

## Cyto- and histoarchitectonics of the chicken spleen in the post-vaccination period

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### Abstract

One of the main functions of the spleen is participation in many immunological processes, protecting the body from various harmful agents. It is a secondary organ of lymphopoiesis and the only organ of immunogenesis, which provides immune control of blood and detects genetically foreign agents in it, and performs the role of a biological filter. Therefore, the study of its histo-architectonics has theoretical and practical significance. The histostructure of the spleen of chickens has significant differences, particularly the absence of trabeculae, a small amount of connective tissue based around large vessels. The structure of the spleen of one-day-old vaccinated and non-vaccinated chickens is not formed, as there is no differentiation of the white pulp from the red pulp, which is related to the biological features of the chickens' body. According to our research, the presence of lymphoid nodules is observed for the first time in the 25-day age of the vaccinated group chickens. According to morphometric studies, it was established that their number was  $2.33 \pm 0.42$  pcs. Comprehensive farm vaccination programs include ten vaccinations of chickens up to 100 days of age. Under the influence of multiple antigenic stimulations in the following age periods of a 50-day-old bird (after six vaccinations: twice against infectious bronchitis and infectious bursal disease, once against Marek's disease and Newcastle disease), the 75-day (after nine times vaccination), 100-day (after ten times vaccination) number and sizes of lymphoid nodules, as well as periarterial lymphoid sheaths and periellipsoid lymphoid sheaths increased concerning chickens of the control group. Our research established that the histostructure of the spleen of one-day-old chickens of the experimental and control groups is incomplete. The bird's spleen, a peripheral organ of immune protection, is formed at 25 days. However, chickens are vaccinated twice (against Marek's disease and infectious bronchitis) in the incubator when they are less than one day old, and 90% of the planned vaccinations are completed by the age of 75 days. Therefore, studying morphological changes in the spleen of birds of different ages can be an essential tool for re-evaluating preventive treatment protocols in modern poultry farming.

**Keywords:** morphology, lymphoid nodules of the spleen, diffuse lymphoid tissue, vaccine prophylaxis of chickens.

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## 1. Introduction

The spleen of chickens is one of the essential organs of the immune system (Zhang et al., 2019; Mustafa & El-Desoky, 2020). According to Kozlu et al. (2011); Zhang et al. (2015), the spleen's resistance to diseases increases due to the absence of lymph nodes in chickens. At the same time, it is sensitive to external and internal influences (Dunaiev'ska, 2018). The effect of adverse factors (Graczyk et al., 2003), including vaccination (Gural'ska, 2016; Stromberg et al., 2018), various diseases (Al-Khatib & Al-Qutbey, 2005; Yang et al., 2006; Sandford et al., 2011) changes the composition of immunocompetent cells and the structure of the organ itself. According to Li et al. (2020), stress caused by vaccination causes lymphocyte apoptosis in the spleen. In addition, stress can alter immune responses (Wilkinson et al., 2011).

The spleen plays an essential role in cellular and humoral immunity. The histoarchitectonics of the spleen is one of

the significant studies identifying lymphocytes responsible for immunity (Yabe et al., 2017).

Even at a young age, chickens are exposed to stressful factors that negatively affect their health and well-being, and one of the most influential factors is stocking density (Bashchenko et al., 2020; Hofmann, 2021; Brezvy'n et al., 2021). Vaccination is also one of these factors. Its use has a weak reactogenic effect. Therefore, in the spleen of the bird, there is a weak hyperplasia of lymphoid cells and a low density of T-lymphocytes in the periarterial lymphoid sheaths (Asrutdinova et al., 2020).

Kotarev et al. (2020) claims that the morphology of the spleen is the leading indicator for establishing an assessment of the organism's state during its formation and adaptation to the external environment. Therefore, the study of the evaluation of the morphological state of the spleen is of great importance.

The research aims to establish cytological and histological features of the formation of the spleen of chickens as an

organ of immune protection at various stages of polyvalvaccinum prevention of infectious diseases.

#### Tasks of the research:

1. To establish the cytological and histological features of the spleen of chickens 1, 15, 25, 50, 75, 100, and 120 days old in the post-vaccination period.

2. To establish the cyto- and histoarchitectonics of the spleen of chickens at different vaccination times.

## 2. Materials and methods

A group of one-day-old high-sex brown cross chickens raised in the conditions of the branch of “Solotvinska Poultry Factory” LLC “Zelenyi Val” was selected for the experiment. Stery Solotvyn of the Berdychiv district of the Zhytomyr region, which was divided according to the principle of analogs into two groups: control (vaccinations were not carried out) and experimental (chickens were vaccinated according to the plan of vaccinations for repairing young animals once against Marek’s disease, the syndrome of reduced laying capacity, twice against Gamboro’s disease, three times – against Newcastle disease, four times – against infectious bronchitis of chickens).

The research was conducted in the educational and scientific clinical and diagnostic laboratory of the Polissia National University. The material was the spleen of chickens aged 1, 15, 25, 50, 75, 100, and 120 days selected from the control and experimental groups.

During the research, the “General Ethical Principles of Experiments on Animals” (Ukraine, 2001) were followed, which is consistent with the Law of Ukraine “On the Protection of Animals from Cruelty Treatment” dated February 21, 2006, No. 3447-IV and the Provisions of the “European Convention on the Protection of Animals, which are used for experimental and other scientific purposes”.

The anatomical level of the Study included: slaughter and exsanguination of the bird, dissection of thoracoabdominal cavities, and preparation of organs followed by removal from the cavity. Slaughter of chickens 1, 15, 25, 50, 75, 100, and 120 days old was carried out by acute exsanguination after ether anesthesia.

Histological studies were carried out according to generally accepted tissue fixation methods and tissue section production.

Morphometric methods were used to obtain objective data on the structural organization of the gland in chickens. The research was carried out using a Primo Star light microscope (Carl Zeiss, Germany) and Image Scope software.

Histological specimens were studied and photographed using a digital camera mounted in a Primo Star microscope (Carl Zeiss, Germany) and connected to a personal computer.

Statistical processing of the research results was performed using variational statistical methods on a personal computer using the Statistica 6.0 program (StatSoft Inc., USA). Fisher’s F-test assessed the reliability of the obtained data. The difference between the two values was considered significant at  $P < 0.05$ .

## 3. Results and discussion

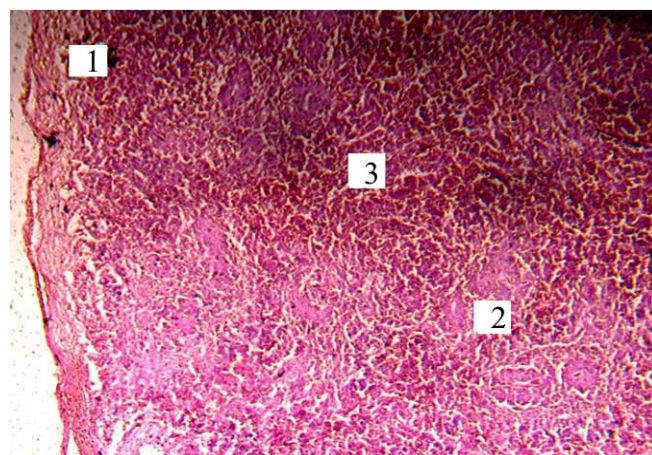
Externally, the spleen is covered with a connective tissue capsule with elastic fibers and myocytes. The thickness of the spleen capsule of one-day-old chickens is  $37.23 \pm 1.704 \mu\text{m}$ . From above, the capsule is covered with mesothelium.

The connective tissue stroma of the organ of day-old chickens is very poorly developed. There are no trabeculae. The basis of the parenchyma of the spleen is formed by reticular tissue, in which blood cells lie. The white pulp was not differentiated from the red pulp (Fig. 1).



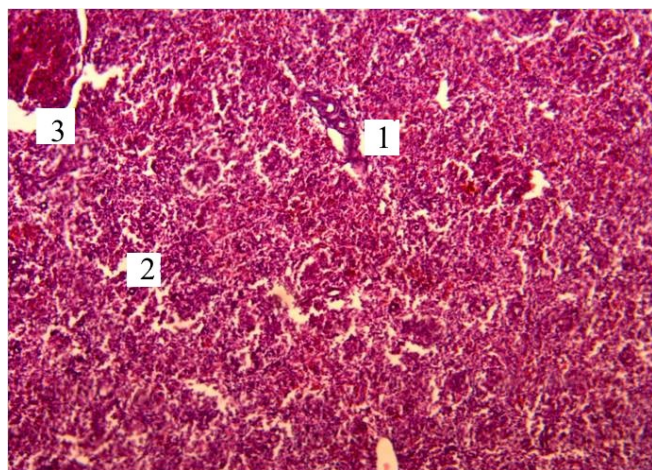
**Fig. 1.** Microscopic structure of the spleen of a vaccinated one-day-old chicken: 1 – capsule; 2 – blood vessel; 3 – red pulp. Hematoxylin and eosin. x 100

In the 15-day-old chickens of the experimental group, we noted only different accumulations of lymphoid tissue that did not have a clear border (Fig. 2).



**Fig. 2.** Microscopic structure of the spleen of a 15-day-old vaccinated chicken: 1 – capsule; 2 – diffuse lymphoid tissue; 3 – red pulp. Hematoxylin and eosin. x 100.

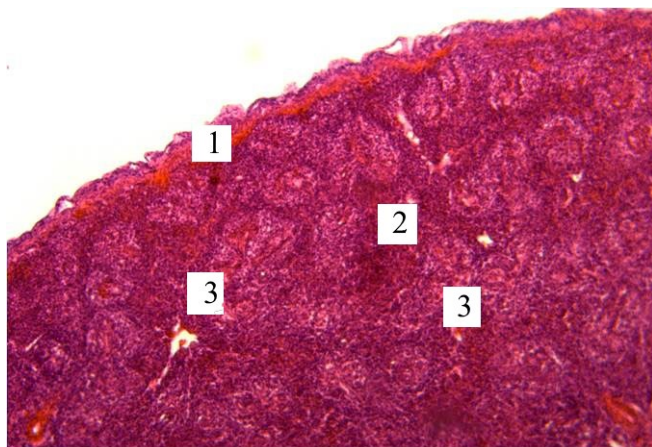
The histoarchitectonics of the spleen of 25-day-old chickens of the experimental and control groups were formed by white and red pulps and connective tissue stroma, which is very weakly developed. There are no trabeculae; only a small amount of connective tissue is found along the course of the vessels. According to histological studies, the WP of the organ of 25-day-old chickens was represented by diffuse lymphoid tissue, periarterial lymphoid sheaths, and the presence of lymphoid nodules (LN) (Fig. 3). For this, the number of LN was  $2.33 \pm 0.42$  pcs. In the 25-day-old chickens of the control group, instead of formed LNs, we noted only different accumulations of lymphoid tissue that did not have a clear border.



**Fig. 3.** Microscopic structure of the spleen of a 25-day-old vaccinated chicken: 1 – blood vessels; 2 – red pulp; 3 – white pulp. Hematoxylin and eosin. x 200.

It should be noted that under the influence of antigenic stimulation, the number of LNs, their sizes, and periarterial lymphoid sheaths increased. In the spleen's red pulp, many erythrocytes, reticulocytes, and lymphocytes were noted. The thickness of the spleen capsule in chickens of this age period of the experimental group practically did not differ from the control.

The structure is preserved in the spleen of 50-day-old chickens (after six vaccinations). A pronounced border between white and red pulp was noted in the spleen of chickens of this age period. A significant number of LNs were small in size, and there were substantial accumulations of lymphocytes around the vessels (Fig. 4).

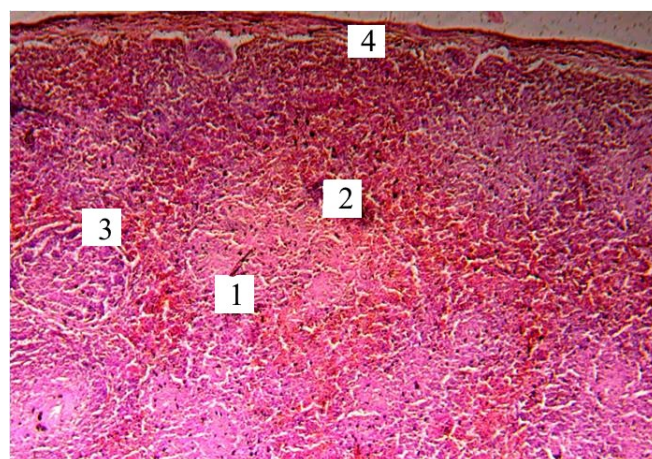


**Fig. 4.** Microscopic structure of the spleen of a 50-day-old vaccinated chicken: 1 – capsule; 2 – red pulp; 3 – white pulp. Hematoxylin and eosin. x 100.

Single macrophages and lymphoblasts were observed in the germinal centers of LN, which were at different stages of mitosis. The number of LN with germinative centers in the spleen of chickens of the research group was up to  $3 \pm 0.44$  pcs. on the conditional unit square (lens 10, vol. 10). Yet, a significant part of the white pulp was located in the periarterial lymphoid sheaths. According to the morphometric Study of the spleen of 50-day-old chickens of the research group, an increase in the diameter of the liver by  $16.70 \mu\text{m}$  was established compared to the control.

Our histological studies of the spleen of 75-day-old chickens (after six vaccinations) revealed the presence of a

large number of LN and significant sizes of periarterial and periellipsoid lymphoid sheaths, due to which the white pulp of the spleen was well-defined and, unlike previous age groups, was differentiated (Fig. 5).



**Fig. 5.** Microscopic structure of the spleen of a 75-day-old vaccinated chicken: 1 – blood vessels; 2 – red pulp; 3 – white pulp. Hematoxylin and eosin. x 100.

The number of LNs with reactive centers was already  $4.33 \pm 0.55$ . on the conditional unit square (lens. 10, vol 10), while in control, this indicator was  $2.0 \pm 0.25$  pcs. The cytopopulation of the diffuse lymphoid tissue consisted of lymphoblasts, dendritic cells, and macrophages, and an increase in the number of blood vessels was observed in the red pulp. Similar changes in the cyto- and histoarchitectonics of the organ of chickens of the research group concerning the control were also noted in chickens 100 and 120 days old.

A significant number of scientific works are devoted to the specific prevention of chickens; among them are studies on prevention programs for infectious bronchitis (Asrutdinova et al., 2020; Guralska et al., 2020), infectious bursal disease (Scanavini Neto et al., 2004; Yasmin et al., 2016; Dey et al., 2019), Newcastle disease (Masum et al., 2014), infectious laryngotracheitis.

Our research confirms and complements our previous results on the effect of vaccination on the development of lymphoid formations in chickens and the formation of splenic lymphoid nodules at 20 days of age (Guralska, 2016).

Al-Zubeady et al. (2018) observed pathological changes in the spleen of vaccinated chickens. However, Eto et al. (2012) noted no histological changes in the spleen in vaccinated laying hens.

Asrutdinova et al. (2020) research observed increased proliferation and differentiation of lymphoid cells in lymphoid nodes of the spleen after vaccination against infectious bronchitis. The authors also noted hyperplasia of periarterial and periellipsoid lymphoid sheaths, as well as the presence of germinal centers in lymph nodes. According to the results of a study by Guralska (2016), there is an increase in T-cells in the spleen of chickens vaccinated against infectious bronchitis. A decrease in the spleen index was observed in vaccinated chickens. Scanavini Neto et al. (2004) noted similar changes in their research.

When conducting a microscopic analysis of the spleen of chickens vaccinated against the infectious bursal disease, Scanavini Neto et al. (2004) observed hyperplasia of reticuloendotheliocytes of periarterial lymphoid sheaths and an increase in the number of germinal centers of lymphoid

nodules. An increase in the number of germinal centers of lymphoid nodules in the spleen of chickens in the post-vaccination period was also noted in our research.

Our findings confirm the findings of [Colombatti \(1989\)](#), [Mustafa & El-Desoky \(2020\)](#) regarding the presence of a thin capsule in the spleen, as well as the data of [Kannan et al. \(2015\)](#), [Guralska \(2016\)](#) regarding weakly developed trabeculae. At the same time, [Akter \(2006\)](#) notes that the bird's spleen is covered by a thick capsule and confirms the data of [Kannan et al. \(2015\)](#), [Guralska \(2016\)](#) regarding the presence of a small number of trabeculae. However, [Mustafa & El-Desoky \(2020\)](#) did not note the presence of trabeculae in their research.

Our results confirm the results of several authors regarding the fact that the spleen is already well-developed in chickens 20–30 days old. In chickens, the periarterial lymphoid sheaths are the most developed in the first week of life. [Reshag & Hamza \(2017\)](#) noted the presence of perilellipsoid lymphoid sheaths, and lymphoid nodules in the spleen of 21-day-old chickens, in which a germinal center was observed at 30 days of age. [Ayman et al. \(2021\)](#) noted the appearance of lymphoid nodules in 14-day-old chickens. Our studies and previous studies by [Budnik & Guralska \(2021\)](#) suggest that the white pulp of the organ consisted of lymphoid nodules, periarterial lymphoid sheaths, and perilellipsoidal lymphoid sheaths. All this indicates the morpho-functional maturity of the immune tissue in the spleen.

#### 4. Conclusions

Thus, the research results we obtained show that when chickens are vaccinated, lymphoid formations of the spleen develop more intensively than in non-vaccinated birds. This is confirmed by the formation of lymphoid nodules with reactive centers in the spleen of chickens at 25 days and is accordingly observed in subsequent age periods

#### Conflict of interest

The authors claim that there is no conflict of interest.

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