.64 ± 0.70%), and the smallest – in the partridge (2.31 ± 0.05%). Its individual structural elements were also found in the muscular and serous membranes of birds of certain species. According to the researchers, the unique anatomical location of the oesophageal tonsil is important for the development of cellular and humoural links of the immune response after oral administration of vaccines.

Materials and Methods

Macro- and microscopic studies of the oesophageal tonsil of turkeys were conducted during 2024 and until July 2025. The resource and facilities were provided by the certified educational, scientific and production laboratory "Centre for Biomorphological Technologies" of the

Department of Vertebrate Biomorphology named after Academician V.G. Kasyanenko of the National University of Life and Environmental Sciences of Ukraine. Research was conducted in compliance with the principles of the European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (1986), ARRIVE recommendations (n.d.) on reporting on experiments with live animals, and Law of Ukraine No. 3447-IV (2006). To euthanise the birds, in accordance with Order of the State Committee of Veterinary Medicine of Ukraine No. 365 (2010), barbiturate anaesthesia was used, namely sodium thiopental at a dosage of 7-20 mg/kg (depending on body weight) by intravenous injection. The decision of the bioethical Commission of the National University of Life and Environmental Sciences of Ukraine of Ukraine on granting consent to the use of animals for scientific purposes (No. 045/2025 of June 26, 2025) was received to conduct research.

Material for the study was selected from 66 female clinically healthy turkeys of the hybrid broiler breed Big-6 in six age groups: 1, 10, 20, 30, 40, and 50 days of age. The day-old birds were divided into experimental (vaccinated) and control (unvaccinated) groups. The experimental group received vaccines in accordance with the scheme of vaccine prevention and therapeutic treatments of the poultry farm LLC "Volodar" in the Bila Tserkva district of the Kyiv Oblast. Turkeys were vaccinated against Newcastle disease and Marek's disease immediately after hatching; against coccidiosis at one day old; against viral rhinotracheitis at 7, 21, and 42 days old; at 14 days old, they were revaccinated against Newcastle disease; and vaccinated against haemorrhagic enteritis at 28 days old. Birds of the second group of preventive vaccinations were not used. Turkeys of both groups were kept in a poultry farm. According to the diet, young animals were fed starter compound feed with a high protein content (up to 28%), and over time it was replaced with mixed feed containing a lower proportion of protein and more grain mixture for more intensive development of poultry.

Macroscopic, microscopic, morphometric, and statistical methods of studying the oesophageal tonsil of turkeys of the experimental and control groups were used to perform these tasks. The initial (anatomical) level of the study was carried out, which provided for the slaughter and exsanguination of the bird, its opening and preparation of the oesophageal tonsil area with its extraction for further morphological studies. Macroscopic studies determined the features of localisation, colour, shape, and linear parameters of the length and width of the oesophageal tonsil of turkeys in the age aspect using a calliper and ruler, followed by sampling for microscopic studies. The samples were placed in histological cassettes and immersed in a 10% aqueous neutral formalin solution for 48 hours. They were then washed for 24 hours in tap water. To remove water from the samples, dehydration with ethyl alcohol of increasing concentration (60°, 70°, 96°, and 100°) was performed for one to three hours. In the future, the material was passed through a mixture of alcohol and chloroform, then kept in pure chloroform, after which the material was placed in a mixture of chloroform-paraffin (at a temperature of 37°C) in and in paraffin (at a temperature of 55-56°C). A stainless steel filling and moulding station was used to produce the paraffin blocks.

Histological sections up to 5-10 microns thick were made from the obtained paraffin blocks using an MPS-2 sled microtome (MedTech-Price, Ukraine). Subsequently, histological sections were stained with haematoxylin and eosin according to Mallory and impregnated according to Kelemen using standard methods. Calculation of the area of lymphoid tissue and its structural and functional levels was carried out by the "point counting" method using a binocular microscope and a measuring grid. The size of round and oval lymphoid nodules was determined using Olympus CX 43 microscope (Labdepo, Japan) and a micrometre eyepiece.

The results obtained during the research were recorded in protocols. Statistical processing of the results (mean, standard deviation) was performed on a personal computer using StatSoft Statistica 13.1 (2016) software suite. The assessment of statistical reliability was determined by the Student's t-test at three probability levels $P < 0.05$, $P < 0.01$, and $P < 0.001$. Micrographs of individual histoses of the location of the oesophageal tonsil of turkeys were performed using a microscope equipped with a Primo Star digital camera (Carl Zeiss, Germany) and connected to a personal computer.

Results and Discussion

Features of the macroscopic structure of the oesophageal tonsil

As the results of studies showed, in the area of the oesophageal tonsil, the mucous membrane formed low, wide longitudinal folds (6-8) and was more thickened compared to the cervical part of the oesophagus. V.T. Khomich *et al.* (2020) noted that the domestic goose and the Canadian black goose at the border of the oesophagus and stomach do not have the folding of the mucous membrane and cone-shaped papillae of the excretory ducts of the glands characteristic of the glandular part of the stomach. According to the researchers, such a transition zone is anatomical and physiological feature that is characteristic of this bird species and is associated with their trophic specialisation.

Macroscopically, the oesophageal tonsil was viewed on the inner surface of the wall of the transition of the oesophagus to the stomach of turkeys of the experimental group, starting from the 10-day age (Fig. 1A), and the control group – from the 20-day age. It had the appearance of a thin whitish ring-shaped strip with a bumpy surface and holes of crypts, which was located around the perimeter of the site at the base of the folds of the mucous membrane and between them. In older birds, the folds became more visible visually, and the colour of the tonsil changed to pinkish-whitish with a yellowish tinge (Fig. 1B). This is consistent with data by V.T. Khomych & S.I. Usenko (2013), who, when studying the oesophageal tonsil in ducks, noted that the corresponding colour and bumpy surface of such an immune formation are associated with the localisation of significant accumulations of lymphoid tissue in it.

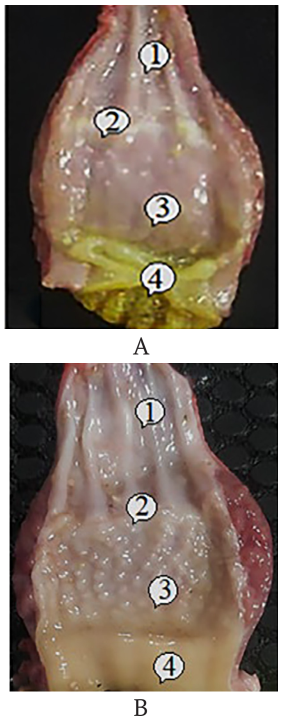

Figure 1. Oesophageal tonsil in turkeys of the experimental group aged 10 (A) and 30 (B) days

Note: *1 – oesophagus; 2 – area of localisation of the oesophageal tonsil; 3 – glandular part of the stomach; 4 – muscular part of the stomach with cuticle (A) and without cuticle (B). Native drugs*

Source: *photo by the authors*

With increasing age of turkeys, macroscopic indicators of the length and width of the oesophageal tonsil increased (Table 1). In the poultry of the experimental group, the tendency to increase these indicators was observed from the 10-day age, and the control group – from the 20-day age. The length and width of the oesophageal tonsil reached maximum values in a 50-day-old bird. In addition, they were slightly larger in the group of turkeys that were vaccinated.

Table 1. Macroscopic parameters of the oesophageal tonsil in turkeys, mm, M±m, n=6

Poultry group	Length	Width
	Day 10	
Experimental	11.5±0.28	2.08±0.15
Control	-	-
	Day 20	
Experimental	16.80±0.15	3.80±0.21
Control	15.22±0.48	3.16±0.15
	Day 30	
Experimental	23.22±0.34	4.87±0.08***
Control	21.37±0.23	4.50±0.28*
	Day 40	
Experimental	26.72±0.32	6.03±0.21*
Control	23.76±0.14*	5.10±0.07**
	Day 50	
Experimental	28.75±0.17*	6.80±0.15**
Control	27.28±0.19	5.92±0.13**

Note: *Macroscopic parameters of the oesophageal tonsil in turkeys, mm, M±m, n=6*
Source: *developed by the authors*

On average, in birds of the experimental group from 10 to 50 days, linear indicators of the length and width of the oesophageal tonsil increased by 150.0% (2.5 times) and 226.92% (3.2 times), respectively. In the comparative aspect of turkeys in both groups, an increase in these indicators was observed from 20 to 50 days of age. Thus, the length and width of the oesophageal tonsil in the experimental group increased by 71.13% (1.71 times) and 78.95 % (1.78 times) during this period, and in the control group – by 79.24% (1.8 times) and 87.34% (1.9 times), respectively. The growth of these indicators was uneven. The length and width of the oesophageal tonsil in turkeys of both groups increased most intensively from 20 to 30 days: in the experimental group, respectively, by 38.21% and 28.16% ($P < 0.001$), and the control group – by 40.41% and 42.41% ($P < 0.05$). With a lower intensity, these indicators increased in poultry of the experimental group from 40 to 50 days (by 7.59%, $P < 0.05$ and 12.77%, $P < 0.01$, respectively) and the control group from 30 to 40 days (by 11.18%, $P < 0.05$ and 13.33%, $P < 0.01$, respectively). Thus, macroscopically, from day 10, the oesophageal tonsil in turkeys of the experimental group was detected in the form of a whitish stripe and had slightly higher indicators of length and width, compared to the tonsil in the control group, which was observed starting from the age of 20 days. The most intense increase in these indicators in birds of both groups was observed at 3-4 weeks of their development.

Histotopography and microstructure of the oesophageal tonsil

The microscopic structure of the oesophageal tonsil in turkeys of both groups is similar to the thoracic-abdominal part of the oesophagus, but it is somewhat thickened. The wall of this area was formed by three membranes: mucous, muscular, and serous (Fig. 2). The mucous membrane had a characteristic structural relief, the elements of which were longitudinal folds separating crypt-like formations, the development of which is conditioned by the deepening of the surface epithelium into its own plate. According to research data by A.A. El-Mansi *et al*. (2025), the number of folds in birds depends on their species characteristics, and the size of the folds in the area of the oesophageal tonsil is larger than in other parts of the oesophagus, which indicates the presence of lymphoid tissue in them.

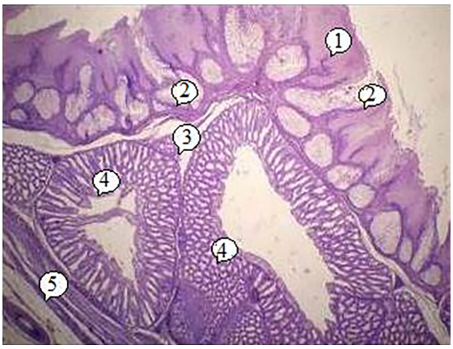

Figure 2. Area of transition of the oesophagus to the glandular part of the stomach in a day-old turkey

Note: *1 – oesophageal epithelium; 2 – oesophageal glands; 3 – submucosal base; 4 – lobules of deep glands of the glandular part of the stomach; 5 – muscle membrane. Staining with haematoxylin and eosin ×40*

Source: *developed by the authors*

The epithelial layer of the mucous membrane passed from a multilayer flat slightly keratinised to a single-layer prismatic glandular one. The multi-layered epithelium is characteristic of the oesophagus and consisted of epithelial cells of the basal, spinous, or spiny and superficial layers. The cells of the basal layer are arranged in a single row and had a cylindrical shape with nuclei pronounced in the area of the basal pole. The spiny layer is represented by many rows of polygonal epithelial cells with processes, and the cells of the surface layer were placed in 2-5 rows. The latter had a pronounced flat shape, and destroyed nuclei were recorded in individual cells. The single-layer prismatic epithelium is characteristic of the glandular part of the stomach and is represented by cylindrical epithelial cells, which are arranged in a single row. Their surface was covered with mucus. In its own plate of the mucous membrane, the glands characteristic of the oesophagus were preserved, and the muscle plate, which was formed by bundles of smooth muscle cells, was intermittent. Lobules of deep glands, which are characteristic of the glandular part of the stomach, were observed in the submucosal base.

The muscle membrane of the oesophageal tonsil site was formed by undisturbed smooth muscle tissue that was adjacent to the submucosal base. Bundles of its cells formed the inner and outer longitudinal and middle – circular layers, which had different development. The circular layer was best developed, the inner longitudinal layer was slightly smaller, and the outer longitudinal layer was weaker. Between the muscle layers were small layers of loose fibrous connective tissue with pronounced blood vessels, nerve nodes and fibres. Outside, the area of the oesophageal tonsil of turkeys is covered with a serous membrane, which passed to the glandular part of the stomach. It was formed by a single-layer flat epithelium and loose fibrous connective tissue with a small number of blood vessels.

According to the conclusions of N. Hamoda & A. Farag (2018), changes in the epithelium from multi-layered to single-layered, and feed retention in the area of the oesophageal tonsil leads to increased antigenic effects, and the development of lymphoid tissue in this area. A.R.H. Al-Fartwsy *et al.* (2025) reported that in guinea fowl, the muscle membrane of the oesophageal tonsil location is formed by two layers: circular (inner) and longitudinal (outer). According to research data by N. Dyshliuk *et al.* (2024) in the common blackbird, the inner layer of the muscular membrane was buried in separate large folds and was involved in their formation, and in the grey heron, this membrane was somewhat thickened and had significant vascularisation and innervation, which, according to L.P. Kharchenko *et al*. (2001), indicates the presence of a sphincter between the oesophagus and stomach.

The process of morphofunctional differentiation and specialisation of lymphoid tissue in the oesophageal tonsil of turkeys of both groups arose in the following sequence: at the beginning, diffuse clusters of lymphocytes placed in their own plate of the mucous membrane appeared, then – pre-nodules, primary lymphoid nodules were formed from the latter, and secondary lymphoid nodules with light centres developed on their base due to the action of antigens. Such a full range of levels of structural organisation of lymphoid tissue, according to I. Oláh *et al.* (2014), may indicate the functional maturity of the oesophageal tonsil in birds, i.e., its ability to destroy and remove the antigen recognised by lymphocytes.

At day-old age, the lymphoid tissue in the mucous membrane of the oesophageal tonsil area was represented only by separate, structurally homogeneous clusters of diffusely located lymphocytes, without noticeable rarefactions or compactions in the centre (Fig. 3). Foci of diffuse lymphoid tissue were located mainly under the surface epithelium in its own plate of the mucous membrane. Some of the lymphocytes migrated to its lower layers and established a "lymphoepithelium", which had tight contacts of lymphoid and epithelial cells. Individual lymphocytes were in a mitotic state.

Figure 3. Diffuse lymphoid tissue at the junction of the oesophagus to the glandular part of the stomach in a day-old turkey

Note: *histological preparation: 1 – mucous membrane; 2 – oesophageal epithelium; 3 – oesophageal glands; 4 – diffuse lymphoid tissue; 5 – lobule of the deep gland of the glandular part of the stomach; 6 – muscle membrane. Staining with haematoxylin and eosin ×40*

Source: *developed by the authors*

During lymphocyte infiltration, the epithelium was locally disturbed and became spongy. Minor accumulations of diffuse lymphoid tissue were also recorded near blood vessels, with the terminal parts of the oesophageal glands and their ducts, and near the lobules of deep glands that belong to the glandular part of the stomach. In addition to elastic and collagen fibres, reticular cells were recorded in the areas of lymphocyte clusters. The latter had different orientations and, together with reticulocytes, formed a structure similar to a "grid", in the loops of which there were phagocytic macrophages and lymphoid cells of varying degrees of maturity.

According to I. Oláh *et al.* (2014), among lymphocytes migrating to the epithelium, small forms predominated, which were characterised by a high nuclear-cytoplasmic ratio. According to the researchers, such movement and accumulation of lymphocytes towards the lumen (external environment), which is enriched with microflora with its diverse antigenic spectrum, indicates that lymphocytes are immunocompetent cells that maintain the constancy of the internal environment and protect against infections. As noted by L.V. Chernyshenko & S.T. Chernokulskiy (1986), the location of lymphoid tissue along the course of the lymph and haemomicrocirculatory bed is a pattern and indicates close contact of the immune system organs with the microcirculation system and blood enrichment throughout their entire length with lymphocytes.

At 10-day age, the area of localisation of lymphoid tissue in the oesophageal tonsil mucosa increased by 82.05% (1.8 times) in turkeys of the experimental group and by 46.38% (1.4 times) in the control group compared to this indicator in day-old poultry (Table 2). Accumulations of diffuse lymphoid tissue were found in the area of the base and apex of the folds of its own plate and partially submucosal base of the mucous membrane. The area of lymphoid infiltration of the epithelial layer, oesophageal gland packets and their excretory ducts also increased.

Table 2. Area of the mucous membrane in the area of the oesophageal tonsil in turkeys, %, M ± m, n = 6

Poultry group	Mucosal area	
	without lymphoid tissue	**with lymphoid tissue**
Day 1		
Hatching	91.70 ± 0.25	8.30 ± 0.25
Day 10		
Experimental	84.89 ± 0.27	15.11 ± 0.27
Control	87.85 ± 0.14	12.15 ± 0.14
Day 20		
Experimental	74.83 ± 0.34	25.17 ± 0.34
Control	77.79 ± 0.20	22.21 ± 0.20**
Day 30		
Experimental	67.83 ± 0.11	32.17 ± 0.11
Control	70.73 ± 0.19**	29.27 ± 0.19
Day 40		
Experimental	61.86 ± 0.12	38.14 ± 0.12
Control	66.29 ± 0.10	33.71 ± 0.10*
Day 50		
Experimental	59.21 ± 0.28**	40.79 ± 0.28
Control	63.88 ± 0.12**	36.12 ± 0.12*

Note: **P < 0.05; **P < 0.01; ***P < 0.001 compared to the corresponding previous indicator*
Source: *developed by the authors*

In the 10-day age period, in both groups, active development of pre-nodules was observed in diffuse lymphoid tissue, which were denser clusters of lymphocytes without a pronounced shell and clear contours (Fig. 4A), and in the poultry of the experimental group, the third level of structural organisation was also recorded – primary lymphoid nodules with dense cell cooperation. The reticular base of the latter had large-and small-sized architectonics in their central part due to the interweaving of reticular fibres, while collagen fibres were not found there. At the periphery of the nodules, reticular fibres, together with collagen fibres, were part of the shell and were oriented in a circle.

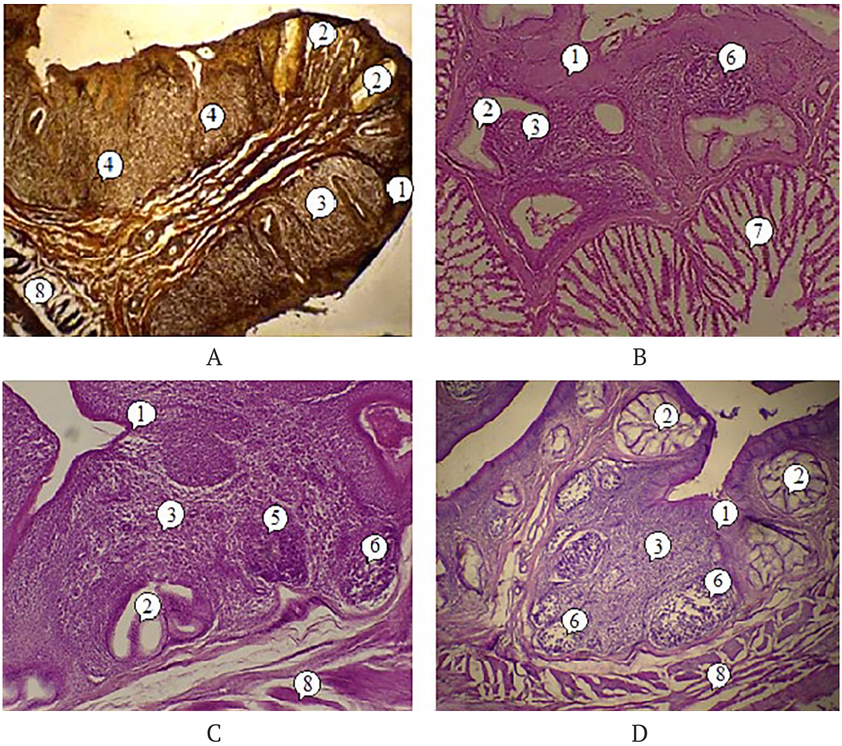

Figure 4. Area of transition of the oesophagus to the glandular part of the stomach of turkeys
Note: *control group: A – 10-day and B – 20-day age; experimental group: C – 20-day and D – 30-day age. 1 – superficial epithelium; 2 – oesophageal glands; 3 – diffuse lymphoid tissue; 4 – pre-nodular form; 5 – primary lymphoid nodule; 6 – secondary lymphoid nodules; 7 – lobule of the deep gland of the glandular part of the stomach; 8 – muscle membrane. Impregnation with silver nitric acid according to Kelemen ×80 (A). Staining with haematoxylin and eosin ×80 (B, C, D)*
Source: *developed by the authors*

In 20-day-old turkeys, the area of lymphoid tissue in the oesophageal tonsil mucosa increased by 66.58% (1.7 times) in the experimental group and by 82.80% (1.8 times, $P < 0.01$) in the control group compared to the corresponding indicators of the previous group (Table 2). Clusters of lymphoid tissue had different shapes and sizes and were separated from each other by structural elements of loose fibrous connective tissue. They included significant accumulations of diffuse lymphoid tissue, pre-nodules, and primary and secondary lymphoid nodules in birds of both groups (Fig. 4B, 4C). The latter are the fourth level of structural organisation with noticeably pronounced germinal (light) centres, which according to V.R. Indu *et al.* (2020), can increase in size with a significant antigenic effect on the body. Their appearance, according to the researchers, indicates a high immunological competence of the lymphoid tissue and, accordingly, the oesophageal tonsil. Individual secondary lymphoid nodules were in the form of longitudinally arranged chains. Such chains lay in the thickness of the mucous membrane and were located both on the tops of the longitudinal folds and between them. Perivascular lymphoid nodules were registered in the submucosal membrane around the blood vessels. In numerous mitotic figures of lymphoid cells, mainly with light cytoplasm and macrophages, were observed in the breeding centres.

On the periphery of the secondary nodules, a mantle with densely arranged lymphocytes, mostly small in shape, was clearly visible, which were arranged in several layers and formed a thin strip around the breeding centre, which was also reported by C. Casteleyn *et al.* (2010) when studying the immune formations of the digestive tube of chickens. The researchers noted that small lymphocytes of the mantle zone have an immunological memory, that is, when they meet the antigen again, they react much faster and stronger, so they can fight bacteria and viruses. In the current study, in individual nodules, the area of the mantle that was turned to the epithelial layer of the mucous membrane was expanded and had the appearance of a dome. The latter was one of the sources of lymphocytes that migrated to the thickness of the surface epithelium. The formed secondary lymphoid nodules of the oesophageal tonsil of turkeys are limited to collagen, elastic and reticular fibres that formed their shell (capsule). Individual fibres had a connection with such fibres of their own plate of the mucous membrane. In the central part of the secondary nodules, the fibres were sparse and fragmented, and in most of them they were absent altogether. According to research data by C. Ceccopieri & J.P. Madej (2024), proliferation centres in lymphoid nodules occur only when the body's immunological activity increases in response to antigen penetration and the need for the production of lymphocyte populations increases.

On day 30, the area of lymphoid tissue in the mucous membrane continued to increase by 27.81% (1.2 times) in the experimental group and by 31.79% (1.3 times) in the control group compared to the previous age period (Table 2, Fig. 4D). The growth of infiltrated areas of the surface epithelium of the mucous membrane by lymphoid cells was observed. Individual lymphoid nodules were registered in the wall of the terminal parts of the oesophageal glands and their excretory ducts. Moreover, infiltration of glandular epithelial lymphocytes and their active migration as part of secretions to the surface of the oesophagus were observed. These data are consistent with the conclusions by I. Oláh *et al.* (2014) and A.M. Abdellatif1*et al.* (2022), that the replacement of the oesophageal glands with lymphoid tissue is not accidental, since this connection causes the production of antibodies in the form of immunoglobulin A, which together with the secretory component of the glands provide local resistance to infections on the surface of the mucous membranes.

In turkeys on days 40 and 50, the area of accumulations of lymphoid tissue in the mucous membrane increased by 18.55% (1.2 times) and 6.95% (1.0 times) in the experimental group and by 15.17% (1.1 times, $P < 0.05$) and 7.15% (1.0 times, $P < 0.05$) in the control group compared to the corresponding indicators of the previous period (Table 2). In these age groups, all levels of structural organisation of lymphoid tissue were observed, among them a significant number of secondary lymphoid nodules with well-defined light centres were noted (Fig. 5). The area of interepithelial infiltration by lymphocytes of epithelial tissue (superficial and glandular) also increased.

As noted above, with increasing age of turkeys, the area of lymphoid tissue in the mucous membrane of the oesophageal tonsil area increased. During the entire study period of poultry (from day-old to 50-day-old), its content increased by 391.44% (4.9 times) in the experimental group and by 335.18% (4.3 times) in the control group, while the area of the mucous membrane without lymphoid tissue decreased (by 35.43%, 1.5 times in the experimental group and by 30.34%, 1.4 times in the control group) (Table 2). The most intensive increase in the area of lymphoid tissue was observed in birds of the experimental group from daily to 10-day age (by 82.05%, 1.8 times), and in the control group – from 10 to 20 days (by 82.80%, 1.8 times). With a lower intensity, this indicator increased in poultry of both groups from 40 to 50 days by 6.95% (1.0 times) in the experimental group and by 7.15% (1.0 times) in the control group.

A

B

Figure 5. Area of transition of the oesophagus to the glandular part of the stomach in 50-day-old turkeys

Notes: *A – control group; B – experimental group. 1 – superficial epithelium; 2 – oesophageal gland; 3 – diffuse lymphoid tissue;4 – primary lymphoid nodule; 5 – secondary lymphoid nodules; 6 – lobule of the deep gland of the glandular part of the stomach. Staining according to the Mallory method ×80 (A, B)*

Source: *developed by the authors*

The content of individual structural and functional levels of oesophageal tonsil lymphoid tissue in turkeys of both studied groups changed with increasing age (Table 3). The largest area was the diffuse form of lymphoid tissue, which on the first day (after hatching) was 100%. With an increase in the age of turkeys (from day-old to 50-day age), the area of diffuse lymphoid tissue decreased by 47.83% (1.9 times) in the experimental group and by 41.83% (1.7 times) in the control group. The sharpest decrease in this indicator was observed in poultry from 10 to 20 days by 24.33% (1.3 times) in the experimental group and by 22.94% (1.2 times) in the control group.

Table 3. Content of structural levels of lymphoid tissue in the oesophageal tonsil in turkeys, %, M ± m, n = 6

Poultry group	Diffuse form	Pre-nodes	Lymphoid nodules	
			primary	secondary
Day 1				
Hatching	100	-	-	-
Day 10				
Experimental	88.50 ± 0.67	5.33 ± 0.50	6.17 ± 0.56	-
Control	94.82 ± 0.29	5.18 ± 0.29**	-	-

Table 3. Continued

Poultry group	Diffuse form	Pre-nodes	Lymphoid nodules primary	Lymphoid nodules secondary
		Day 20		
Experimental	66.97 ± 0.97	5.98 ± 0.33*	8.32 ± 0.50	18.73 ± 1.05
Control	73.07 ± 0.33	5.57 ± 0.15**	7.10 ± 0.34*	14.26 ± 0.23
		Day 30		
Experimental	62.38 ± 1.27	6.08 ± 0.42**	9.08 ± 0.21*	22.46 ± 0.88
Control	70.13 ± 0.44	6.03 ± 0.17***	7.76 ± 0.25**	16.08 ± 0.16*
		Day 40		
Experimental	56.35 ± 0.89	6.53 ± 0.28***	12.94 ± 0.59	24.18 ± 0.58**
Control	65.00 ± 1.72	6.20 ± 0.28**	9.76 ± 0.91***	19.04 ± 1.11*
		Day 50		
Experimental	52.17 ± 0.66	7.05 ± 0.52*	13.73 ± 0.28*	27.05 ± 0.59
Control	58.17 ± 0.50	6.87 ± 0.23**	11.92 ± 0.22	23.04 ± 0.75

Note: **P < 0.05; **P < 0.01; ***P < 0.001 compared to the corresponding previous indicator*
Source: *developed by the authors*

Against the background of a decrease in the area of diffuse lymphoid tissue, an increase in the content of pre-nodules and lymphoid nodules was observed. As noted above, in turkeys of the experimental group, pre-nodules and primary lymphoid nodules were detected from 10-day age, and in the control group, pre-nodules were recorded from 10-day age, primary nodules – from 20-day age of the bird. In both groups, secondary lymphoid nodules were recorded from day 20. Moreover, in the poultry of the experimental group, the area of pre-nodules, primary and secondary lymphoid nodules was slightly larger than in the turkeys of the control group. The content of pre-nodules (from 10 to 50 days) increased by 1.3 times, or by 32.27% – in the experimental and 32.63% – in the control groups. This process was most intense in birds of the experimental group from 10 to 20 days by 12.20% (1.1 times, P < 0.05), and in turkeys of the control group – from 40 to 50 days by 10.81% (1.1 times, P < 0.01). The content of lymphoid nodules (primary and secondary) reached its maximum values in 50-day-old poultry. Notably, there are slightly more secondary lymphoid nodules in turkeys of both groups than in primary ones. A particularly significant increase in the area of primary lymphoid nodules was observed in poultry from 30 to 40 days (by 42.51% in the experimental and 25.77%, P < 0.001 in control groups), and with a lower intensity, this indicator in the experimental bird increased from 40 to 50 days by 6.11% (P < 0.05), and in the control – from 20 to 30 days at 9.29% (P < 0.01). The maximum increase in the content of secondary lymphoid nodules was recorded from 20 to 30 days in the experimental group (by 19.91%, 1.1 times) and from 40 to 50 days in poultry of the control group (by 21.01%, 1.2 times). With less intensity, this indicator increased from 30 to 40 days of age of experimental turkeys (by 7.66%, P < 0.01) and from 20 to 30 days (by 12.76%, P < 0.05) in control group.

The lymphoid nodules of the oesophageal tonsil of turkeys were mainly rounded and oval in shape, and some with were pear-shaped, egg-shaped, etc. (Fig. 6). They clearly showed the shell that separated the cellular composition of nodules from diffuse lymphoid tissue. Nodules had different sizes and formed groups that are best expressed in birds of 40-50 days. Moreover, in both groups, the size of secondary nodules prevailed above the primary ones (Table 4).

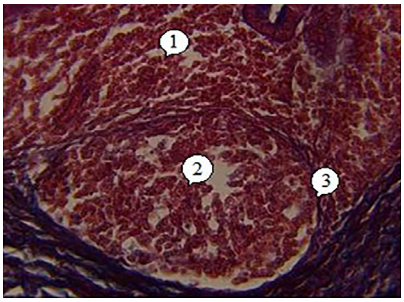

Figure 6. Lymphoid nodule of the oesophageal tonsil in the control group in 50-day-old turkeys
Notes: *1 – diffuse lymphoid tissue; 2 – secondary lymphoid nodule; 3 – collagen fibres. Staining according to the Mallory method ×100*
Source: *developed by the authors*

Table 4. Size of lymphoid nodules of the oesophageal tonsil in turkeys, M ± m, μm (n = 6)

Poultry group	Lymphoid nodules					
	Primary			Secondary		
	Rounded	Oval		Rounded	Oval	
		Length	Width		Length	Width
Day 10						
Experimental	118.83 ± 1.58	225.83 ± 3.86	149.83 ± 6.44	-	-	-
Control	-	-	-	-	-	-
Day 20						
Experimental	169.50 ± 1.96	250.33 ± 4.85	163.50 ± 7.38	174.83 ± 1.99	262.33 ± 2.98	177.17 ± 5.29
Control	158.17 ± 3.30	221.50 ± 5.97	144.83 ± 2.55	164.17 ± 4.92	236.83 ± 3.14	147.67 ± 3.55
Day 30						
Experimental	183.83 ± 4.02	263.00 ± 2.24	170.50 ± 1.21	187.33 ± 1.43	274.33 ± 4.79	179.00 ± 3.74*
Control	167.33 ± 4.42	237.83 ± 5.17	156.83 ± 4.02	179.5 ± 1.96	272.5 ± 3.27	160.17 ± 3.67
Day 40						
Experimental	207.5 ± 7.56	274.17 ± 3.42	177.33 ± 5.67	221.83 ± 5.11	277.5 ± 4.76*	180.17 ± 4.20
Control	194.67 ± 2.80	269.5 ± 2.71	161.83 ± 4.57*	210.0 ± 2.24	274.0 ± 5.04*	171.67 ± 4.29
Day 50						
Experimental	244.33 ± 9.71	286.17 ± 2.30	189.83 ± 5.14	250.17 ± 7.41	301.17 ± 4.76	191.17 ± 6.07
Control	201.83 ± 3.83*	277.67 ± 2.05	173.83 ± 10.55	233.17 ± 4.76	294.17 ± 2.65	178.67 ± 5.48

Note: **P < 0.05; **P < 0.01; ***P < 0.001 compared to the corresponding previous indicator*
Source: *developed by the authors*

With an increase in the age of the bird, a gradual increase in the values of these indicators was observed. Over the period from 10 to 50 days, linear measurements (diameter of rounded, length and width of oval) of primary lymphoid nodules of experimental turkeys increased by 105.61% (2.1 times), 26.72% (1.3 times), and 26.69% (1.3 times), respectively, and the same indicators in poultry of the control group (20 to 50 days), respectively, by 27.60% (1.3 times), by 25.36% (1.2 times), and 20.02% (1.2 times). The diameter of rounded and the length and width of oval primary lymphoid nodules in the experimental group increased with greater intensity from 10 to 20 days (corresponding to 42.64%, 10.85%, and 9.12%), while in the control group the width of oval nodules increased most intensively from 20 to 30 days (by 8.29%), and the diameter of rounded and oval length from 30 to 40 days (by 16.34% and 13.32%, respectively). Linear indicators of secondary lymphoid nodules of the oesophageal tonsil also increased with increasing age. Thus, during the period from 20 to 50 days of age, the diameter of rounded, length and width of oval nodules in the experimental group of poultry increased by 43.09% (1.4 times), 14.81% (1.1 times), and 7.90% (1.1 times), respectively, and the same indicators in turkeys of the control group, respectively, by 42.03% (1.4 times), 24.21% (1.2 times), and 20.99% (1.2 times). With a higher intensity of growth in the diameter of rounded secondary lymphoid nodules in poultry of the experimental group occurred from 30 to 40 days (by 18.42%), and the length and width of oval ones – from 40 to 50 days (by 8.53% and 6.11%, respectively), while in the control group the length and width of oval ones increased most intensively from 20 to 30 days (by 15.06% and 8.46%, respectively), and the diameter of rounded ones from 30 to 40 days (by 16.99%). Thus, the paper presented the features of the morphology of the oesophageal tonsil in vaccinated and unvaccinated turkeys, the stages of development of lymphoid tissue, and the established age period of the bird, in which its morphofunctional maturity occurs, which should be taken into consideration by specialists when drawing up treatment and preventive plans at poultry complexes.

Conclusions

The topography and structural and functional organisation of the lymphoid tissue of the oesophageal tonsil of vaccinated and unvaccinated Big-6 turkeys aged from one to 50 days old, and the timing of its morphofunctional maturity was determined, which specialists should consider when developing new vaccine prevention strategies. However, secondary lymphoid nodules were detected on day 20 in turkeys of both groups under study, which indicated that the oesophageal tonsil acquired morphofunctional maturity and the ability to exert a specific immune response to the action of foreign antigens. Secondary lymphoid nodules had well-defined light centres, in which lymphoid cells were recorded, including immunoblasts with light cytoplasm and macrophages. The lymphoid tissue of the oesophageal tonsil of birds of both groups was found in its own mucosal plate and submucosal base, while it was absent in the muscular and serous membranes. Some of the lymphocytes migrated to the lower layers of the surface epithelium and ended up around the blood vessels, in the wall of the secretory parts of the oesophageal glands between the glandulocytes and their excretory ducts. In this case, active migration of these cells as part of the secretory component to the surface of the mucous membrane was observed. With an increase in the age of turkeys in both groups, there was an uneven increase in the area of the mucous membrane with lymphoid tissue, which in 50-day vaccinated poultry was 11.45% (1.1 times) more than in unvaccinated poultry. The content of diffuse form in the lymphoid tissue decreased, and pre-nodules and lymphoid nodules (primary and secondary) increased. With increasing age of turkeys, the size of lymphoid nodules increased, which acquired maximum values in 50-day-old poultry and were 17.39% (rounded primary) and 6.79% (rounded secondary) in the vaccinated group were larger than in the unvaccinated one. In addition, in turkeys of both groups, the linear indicators of secondary nodules exceeded those of primary nodules.

Thus, it was found that vaccination of poultry against bacterial and Infectious diseases stimulates the development of structural levels of the lymphoid tissue of the oesophageal tonsil, which accelerates its morphofunctional maturity. Morphological and morphometric data also indicate a close relationship between age-related changes and vaccination with the formation of immunocompetent structures of the oesophageal tonsil of turkeys and confirm its important role in ensuring local immunity. Prospects for further research can be aimed at investigating the structural and functional organisation of the oesophageal tonsil of older turkeys, the impact of vaccination on it, and comparative assessment with other representatives of the animal world.

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Conflict of Interest

None.

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Макро- та мікроструктура стравохідного мигдалика індиків у поствакцинальний період

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Анотація. Успішний розвиток птахівництва залежить від стану імунної системи птиці, яка з перших діб життя контактує зі значною кількістю антигенів, що потрапляють в організм переважно через травний канал і провокують розвиток захворювань. Затримка корму в перехідній зоні між стравоходом і шлунком призводить до формування стравохідного мигдалика, як одного з найбільш розвинутих імунних утворень. Метою цієї роботи було з'ясувати розвиток стравохідного мигдалика індиків і встановити строки його повної морфофункціональної зрілості за вакцинопрофілактики. Матеріал для проведення макро- та мікроскопічних досліджень відібрали від 66 особин індиків породи Біг-6 на ранніх етапах постнатального періоду онтогенезу, яких розділили на дослідну і контрольну групи. При виконанні роботи використовували класичні методи морфологічних досліджень. Показано, що в обох групах індиків рівні структурної організації лімфоїдної тканини, яка формує основу стравохідного мигдалика, виникають у певній послідовності, але з різною інтенсивністю. У добової птиці виявляється перший її рівень – дифузна лімфоїдна тканина, яка представлена локальними скупченнями дифузно розташованих лімфоцитів, частина яких мігрує у поверхневий епітелій і має тісний контакт з епітеліоцитами. На 10 добу індиків дослідної групи з'являлися передвузлики із щільним розташуванням лімфоцитів без оболонки (другий рівень) і первинні лімфоїдні вузлики з компактним розташуванням клітин лімфоїдного ряду, що обмежені оболонкою (третій рівень), а з 20 доби – вторинні лімфоїдні вузлики зі світлими центрами і добре вираженою мантійною зоною (четвертий рівень), тоді як у контрольної групи – передвузлики реєструвалися з 10-добового, а первинні і вторинні лімфоїдні вузлики – з 20-добового віку. Це може свідчити про те, що на 20 добу стравохідний мигдалик як імунне утворення набуває морфофункціональної зрілості, а його клітини здатні розпізнавати і знищувати конкретні антигени. Отримані результати сприяють з'ясуванню природних механізмів розвитку імунологічних процесів у птиці в онтогенезі, що слід враховувати ветеринарним лікарям при розробці нових стратегій вакцинопрофілактики

Ключові слова: ділянка переходу стравоходу у шлунок; лімфоїдна тканина; лімфоїдні вузлики; морфологічні дослідження; лімфоцити; птиця