

Pathomorphological changes in the bursal sac of chickens vaccinated against Newcastle disease using adjuvants

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Abstract. The article presents the results of a study of pathomorphological changes in the bursal sac of chickens vaccinated against Newcastle disease. The experiment was carried out in the conditions of Sabinsky IPS LLC in the Sabinsky district of the Republic of Tatarstan during the period of raising chickens of the Lomann LSL cross. For research, 60 chickens of 8 weeks of age were taken, which were divided into 4 groups of 15 chickens in each. The birds of the first group were immunized with a once inactivated emulsified vaccine against Newcastle disease with the addition of squalene in the form of emulsion at a dose of 0.5 cm³. The second group was inactivated by the emulsified vaccine against Newcastle disease containing Montanide ISA-71VG adjuvant. The third group was immunized with a vaccine against Newcastle disease without adjuvant, and the remaining 15 chickens were intact. The vaccine in various embodiments was administered to the chickens in a volume of 0.5 cm³ subcutaneously in the dorsal surface of the middle part of the neck. Slaughter of experimental birds of groups 1, 2, 3, and 4 was carried out on the 7th, 14th, and 21st days after vaccination, 6 animals for each study period. Pieces of the bursal sac were preserved in a 10% solution of neutral formalin. After dehydration and compaction of the taken material, the prepared histological sections with a thickness of 8 μm were stained with hematoxylin and eosin and examined under a microscope. Studies of involutinal changes in the microstructure of the bursal sac of chickens showed its dependence not only on hereditary mechanisms, but also on additional antigenic stimulation. It has been established that the vaccine against Newcastle disease has reactogenic and immunogenic qualities. In this connection, the most pronounced involutinal processes were noted in the bursal sac in chickens of the 3rd group, and the maximum immunogenic qualities of the vaccine preparation were manifested in the structure of the bursal sac in chickens of the 2nd and especially the 1st group. The lowest level of alterative changes in the bursal sac was observed in birds of the 4th group. Involutinal processes in these birds were determined by hereditary factors, while the chickens of the remaining groups noted the incidental nature of the manifestation of involution.

1 Introduction

The immune system of birds is a combination of organs and tissues that maintain the constancy of the internal environment and create the body's immunity to foreign agents [1–4]. In the process of evolution, it has been constantly evolving and changing, from invertebrates to mammals [5, 6]. An important role in the implementation of the protective function of the body is played by lymphoid organs, lymphoid tissue and lymphoid cells. [7–9]. The organs of the immune system of birds in accordance with their function and role are divided into central and peripheral [10, 11]. The central organs of the immune system include the thymus gland (thymus), the bursal sac, and the bone marrow, and the peripheral organs include the spleen, lacrimal and Gardera gland, Meckel diverticulum, Peyer's patches, tonsils, esophageal tonsils and various assemblies of lymphoid tissue. [12–21].

The aim of our research was a comparative study of the pathomorphology of the post-vaccination process in

the bursal sac of chickens vaccinated against Newcastle disease using various vaccine variants.

2 Materials and methods

The experiment was carried out at the Sabinsky IPS LLC in the Sabinsky district of the Republic of Tatarstan during the period of raising chickens of the Lomann LSL cross. Determination of the effectiveness of Squalene hydrocarbon in the Newcastle disease vaccine was carried out on 60 chickens of 8 weeks of age, which were divided into 4 groups, 15 chickens in each. The birds of the first group were immunized with a once inactivated emulsified vaccine against Newcastle disease with the addition of squalene emulsion in a dose of 0.5 cm³. The second group was vaccinated by an inactivated emulsified vaccine against Newcastle disease containing Montanide ISA-71VG adjuvant. The third group was immunized with a vaccine against Newcastle disease without adjuvant, and the remaining 15 chickens were intact.

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The vaccine in various embodiments was administered to the chickens in a volume of 0.5 cm³ subcutaneously in the dorsal surface of the middle part of the neck. The slaughter of the experimental birds of the 1st, 2nd, 3rd and 4th groups was carried out on the 7th, 14th and 21st day after vaccination, 6 animals for each study period. Pieces of the bursal sac were preserved in a 10% solution of neutral formalin. After dehydration in ethanol and compaction of the material in chloroform-paraffin, paraffin, histological sections were prepared with a thickness of 8 μm. Histological sections were stained with hematoxylin and eosin and examined under a microscope.

3 Results and discussion

In chickens of the first group, on the 7th day after the introduction of the virus vaccine with the addition of squalene emulsion, numerous large, polymorphic, cell-saturated lymphatic follicles were found in the mucous membrane of the bursal sac. The interfollicular epithelium of the mucous membrane was distinguished by the evenness in height of the epithelial cells, as well as the lack of folding in them. With a large increase in the cells of the multi-row prismatic follicle epithelium, the polar structure was well marked. A significant part of the apical region of these epithelial cells had an oxyphilic color and a fine-meshed structure.

The basally located round-oval nuclei of these cells were distinguished by saturation with finely dispersed chromatin and the designated nucleolus. In places near the basal membrane of the interfollicular epithelium, compact small clusters of small, round-oval-shaped cambial cells with a small rim of the cytoplasm around the hyperchromic nuclei were located with a basophilic cytoplasm stain. Caliciform cells in the interfollicular epithelium were small and barely detectable due to weak secretory activity.

In the areas of the closest location of the lymphoid follicles to the interfollicular epithelium (the dome of the lymphatic follicles) there was a region of follicle-associated epithelium, represented by a low, rounded shape with a basophilic cytoplasm staining with epithelial cells. These sections of the epithelium had a small extent. The portion of the dome of the lymphoid follicle under the follicle-associated epithelium did not have a marked basement membrane and the network of capillaries itself, creating a minimum of obstacles for the penetration of antigenic material into the medullary substance of the lymphatic follicles.

In the remaining areas, the cortex-medullary border was well defined and created a histoematological organ barrier. In the submucosal layer of the interfollicular epithelium, as well as between the lymphoid follicles of the bursa, there were small lymphoid cells, single fibroblasts, fibrocytes and thin bundles of collagen fibers that did not cause deformation of the lumen profiles of adjacent small blood vessels. The cortical substance of the lymphatic follicles had a higher density of arrangement of small lymphocytes than the medullary, due to which the reticular base of this zone was largely

hidden. Cells with signs of apoptosis were not found in the cortical substance.

Due to the preservation of the structure of the basement membrane and cells of the capillary network, the cortex-medullary border of the lymphoid follicles reliably impeded the penetration of lymphoid cortical cells into the brain. Numerous large, medium lymphocytes, lymphoblasts were evenly distributed in the medullary substance of the lymphoid follicles, and plasma cells of varying degrees of maturity were found in small numbers, in the vicinity of which single polygonal process dendritic secretory cells were found. The latter were surrounded by large lymphocytes, lymphoblasts and plasma cells.

With a large increase among dendritic cells, long processes were found that were in contact with the surfaces of plasma cells, significantly exceeding the size of the cytoplasm and small processes ending freely. The presence of dendritic lymphoid follicles in the medulla indirectly indicated the active process of transformation of large lymphocytes in the population of immunoglobulin synthesizing cells. Consequently, the lymphoid follicles of the bursal sac on the 7th day of the experiment corresponded to the maximum level of their relative development in the postnatal period and had cellular signs of an active process of immunoglobulin production. The ratio of the thickness of the cortical and brain substances for this period of the experiment was 1:2.

On the 14th day after the introduction of the vaccine with the addition of squalene emulsion, the structure of the bursal sac of chickens was still distinguished by the presence of large polymorphic lymphoid follicles with an increased cell density in both the cortical and brain substances. The process of hyperplasia of cells of lymphoid tissue affected the medullary substance of lymphoid follicles. It noted an uneven distribution of cells due to the appearance of many foci of plasmocyte hyperplasia. Significant changes in the profile of the population composition of lymphoid follicles was due to the predominance of large accumulations of plasma cells (plasmoblasts and especially plasmocytes) over lymphoid cells.

In close proximity to lymphocytes and plasma cells, an increased number of process dendritic secretory cells was detected. They retained their well-defined processes among the newly formed cells. The density of small lymphocytes in the cortical substance increased sharply, but among them appeared cells with signs of karyo- and cytopenosis and the formation of apoptotic bodies. The ratio of the thickness of the cortical and brain substances in the lymphatic follicles, due to the alignment of the values of the latter due to hyperplasia of lymphoid cells, was 1:2.5–3.0.

The cortex-medullary border was maintained throughout, except for the area located near the dome of the lymphoid follicles. In the interfollicular epithelium of the mucous membrane, folds of prismatic epithelium appeared. Cambial epithelial cells persisted, but had an even more pycnomorphic appearance. The length of the location of the follicle-associated epithelium persisted, increased in some places, which correlated with the

increased density of the cellular composition of lymphoid follicles. The noted changes in the follicle-associated epithelium and in the lymphoid follicles themselves were the morphological equivalent of the increased level of exposure to vaccine antigens and the response of the lymphoid tissue of the bursal sac. The sclerotic and atrophic processes in the submucous membrane of the interfollicular epithelium, as well as between the lymphoid follicles, remained poorly marked and had focal manifestations.

On the 21st day, the post-vaccination process in the bursal sac of chickens was accompanied by a decrease in the volume of individual lymphoid follicles, mainly due to rarefaction of the brain cells. As a result, lymphoid follicles acquired round or oval shapes. The ratio of the thickness of the cortical and brain substances in them was 1:2.5. The cortical substance of the lymphoid follicles, which became uneven in thickness, maintained a high density of small lymphocytes, among which deformed cells with karyocytopycnosis and residual apoptotic bodies were increasingly found. The structure of the basement membrane and capillary network cells forming the cortex-medullary border was preserved in some places; in some places, lymphoid follicles were not visible as a result of infiltration of small lymphocytes into the peripheral brain substance.

The processes of apoptosis and infiltration of lymphocytes of the cortical substance into the medullary region were maximally marked in the region of lymphoid follicles, adjacent to the interfollicular epithelium. In the medulla of the lymphoid follicles, the density of the arrangement of the cells of the lymphoid tissue decreased. Differentiated cells of the plasmacytic series still prevailed in it, near which dendritic secretory cells were constantly present, as well as dying forms of lymphocytes. Due to the dilution of cells in the brain, reticular cells are well defined. The content of dendritic forms of cells in the brain has changed little. They still retained their process shape and were surrounded by plasmocytes.

In the submucosa of the interfollicular epithelium, as well as between the lymphoid follicles, an increase in the content of fibroblasts, single fibrocytes and thin bundles of collagen fibers was noted as a moderate manifestation of sclerotic processes that do not cause sharp disturbances of local hemocirculation. For this period of the study, as a result of the appearance of moderate signs of involution of the lymphoid tissue of the organ in the interfollicular epithelium, folding sites arose. The epithelial cells in them markedly decreased in volume, shortened, but in the majority retained the prismatic shape of the cell. Despite involutional changes in the lymphoid follicles in the interfollicular epithelium persisted, but with a smaller extension of the location of the follicle-associated cells. Cambial cells became poorly marked.

Consequently, on the 21st day of the experiment in the bursal sac, against the background of a weak manifestation of involution of lymphoid tissue and the appearance of the initial signs of sclerotic, atrophic changes, the potential for a full-fledged immune response remained. This was indicated by the

preservation of the population of plasma, dendritic secretory and follicle-associated epithelial cells.

In experimental chickens of the second group vaccinated with the addition of adjuvant Montanide ISA-71VG. immunomorphological changes in the lymphoid follicles of the bursal sac were less pronounced, and involutional manifestations were indicated earlier. On the 7th day of the experiment, along with large wedge-shaped, more numerous small round-shaped lymphoid follicles were found in the bursal sac. The density of the cells of the lymphoid tissue in the cortical and medulla of the lymphoid follicles was noticeably lower in comparison with similar indicators of chickens of the previous first group.

Due to the dilution of the cells of the cortical substance of the lymphoid follicles, the ratio of the thickness of the cortical and brain substances was 1:5. Signs of increasing involution of lymphoid tissue in these birds were combined with the process of metamorphosis of the cell population of the medullary substance of lymphoid follicles. Among its cells, the presence of small plasmoblasts located in close proximity to dendritic process secretory cells was detected. The fuzzy line of the cortex-medullary border, which arose due to infiltration with small lymphocytes, indicated violations of the histoematological barrier already on the 7th day after the vaccine was administered and indirectly characterized its more pronounced reactogenicity in this embodiment than in chickens of the first experimental group.

On the 14th day, involutional processes in lymphoid follicles were associated with rarefaction of cells of the predominantly medullary region. The content of small, medium lymphocytes sharply decreased in the medulla, although the plasmacytic reaction retained a moderate manifestation. The ratio of the thickness of the cortical and brain substances for this period was 1:2-2.5. On the 21st day, the involutional processes in the cells of the cortical substance sharply intensified. As a result of atrophy and necrobiosis of its cells, the ratio of the thickness of the cortex and medulla on the 21st day of the experiment reached 1:7. To a large extent, the basement membrane and capillary network of the histoematological barrier of the follicles were destroyed. Sclero-atrophic processes with hemocirculation disorders in the connective tissue basis of the organ were also expressed at the end of the experiment. The interfollicular epithelium in these birds, during the entire study period, was distinguished by focal atrophy of epithelial cells and the length of the sites occupied by follicle-associated epithelial cells was reduced.

Consequently, changes in the intensity of the growth of signs of incidence of involution in the lymphoid, epithelial and connective tissues noted in all periods of the experiment were combined with a reduced potential of immune reactivity of the bursal sac in these chickens even against the background of vaccine stimulation.

In chickens of the third group, immunomorphological changes in the bursal sac differed in all periods of the study by the formation of the smallest volume of lymphoid follicles. On the 7th day between the small,

round-shaped lymphoid follicles, as well as in the submucous membrane, extensive areas with significant accumulations of lymphoid cells, fibroblasts, forming fibrous connective tissue, were noted. Lymphoid follicles of the bursal sac were secreted by a small volume of brain substance, as a result of which the ratio of the thickness of the cortical and brain substance was 1:1.5.

Compared to the chickens of the previous second and especially the first group, the lymphoid follicles in these birds were significantly rarefied due to active autoselection. Many cells of the cortical and especially brain substance were distinguished by signs of apoptosis and necrosis. The interfollicular epithelium of the mucous membrane has acquired many folds with the formation of numerous outgrowths of epithelial cells. Follicle-associated epithelial cells reduced with sharply basophilic coloration were barely indicated in the interfollicular epithelium.

On the 14th day after vaccination, the lymphoid follicles sharply differing in form and the appearance of edema in them significantly increased in volume and were saturated with lymphoblasts and plasma cells. The content of dendritic cells in the brain remained unchanged. In the interfollicular epithelium uneven in height of prismatic cells, numerous outgrowths and folds arose. The early signs of sclerotic changes in the submucosal and interfollicular connective tissue revealed on the 7th day of the experiment sharply intensified on the 14th day.

As a result of squeezing the gaps of small blood vessels, areas of local hemocirculation disturbance arose, which contributed to a rarefaction of the cellular composition of the lymphoid tissue of the cortical and especially brain substance, and the appearance of pronounced edema in it. As a result, the ratio of the thickness of the cortical and medulla in the lymphoid follicles was 1: 2.

On the 21st day after vaccination, the lymphoid follicles in the cortical region completely or partially lost their cellular composition and with it the structures that provide the histohematological barrier of the organ. The ratio of the thickness between the cortical substance preserved in individual lymphoid follicles and the sharply edematous, dilute medullary substance was 1: 8. The vast majority of cortex and medulla cells showed signs of karyocytopenosis. Plasmoblasts, plasmocytes and process dendritic secretory cells were completely absent in the follicular brain substance, only single lymphoid cells and macrophages with signs of necrobiosis remained with hyperchromic nuclei. Cells of the interfollicular epithelium became sharply reduced in volume. The regenerative processes of cells in the interfollicular epithelium also completely stopped. Follicle-associated epithelium was not detected. The processes of production of components of the fibrous connective tissue in the submucosa and between the lymphoid follicles, accelerating sclero-atrophic processes in the organ, reached maximum severity.

Consequently, in chickens of the third group, post-vaccine restructuring without the use of adjuvant, structural changes in the bursal sac were manifested by a

sharp increase in the processes of case-related involution of the organ at all observation times and were most pronounced compared to chickens of the second and especially the first group, due to the severity of the reactogenic properties of the applied vaccine preparation. Immunogenic qualities vaccine preparation due to lack of adjuvant appeared less pronounced compared with chickens of the previous experimental groups.

In control unvaccinated chickens of the fourth group, structural changes in the bursal sac at the same time were characterized by a moderate course of involutational processes without immunomorphological reactions, due to the absence of immunogenic vaccine load.

On the 7th day of the experiment, it was noted that a sequential gradual decrease in the volume of lymphoid follicles and rarefaction of lymphoid tissue cells occurred in these chickens mainly due to the mechanisms of apoptosis and atrophy. Preserved numerous round-shaped lymphatic follicles of the bursal sac were distinguished by a small size and moderate density of the content of lymphoid tissue cells. Due to the thinning of the cortical substance and the edema of the also rarefied medullary region, the ratio of the thickness of the cortical and medulla was 1:4.5.

Hyperchromic small deformed lymphoid cells and reticulocytes predominated in the cortical substance. In some places in the follicles, as a result of the destruction of the basement membrane and capillary network, lymphoid cells penetrated into the peripheral region of the rarefied medulla, thereby indicating areas of violation of the histohematological barriers of the bursal sac.

Rare plasma and process dendritic secretory cells were located in the medulla. The most pronounced signs of atrophy of lymphoid tissue were noted in the follicles located as close as possible to the follicle-associated epithelium. It should be noted that the growth of fibrous connective tissue in the submucous membrane and between the follicles, which leads to earlier manifestations of violations of intraorgan hemocirculation, was intense by this time of the study. In the subsequent 14th and 21st days of the experiment, the ratio of the thickness of the cortical and brain substance gradually changed to 1:3 and 1:2 and was combined with an increase in sclerotic and atrophic processes in the submucosal, interfollicular connective tissue.

4 Conclusion

The study has shown that the vaccine used against Newcastle disease has pronounced immunogenic and reactogenic qualities that actively affect the structure of the bursal sac of vaccinated chickens. The use of adjuvants in vaccination significantly contributes to the correction of the quality of the vaccine preparation indicated above. The highest severity of immunomorphological rearrangement of lymphoid tissue and the lowest level of manifestation of the incidence of involution of the bursal sac were observed in birds of the first group with a combination of the vaccine preparation

and adjuvant in the form of squalene emulsion, compared with chickens of the second and third groups. It should be noted that involutory processes in the bursal sac in chickens of the first and fourth groups were manifested with a predominant activation of the mechanism of apoptosis and atrophy. Whereas in the second and especially the third group, destructive changes were also manifested with the mechanisms of necrobiosis and necrosis. In this connection, the maximum severity of involutory changes was noted in the bursal sac in chickens of the third group. The lowest level of alternative changes in the bursal sac was observed in the birds of the fourth group. The involutory processes in these birds were determined by hereditary factors, while in the chickens of the other experimental groups the incidental nature of the manifestation of the involution of this organ was noted.

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