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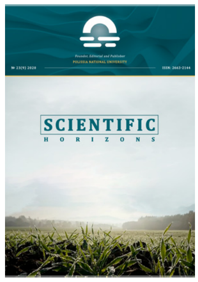

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Effect of chicken infectious bronchitis vaccine on morphogenesis and differentiation of cells in caecal tonsils

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Abstract. The study of the chickens' immune system morphofunctional state allows assessing critical periods of their development and the body as a whole, as well as the effectiveness of vaccine prevention methods. The purpose of this study was to identify morphological and immunohistochemical changes in the caecal tonsils of chickens aged 8, 20, 40, 90, 110 days for vaccine prevention of infectious bronchitis. During the study, the following research methods were used: cytological, histological, immunohistochemical, morphometric, light-optical, statistical. Histological preparations

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of caecal tonsils of poultry aged 8, 20, 40, 90, 110 days of vaccinated and unvaccinated groups were analysed and studied. Up to 20 days of age, no lymphoid nodules were detected in the caecal tonsils of chickens, both vaccinated and non-vaccinated groups. Histological and morphometric studies were conducted, which showed that vaccination of poultry accelerates the development of lymphoid formations in the early stages of the postnatal period of ontogenesis, especially in chickens aged 20 days, which is manifested by an increase in the number and size of lymphoid nodules of caecal tonsils relative to the control group. Immunohistochemical studies have established that the placement and accumulation of T-lymphocytes with markers $CD4^+$, $CD8^+$, as well as B-lymphocytes ($CD20^+$) and $CD45RA^+$ cells in caecal tonsils, namely in lymphoid nodules and diffuse accumulation of lymphoid cells, depend on the age of chickens and multiplicity of vaccinations. It was proved that in chickens aged 8 days, the number of T-lymphocyte subpopulations with surface markers $CD4^+$, $CD8^+$ prevailed over $CD45RA^+$ and $CD20^+$. With the increase in the number of immunizations (after three-fold vaccination against infectious bronchitis of chickens), immunocytometric studies indicated that in vaccinated chickens aged 40 and 90 days, there was a clear increase in the number of mature B-lymphocytes by 1.58 and 1.37 times, respectively. Considering the fact that the number of $CD8^+$ lymphocytes in vaccinated chickens aged 40 days was 1.49 times greater than the number of $CD4^+$ cells, this led to a sharp decrease in the immunoregulatory index of the caecal tonsils, which must be factored in when carrying out preventive vaccinations

Keywords: poultry; histological changes; lymphoid formations; T-lymphocytes; B-lymphocytes; vaccine prevention

INTRODUCTION

Chicken infectious bronchitis virus (IBV) is a coronavirus that reduces meat and egg production in poultry (Gallardo, 2021; Chandrasekar *et al.*, 2023). The CIB virus has been known since 1930, but even now, it causes significant economic damage to farms (Bande *et al.*, 2017; Legnardi *et al.*, 2020). Live and inactivated vaccines are used to prevent infectious bronchitis, but due to the emergence of IBV new strains, the risk of this disease outbreak increases (Bhuiyan *et al.*, 2021). The prevalence of the CIB virus is growing rapidly (Karimi *et al.,* 2019). Infectious bronchitis has three forms: respiratory, nephroso-nephritic, and reproductive (Guralska *et al.*, 2019; Zhang *et al.*, 2020). External factors that reduce the immune response also affect production parameters. Many factors, including infectious ones, cause immunosuppression in poultry (Gimeno & Schat, 2018; Nair, 2022; Schat & Skinner, 2022).

Poultry farming is the most intensive and fast-growing industry among other livestock production. Presently, it has expanded considerably due to the demand for both meat and eggs (Stamilla *et al.*, 2020). Vaccination is used in poultry farms to prevent infection, reduce morbidity and mortality (Habibi *et al.,* 2017; Yang *et al.,* 2023). According to Stamilla *et al.* (2020), vaccination is a vital step to maintain the necessary biosecurity, especially in the first weeks of poultry life. However, repeated vaccinations significantly inhibit the body's immune functions (Kaab *et al.,* 2018). Vaccines are often used repeatedly, and this, as a result, causes immune stress disorder. Mass use of vaccines, specifically against infectious bronchitis, is to some extent ineffective and can lead to ineffective vaccination (Jordan, 2017). Despite the fairly widespread use of vaccination against infectious bronchitis, this disease causes considerable economic damage to poultry farms (Icochea *et al.*, 2023). M.W. Jackwood and D.H. Lee (2017) argue that the use of live attenuated vaccines for immunization affects the genetic profile of chicken infectious bronchitis strains. According to the results of the study, the use of the vaccine has a weak reactogenic effect (Asrutdinova *et al.,* 2020). And yet, even though there may be outbreaks of the disease in immunized poultry, vaccination is still one of the key methods of preventing infectious bronchitis in chickens (Ali *et al.,* 2023). For vaccine prevention of infectious bronchitis in chickens, it is necessary to first consider the immunosuppressive effect of vaccines on the poultry body (Guralska & Budnik, 2020).

Logvinova & Oliyar (2021) indicate the need to consider the morphogenesis of the lymphoid structures of the intestine of poultry during intensive poultry farming. The gastrointestinal tract plays a major role in immune homeostasis (Ahluwalia *et al.,* 2017; Nochi *et al.,* 2018). Peripheral organs of the immune defence of birds include lymphoid tissue associated with the mucous membrane of the digestive canal (Montalban-Arques *et al.,* 2018). It occupies one of the prominent places in the system of the immune defence organs of birds (Mazurkevych, 2017). The intestine-associated lymphoid tissues of the digestive tract, which consist of lymphoid cells contained in Peyer's patches, caecal tonsils, and also in the own lamina of the intestinal mucosa, are quite well-developed in the bird's body. The cacecal tonsils consist of an aggregated mass of lymphocytes and form numerous nodules (Abd El-Wahab *et al.,* 2017). They trigger a protective immune response against both bacterial and viral pathogens (Alqazlan *et al.,* 2021). The caecal tonsils are analogous in structure to Peyer's patches, have the appearance of two large lymphoid aggregates and are located at the border between the caecum and rectum (Abd El-Wahab *et al.*, 2017). These lymphoid formations are secondary lymphoid tissue that takes part in humoral immune responses (Nnadozie *et al.,* 2019). Lymphoid formations that are located in the digestive organs of poultry protect the body from foreign antigens (Khomich *et al.,* 2021).

The immune formations of the poultry caecum are quite well-developed. The area of the caecal tonsils contains a significant amount of lymphoid tissue (Mazurkevych, 2017). Morphologically, two types of lymphoid tissue are found in them: diffuse (T-zone) and nodular (B-zone) (Nnadozie *et al.*, 2019). Lymphoid tissue is present in the mucosa and submucosa of the caecal tonsils (Mazurkevych, 2017). Lymphoid formations of the digestive system support the genetic constancy of the internal environment of the body (Khomich *et al.,* 2021).

The caecum is constantly exposed to bacterial or other antigens. Therefore, cochlear lymphoid formations play an important role in the immunological response to foreign microorganisms. Cellular immune responses in lymphoid formations are a prerequisite for humoral responses, the deficiency of which in birds in the first weeks of life is associated with the immaturity of T- and B-lymphocytes (Junior *et al.*, 2018). In caecal formations, under the antigen, lymphocytes turn into effector cells, the secretory substances of which determine the development of cellular and humoral immunity (Mazurkevych, 2017). The study of basic structures is a necessary condition for understanding the physiology and immunomorphology of immune defence organs (Ayman *et al.,* 2021). According to Thomrongsuwannakij *et al.* (2021), before using vaccines, it is necessary to investigate the immune status of chickens.

The purpose of this study was to establish the immunocytological and histological features of the formation of caecal tonsils in chickens for vaccine prevention against infectious bronchitis. Objectives of the study: to establish cyto- and histological features of the chickens caecal tonsils during vaccination; to determine the content, localization, and quantitative ratio of subpopulations of $CD8^+$, $CD4^+$, $CD20^+$, CD45RA-lymphocytes of the chickens caecal tonsils during vaccination.

MATERIALS AND METHODS

For the study, Hisex Brown cross chickens of the same age were selected, which were kept at the branch "Solotvyn poultry farm" of the limited liability company "Zelenyi Val" in the village of Stary Solotvyn, Berdichevskyi district, Zhytomyr region. The poultry was divided into two groups: control (intact) and experimental (vaccinated).

Histological studies were performed in the educational-scientific clinical-diagnostic laboratory of the Polissia National University. Histological material was the caecal tonsils of chickens aged 8, 20, 40, 90 and 110 days selected from the control and experimental groups.

Anatomical examination included slaughter and exsanguination of chickens, preparation and removal of organs. Histological studies were carried out according to generally accepted methods of tissue fixation and production of histological tissue sections. For histological examination, caecal tonsils of chickens were selected, which were fixed in a 10-12% aqueous solution of neutral formalin for 48 hours, then washed with tap water for 24 hours, dehydrated with ethyl alcohol of increasing concentration (40%, 50%, 70%, 96%, 100%) followed by pouring in paraffin. Histosections were made on a sled microtome, which were subsequently stained with haematoxylin and eosin, as well as according to the Van Gieson method.

Immunohistochemical studies revealed T-lymphocytes in caecal tonsils: helpers and cytotoxic cells with $CD4^+$ and $CD8^+$ surface markers, respectively; mature B-lymphocytes ($CD20^+$), as well as naïve T-helpers, B-lymphocytes and monocytes ($CD45RA^+$). Monoclonal antibodies were used for this purpose. For better visualization, the histosections were additionally stained with Mayer's haematoxylin (DAKO, Denmark).

A Primo Star light microscope (Carl Zeiss, Germany) and Image Scope software were used for the morphometric study of caecal tonsils of chickens. The immunoregulatory index (IRI) was determined – the $CD4^+$:$CD8^+$ ratio. For microphotography, a digital camera mounted in a Primo Star microscope (Carl Zeiss, Germany) was used. Statistical processing of the study results was performed using the Statistica 6.0 software (StatSoft Inc., USA). Fischer's F-criterion was used to assess statistical significance. The difference between the obtained data was recognized as probable at $P<0.05$.

During the research, the "General Ethical Principles of Experiments on Animals" (Reznikov, 2003) were observed, which is consistent with the Law of Ukraine No. 3447-VI "On the Protection of Animals from Cruelty" dated 10.16.2012 (Law of Ukraine No. 3447-IV, 2006) and the Provisions of the "European Convention for the Protection of Animals Used for Experimental and Other Scientific Purposes" (European Convention, 1986), as well as the Declaration on the Humane Treatment of Animals (Universal Declaration, 2007).

RESULTS AND DISCUSSION

Histological examination of the caecum revealed that the histoarchitectonics of the chickens experimental group organ practically does not differ from the control one. Notably, no lymphoid formations were observed in the control and experimental chickens aged 8 days in their own plate of the caecum mucosa. However, in chickens aged 20 days (with repeated vaccination), the lymphoid tissue was diffuse clusters of lymphoid cells and lymphoid nodules (LN) were also detected, the average diameter of which increased 1.13 times in the experimental and control chickens and amounted to 59.8±2.44 μm ($P<0.05$), respectively (Fig. 1). This indicated the LN hyperplasia in the poultry of the experimental group due to antigenic stimulation.

Therewith, in chickens of this age in the experimental group, a slight increase in the number of lymphocytes in the caecum submucosal base was observed, especially B-lymphocytes and plasmocytes with a low content of macrophages, with a considerable number of lymphocytes concentrated in the area of the caecal tonsils (CTs).

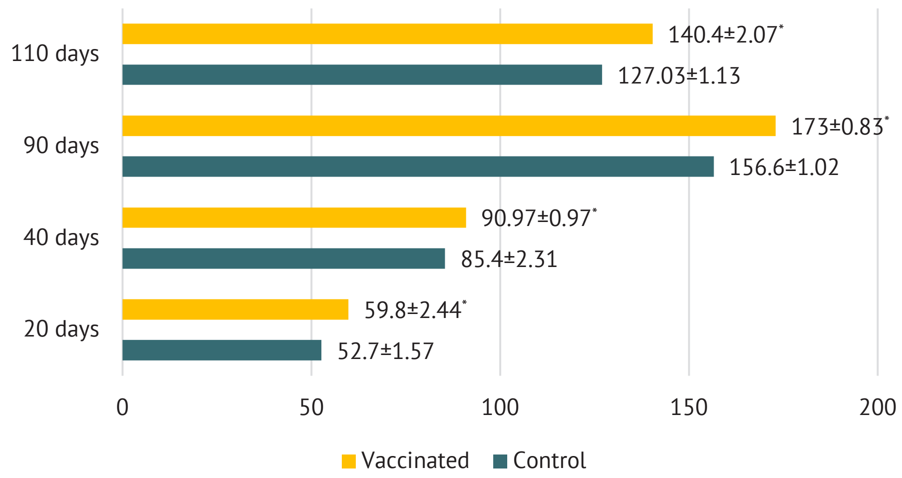

Figure 1. *Diameter of vaccinated chickens' caecal tonsils lymphoid nodules, μm*
Note: ** – P<0.05 for chickens in the control group*
Source*: compiled by the authors*

The detected hyperplasia of lymphoid nodules (Fig. 2) in the CTs area of the experimental group chickens occurred regardless of the vaccine administration method. That is, the systemic course of the processes that took place was noted. The submucosal base of the caecum was infiltrated by lymphocytes, and as the number of antigenic stimulations increased, an intense plasmocytic reaction occurred in the intestinal wall. The chickens after the first vaccinations were dominated mainly by plasmoblasts, as well as immature plasmocytes. In poultry aged 90 and 110 days, the main plasma cells were already mature plasmocytes.

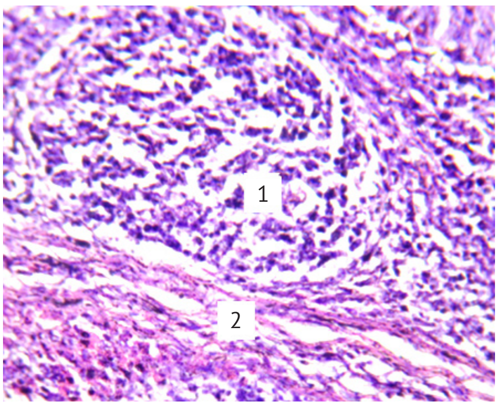

Figure 2. *Fragment of the microscopic structure of the vaccinated chicken aged 40 days caecal tonsils: 1 – lymphoid nodule; 2 – trabecula*
Note*: Haematoxylin and eosin. ×400*
Source*: photographed by the authors*

Lymphoid cells and macrophages were detected in the submucosal base of chickens aged 40 and 90 days in the experimental group. Lymphocyte accumulations were insignificant. Therewith, the presence of LN wrapped in collagen fibres was noted (Fig. 3).

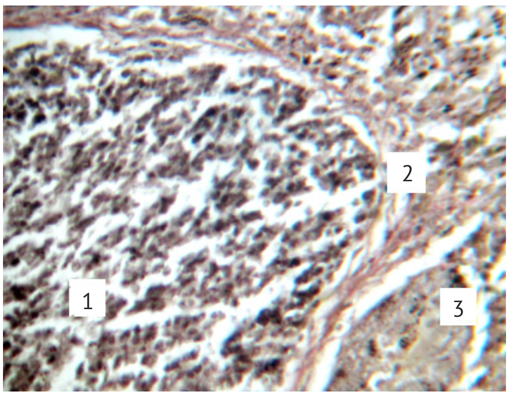

Figure 3. *Fragment of the microscopic structure of the caecal tonsils of a vaccinated chicken aged 40 days: 1 – lymphoid nodule; 2 – trabecula; 3 – crypt*
Note: *The Van Gieson's stain. ×400*
Source*: photographed by the authors*

Analysis of cytomorphometric results indicated that in the experimental group of chickens aged 40 days, the amount of LN was 12.06±0.38 pcs., while in the control – 10.83±0.28 pcs. (Fig. 4). The LN diameter in vaccinated chickens aged 40 days also significantly increased by 1.07 times (P<0.05) and was 90.97±0.97 μm (Fig. 1).

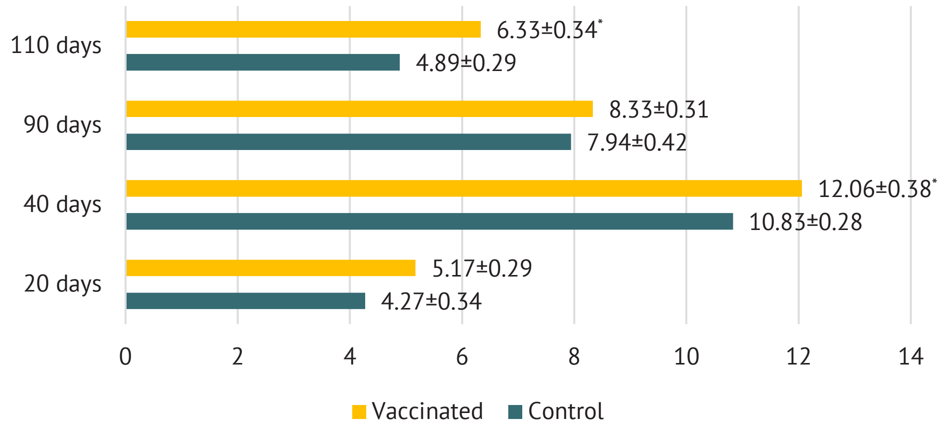

Figure 4. *Number of lymphoid nodules of the vaccinated chickend caecal tonsils, pcs.*
Note: ** – P<0.05 for chickens in the control group*
Source*: compiled by the authors*

Similar cyto- and histoarchitectonics of CTs lymphoid nodules were observed in experimental chickens aged 90 and 110 days. Moreover, there was a stable predominance of the number and size of CTs nodules in vaccinated chickens over the control ones, as evidenced by the presented histometric studies.

During the immunohistochemical analysis of CTs in experimental chickens compared to control ones, an increase in the processes of formation and differentiation of lymphocytes with $CD4^+$ and $CD8^+$ markers, which provide a cellular immune response, as well as $CD20^+$, which form the antibodies, was observed. According to the results of immunohistochemical studies, it was established that T-lymphocytes ($CD4^+$) in the CTs area of vaccinated chickens aged 8 days are located in the mucous membrane. They form small clusters that are located near the crypts. On individual preparations of the CTs of chickens aged 20 and 40 days of the experimental group, these T-lymphocytes are localized singly in the places where the crypts are located (Fig. 5).

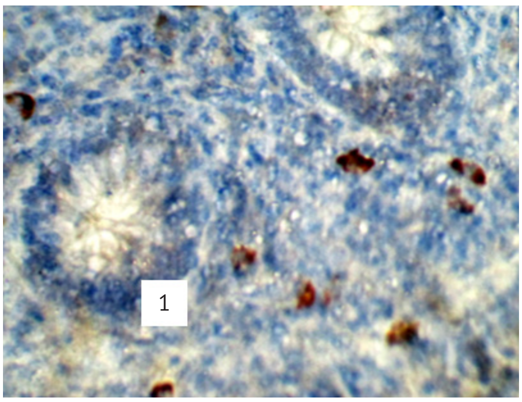

Figure 5. *$CD4^+$ lymphocytes in the caecal tonsils of a vaccinated chicken aged 20 days: 1 – crypts*
Note: *Histopreparations using monoclonal antibodies. ×600*
Source*: photographed by the authors*

The presence of lymphocytes was also noted in the intestinal villi (Fig. 6). In chickens of older age groups, after vaccination, T-lymphocytes with surface markers $CD4^+$ had an analogous location as in a poultry aged 40 days, and they also formed clusters in lymphoid nodules.

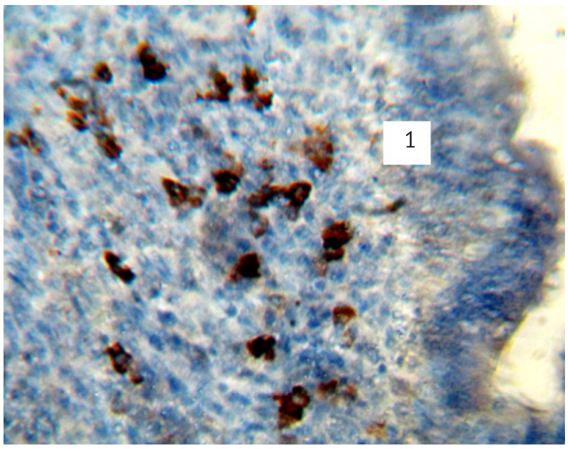

Figure 6. *$CD4^+$ lymphocytes in the caecal tonsils of vaccinated chicken aged 40 days: 1 – villi*
Note: *Histopreparations using monoclonal antibodies. ×600*
Source*: photographed by the authors*

Analysis of the quantitative study of lymphocyte subpopulations in CTs showed clear cytomorphometric manifestations of changes in $CD4^+$ lymphocytes in vaccinated chickens compared to control ones. Thus, in experimental chickens aged 8 days, an increase (P<0.05) in the number of T-helpers was observed by 1.19 times compared to the control. Analogous results regarding the increase in the number of $CD4^+$ lymphocytes were also observed in experimental chickens of a different age (Fig. 7).

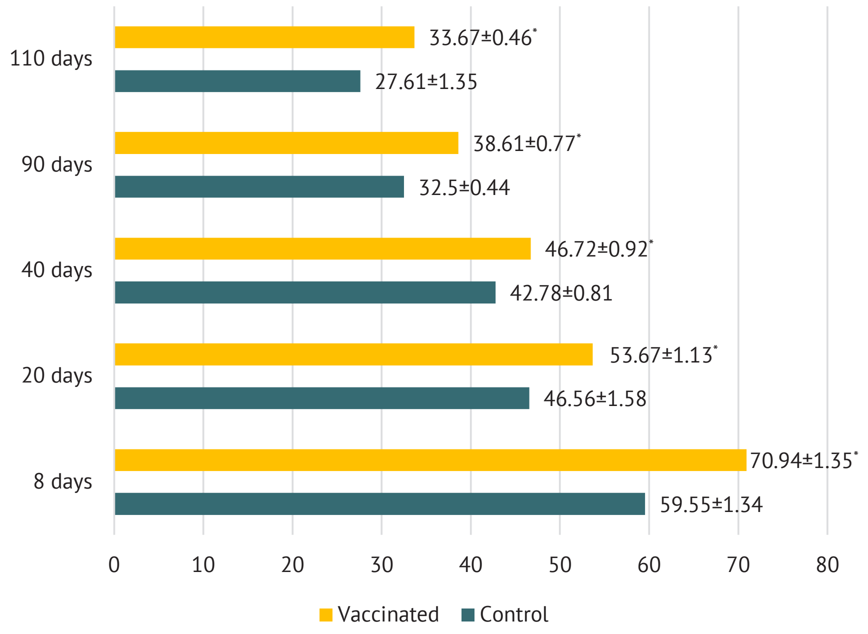

***Figure 7**. The number of CD4⁺ lymphocytes in caecal tonsils of vaccinated chickens, pcs.*
***Note:** * – P<0.05 for chickens in the control group*

The results of immunohistochemical studies indicate that the placement of CD8⁺ lymphocytes in the CTs area of vaccinated chickens aged 8 and 20 days is analogous to the control group. A part of lymphocytes with CD8⁺ surface markers in the CTs of chickens aged 40 days, after threefold vaccination, is diffusely located in the mucous membrane (Fig. 8), while other cells form clusters in lymphoid nodules. In vaccinated chickens aged 90 and 110 days, subpopulations of lymphocytes with CD8⁺ clusters in lymphoid nodules form clusters of 4-10 cells on their periphery.

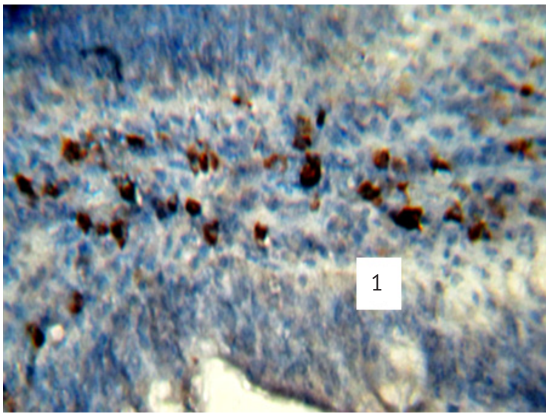

***Figure 8.** CD8⁺– lymphocytes in the caecal tonsils of a vaccinated chicken aged 40 days: 1 – villi*
***Note:** Histopreparations using monoclonal antibodies. ×600*
***Source**: photographed by the authors*

Subpopulations of lymphocytes with CD45RA⁺ clusters in the CTs area of chickens aged 8 and 20 days are individually localized between crypts and in a diffuse cluster of lymphoid cells, their small number was also noted in the villi. In addition, CD45RA⁺ lymphocytes were found in almost every lymphoid nodule of vaccinated chickens aged 40, 90, 110 days. Such cells were also found in the apical region of the epithelial cell cytoplasm, the villi stroma, and the mucosa's own plate (Fig. 9).

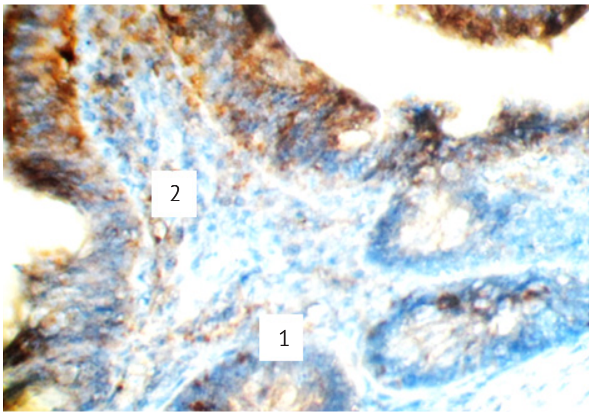

***Figure 9.** CD45RA⁺ lymphocytes in the caecal tonsils of a vaccinated chicken aged 90 days: 1 – crypt; 2 – villi*
***Note:** Histopreparations using monoclonal antibodies. ×400*
***Source**: photographed by the authors*

According to the results of studies, it was found that the number of cytotoxic cells (CD8⁺) in the CTs area of experimental chickens with an increase in the frequency of vaccination changed. Therewith, vaccinated chickens of different age periods showed their growth (Fig. 10).

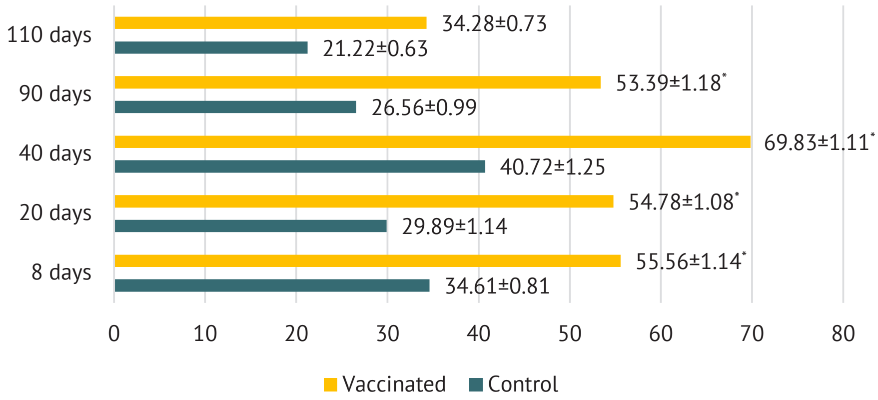

Figure 10. *Number of CD8S⁺ lymphocytes in the caecal tonsils of vaccinated chickens, pcs.*
Note: ** – P<0.05 for chickens in the control group*
Source*: compiled by the authors*

The growth of $CD8^+$ lymphocytes also affected the immunoregulatory index of the CTs of the experimental poultry. This indicator significantly decreased (P<0.05) in all age groups relative to the control (Fig. 11).

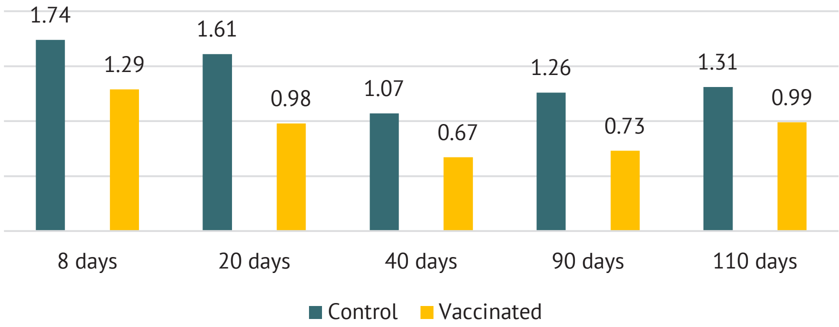

Figure 11. *Immunoregulatory index in the amygdala of vaccinated chickens*
Source*: compiled by the authors*

Cytomorphometric studies also established a significant (P<0.05) increase in the number of cells of the $CD45RA^+$ subpopulation after three- and five-fold vaccination against CIB compared to non-vaccinated ones. At the same time, in vaccinated chickens aged 8, 20, and 90 days, only a tendency to an increase in the number of $CD45RA^+$ cells was observed compared to the control (Fig. 12).

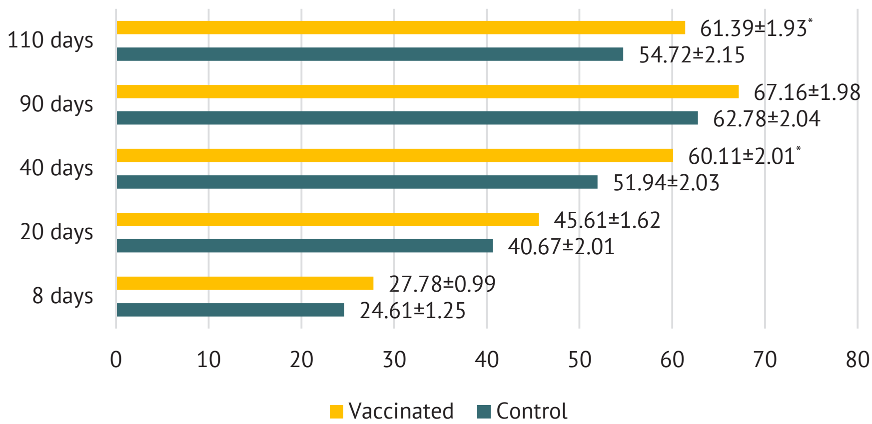

Figure 12. *Number of $CD45RA^+$ lymphocytes in the caecal tonsils of vaccinated chickens, pcs.*
Note: ** – P<0.05 for chickens in the control group*
Source*: compiled by the authors*

In the CTs of vaccinated chickens, a significant increase in the number of CD20+ cells (mature B-lymphocytes) was noted, the presence of which was observed in all structural elements, including the mucous membrane, villous stroma, around the crypts, diffuse lymphoid tissue, and in the LN. Furthermore, in chickens aged 20 and 40 days (after two- and threefold vaccination), B-lymphocytes were detected in the apical region of the cytoplasm of columnar epithelial cells of the epithelial plate of the mucous membrane, as well as in LN. In experimental chickens aged 90 and 110 days, mature B-lymphocytes were localized in LN, the cytoplasm of columnar cells that formed crypts, and a significant number were located in the interfollicular lymphoid tissue (Fig. 13).

At the same time, in vaccinated chickens of all age groups, the number of CD20+ cells increased significantly (P<0.05) compared to intact ones (Fig. 14).

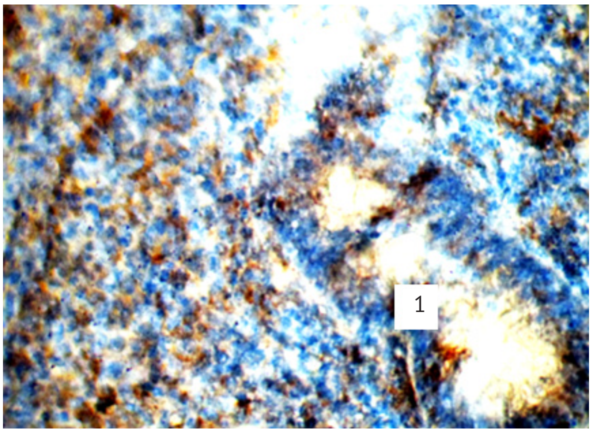

Figure 13. *CD20+ lymphocytes in caecal tonsils of a vaccinated chicken aged 110 days: 1 – crypt*
Note: *Histopreparations using monoclonal antibodies. ×600*
Source*: photographed by the authors*

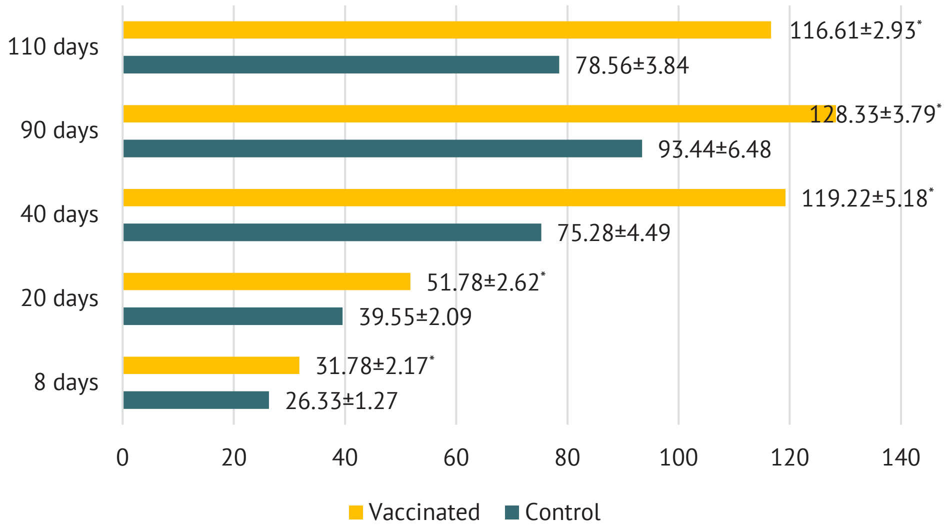

Figure 14. *Number of CD20+- lymphocytes in the caecal tonsils of vaccinated chickens, pcs.*
Note: ** – P<0.05 for chickens in the control group*
Source*: compiled by the authors*

According to the results of histological studies of the caecal tonsils in chickens aged up to 20 days, there were no lymphoid nodules, only diffuse lymphoid tissue was noted, which indicates their functional immaturity. These results confirm the data of B. Song *et al.* (2021) that the immune system of chickens is not developed until 34 days of age. Studies on the morphological characteristics of chickens caecal tonsils at the cellular and tissue levels to some extent complement the data of other authors. The detected postvaccinal morphofunctional changes in the chicken organ depended on the frequency of immunization and their age. According to N. Nagy *et al.* (2022), T-lymphocytes in the tonsils are located mainly in the interfollicular space, but some of them are also in the germinal centres. After hatching, the number of lymphocytes increases, CTs are observed already in the second week of life, and they increase with age. The composition includes specialized epithelium, subepithelial zone, lymphoid nodules and interfollicular areas. In the subepithelial zone, apart from plasma cells, there are also CD4+ and CD8+ lymphocytes, and the interfollicular T-cell-dependent zone consists mainly of CD4+ (Nagy *et al.*, 2022). U. Ayman *et al.* (2021) in chickens aged 1 day, only a small infiltration of lymphocytes was detected in the area of caecal formations; however, in chickens aged 14 days, they already noted the presence of lymphoid nodules, whereas in chickens aged 28 days, encapsulated lymphoid nodules and diffuse lymphoid tissue in the lamina propria and submucosal layer. Caecal tonsils of chickens acquire morphofunctional maturity gradually up to 35 days of life (Udoumoh *et al.*, 2021).

The study results complement the data of A. Hussein and A. Reshag (2019), which indicate that the caecal tonsils contain many B cells. Results of immunohistochemical studies by V. Khomich *et al.* (2021) indicated that the highest number of mature B lymphocytes was observed in poultry aged 180 days. According to the results of the study B. Song *et al.* (2021), the highest levels of specific cellular immunity components, namely the activity of T- and B-cells proliferation, are observed from 30 to 34 life days of broiler chickens. According to A. Hussein and A. Reshag (2019) the size of the caecal tonsils increases with age. In poultry aged 2 months, the presence of mature B-lymphocytes was clearly noted. In Hisex Brown chickens, according to research results, the highest indicators of T-lymphocytes with surface markers $CD4^+$ $CD8^+$ were noted precisely at the age of 40 days, while the indicators of mature B-lymphocytes ($CD20^+$) – at the age of 90 days.

To estimate humoral immunity, it is necessary to consider the level of T-lymphocytes with the surface marker $CD4^+$. $CD4^+$ and $CD8^+$ lymphocytes can have suppressive activity (Dai *et al.*, 2021). According to Lee *et al.* (2019), $CD4^+$ lymphocytes act as regulatory T cells.$CD8^+$ is particularly important for antigen recognition (Liu *et al.*, 2020). According to the study results of E. Aston *et al.* (2019), when immunizing chickens against CIB, the amount of $CD8^+$ lymphocytes grows in relation to unvaccinated poultry. M. Dai *et al.* (2021) point out the importance of cytotoxic T cells in the immune response specifically to the chicken infectious bronchitis virus. These data are confirmed in our research results because starting from the 20-day age of chickens, the number of $CD8^+$ lymphocytes prevailed over $CD4^+$ lymphocytes in caecal tonsils. Thus, if in vaccinated chickens aged 40 days the number of lymphocytes in the CTs with surface markers $CD8^+$ was 69.83±1.11 pcs., then $CD4^+$ lymphocytes – 46.72±0.94 pcs. Based on the results of immunocytochemical studies by R. Zegpi *et al.* (2019), vaccination can induce elevated levels of $CD4^+$ lymphocytes, $CD8^+$ lymphocytes. The presence of CD8 lymphocytes is clearly expressed in young poultry (Hussein & Reshag, 2019). When investigating the cell clusters, results were obtained that indicated a certain dynamics in the development of the immune response, which depended on the age of the poultry, the frequency of vaccination, and the characteristics of the organ under study. Any infection can destroy post-vaccination defences and adversely affect the immune system.

The T-system of poultry is involved in the formation of the immune response and regulates its duration (Hussein & Reshag, 2019). Although an increased number of $CD4^+$, $CD8^+$ T-cells was observed in the peripheral blood during viral infections, according to M. Dai *et al.* (2021), their role, functions, and biological significance are still understudied. An increase in the ratio of $CD4^+/CD8^+$ in the thymus of chickens was observed in the studies of T. Kannan *et al.* (2017): at four weeks to 0.67 and at eight weeks of age to 0.72. According to our research, the ratio of $CD4^+/CD8^+$ in the CTs of vaccinated chickens at 40 days of age was 0.67, and at 90 days it was 0.73. Therewith, K. Acevedo-Villanueva *et al.* (2021) did not note substantial changes in the ratio of $CD4^+/CD8^+$ in CTs of immunized and intact poultry.

The investigation of the immune system organs morphology helps fully understand the immunology of poultry (Nagy *et al.,* 2022). As indicated by T. Mazurkevych *et al.* (2022), it is by determining both the quantitative and qualitative composition of the immune protection organs lymphoid cells subpopulations that the immune status of an animal can be determined at different age periods. Understanding the morphology of caecal tonsils in poultry is particular importance because they perform a protective function (Saleh *et al.*, 2022).

Thus, as a result of the conducted studies, data were obtained indicating the feasibility of using immunohistochemical methods to determine the immune state in vaccinated chickens against infectious bronchitis.

CONCLUSIONS

During histological examination of experimental chickens' aged 110 days caecal tonsils, the size of lymphoid nodules decreases, which may indicate physiological involution. Thus, if at the age of 90 days in vaccinated chickens the nodule diameter was 173±0.83 µm, then at the age of 110 days this indicator decreased to 140.4±2.07 µm. In the control group of chickens, this indicator was 156.6±1.02 µm and 127.03±1.13 µm, respectively.

Immunohistochemical studies of caecal tonsils helped establish the localization and number of lymphocytes with $CD4^+$, $CD8^+$, $CD45RA^+$ and $CD20^+$ differentiation clusters in the structural elements of the organ, which directly depended on the age of the poultry and the frequency of vaccination. Thus, by the age of 20 days, T-lymphocytes with surface markers $CD4^+$ (T-helpers), $CD8^+$ (cytotoxic cells) dominated over mature B-lymphocytes ($CD20^+$).

In vaccinated chickens aged 40 and 90 days, there was a considerable dominance of cytotoxic cells over T-helper cells. Therefore, in the 40-day age of experimental chickens, the number of CD8+ lymphocytes in caecal tonsils was 69.83±1.11 units, and $CD4^+$ lymphocytes – 46.72±0.94 units. As a result, this was reflected in a decrease in the immunoregulatory index of the organ, which in this age period had the lowest indicators and amounted to 0.67.

As the analysis of the immunocytomorphometric study showed, a significant increase in mature B-lymphocytes ($CD20^+$) and naïve T-helpers, B-lymphocytes, and monocytes ($CD45RA^+$) was noted in vaccinated chickens aged 40, 90 and 110 days, both in relation to the previous age groups and to control group. Thus, in control chickens aged 90 days, the number of lymphocytes with the surface marker CD20^{+} was 93.44±6.48 units, while in vaccinated chickens this indicator reached 128.33±3.79 units. It follows from this that stimulation of the immune system through vaccines affects the growth of mature B cells, and the increase in the number of CD45RA^{+} can indicate the presence of an active immune response in the body of the poultry.

In the future, it is planned to conduct a histochemical study of the immune defence organs of chickens for comparative characterization of morphological features.

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CONFLICT OF INTEREST

None.

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Вплив вакцини проти інфекційного бронхіту курей на морфогенез та диференціацію лімфоїдних клітин сліпокишкових мигдаликів

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Анотація. Вивчення морфофункціонального стану органів імунної системи курей дає можливість оцінити критичні періоди їх розвитку та організму в цілому, а також ефективність методів вакцинопрофілактики. Метою дослідження було виявлення морфологічних та імуногістохімічних змін сліпокишкових мигдаликів курей 8, 20, 40, 90, 110-добового віку за вакцинопрофілактики інфекційного бронхіту. В ході роботи використано методи дослідження: цито-, гістологічні, імуногістохімічні, морфометричні, світлооптичні, статистичні. Проаналізовано та досліджено гістологічні препарати сліпокишкових мигдаликів птиці віком 8, 20, 40, 90, 110 діб вакцинованої та невакцинованої групи. До 20-добового віку курей, як вакцинованої, так і невакцинованої групи, лімфоїдних вузликів в ділянці сліпокишкових мигдаликів не виявлено. Було проведено гістологічні та морфометричні дослідження, які показали, що вакцинація птиці прискорює розвиток лімфоїдних утворень на ранніх етапах постнатального періоду онтогенезу, особливо у курей 20-добового віку, що проявляється збільшенням кількості та розмірів лімфоїдних вузликів сліпокишкових мидаликів стосовно контролю. Імуногістохімічними дослідженнями було встановлено, що розміщення та скупчення Т-лімфоцитів з маркерами $CD4^{+}$, $CD8^{+}$, а також В-лімфоцитів ($CD20^{+}$) та клітин-$CD45RA^{+}$ у сліпокишкових мигдаликах, а саме у лімфоїдних вузликах та дифузному скупченні лімфоїдних клітин залежать від віку курей та кратності вакцинації. Було доведено, що у курей 8-добового віку переважала кількість субпопуляцій Т-лімфоцитів з поверхневими маркерами $CD4^{+}$, $CD8^{+}$ над $CD45RA^{+}$ та $CD20^{+}$. Із зростанням кількості проведених імунізацій (після трьохкратної вакцинації проти інфекційного бронхіту курей) імуноцитометричними дослідженнями показано, що у вакцинованих курей віком 40 та 90 діб відбулось чітке зростання кількості зрілих В-лімфоцитів у 1,58 та 1,37 рази відповідно. Зважаючи на те, що у вакцинованих курей 40-добового віку кількість $CD8^{+}$-лімфоцитів переважала у 1,49 рази за кількість $CD4^{+}$-клітин, це призвело до різкого зниження імунорегуляторного індексу сліпокишкових мигдаликів, що необхідно враховувати при проведенні профілактичних щеплень

Ключові слова: птиця; гістологічні зміни; лімфоїдні утворення; Т-лімфоцити; В-лімфоцити; вакцинопрофілактика