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Dynamics of markers of non-specific resistance in trachea of chickens at orthomyxovirus infection

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The purpose. To fix dynamics of factors of non-specific immunoreactivity in mucous membrane of trachea of chickens experimentally infected with low-pathogenic strain of virus of grippe, as a model of pathogenesis of orthomyxovirus infection caused by virus of type A. **Methods.** Biochemical for research of homogenates of mucous membrane of trachea of chickens; statistical. Researches were carried out in 2 groups (n=35) of 45-day's chickens. Birds of the first group were infected with low-pathogenic virus of bird's grippe (strain A/mallard/Ukraine/2007 (H5N2), the second group was a control one. Samples of trachea from 5 chickens from each group were taken on the 1, 3, 5, 7, 10, 14 and 21 day after infection. Markers of non-specific resistance in homogenates of mucous membrane of trachea were determined using methods adapted by us for operation with homogenates of mucous membrane. **Results.** Dynamics is studied of indexes describing the level of inborn mucosal immunity in trachea of a bird at experimental grippe within 21 day. It is determined that infect of chickens with low-pathogenic strain of virus of grippe raises to maximum the level of IgM — up to 48,3% on the third day. On the third day they also observed lowering of production of IgG (on 31,8%), in the period from 7th to 10th day density of IgG increased on 35,5%, and active accumulation of IgA began on the 10th day after infection. In dynamics of experiment they fixed heightening intensity of processes of lipoperoxidation (maximally on the first and the 21st day of experiment), oscillations of activity of catalase (on the 5th day of experiment the increase has made 88%, on the 21st day it lowed on 38,2%, P<=0,05) and lowering of general anti-oxidant activity of lipids (for 21 day on 70,2%). **Conclusions.** The fixed changes in system of non-specific immunity of mucous membrane of trachea at experimental low-pathogenic grippe of birds within 21 day of evolution of infection can be used as a basis for development of strategy of struggle with orthomyxovirus infections and bird's grippe in particular, using special prophylactic means and immunomodulators of direct action.

Key words: *orthomyxovirus, non-specific resistance, trachea, low-pathogenic grippe of birds, chickens.*

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The epizootic situation concerning orthomyxovirus infections of various animal species has worsened significantly last time [1, 2]. In particular, the name of Orthomyxoviridae family includes Greek word myxo (mucous), which reflects the tropism of these viruses to mucous membranes. Orthomyxoviruses cause respiratory or generalized infections of sporadic of epidemic nature in humans, birds and many microorganisms. Orthomyxovirus of A type is the most epidemically, epizootically and economically significant, and it is an etiological causative agent of influenza [3].

It is known that influenza virus reproduces and accumulates in epithelium of the respiratory tract and intestine (portal of the infection). At the same time, according to literature data, the antibodies produced on surface of mucous membrane, may play an important role not only in protection, but also in restriction of primary replication on virus gateway and elimination portals [4], which increases an importance of mucosal immunity against influenza [5, 6].

The importance of avian influenza pathogenesis is that lesion occurs at cellular, subcellular and genetic levels [7, 8]. The production of active oxygen forms, which leads to initiation and development of lipids' peroxidation in microorganism's biological membranes, is one of pathogenetic elements of influenza. Besides, immunoactive substances – immunoglobulins, and various classes of cytokines, are produced in epithelial cells [4, 9]. At the same time, internal lesions and unspecific resistance system's functional status in mucosal membranes of poultry infected with avian influenza, are insufficiently studied.

Objective. To determine the dynamics of unspecific immune reactivity factors in tracheal mucous membrane in chickens, which were experimentally infected with low pathogenic strain of avian influenza virus (LPAI), as pathogenetic model of orthomyxovirus infections caused by influenza A virus.

Materials and methods. Studies were carried out on 45-days old chickens of Hubbard cross (n=35). Chickens of the first group were infected with low pathogenic avian influenza virus, strain A/mallard/Ukraine/2007 (H5N2), which had been isolated by researchers from the department of avian diseases of NSC "IECVM", the second group of chickens was referent.

Samples of trachea from 5 chickens of each group were taken on first, 3rd, 5th, 7th, 10th, 14th, and 21st days after infection. Poultry was euthanized with chloroform narcosis according to norms on bioethics.

The level of A, M, and G immunoglobulins was determined in homogenates tracheal mucous membranes using Mancini method (1965) [10]. To determine the intensity of lipids' peroxidation (POL), the concentrations of diene conjugates (DC) and malonic dialdehyde (MDA) were measured using V.B. Gavrilov and M.I. Mishkorudna method (1985), which we modified. The status of antioxidant protection system (APS) was assessed on catalyze activity as well as on the total antioxidant activity level of lipids (AOA) [11]. All above mentioned common methods we adapted for working with homogenates of tracheal mucosal membranes. Statistical processing of obtained data we carried out using common methods in MS Excel software.

Results and discussion. As the result, the dynamics of immunoglobulin level was determined (table). According to this data, due to infecting with low pathogenic strain of avian influenza, the enhanced induction of immunoglobulins M is occurred. The next day after infection, Ig M level in experimental chickens is 15,4% ($p \leq 0,05$) higher than in referent group. On 3rd, 5th and 10th day of experiment, Ig M most intensively accumulated, and statistically plausible differences between experimental and control groups were 48,3%, 38,7% and 40,0% respectively.

Level of immunoglobulins in tracheal mucosal membranes in poultry with low pathogenic influenza (M±m, n=5)

Day of experiment	Ig M, mg/ml		Ig G, mg/ml		Ig A, mg/ml	
	Experimental group	Referent group	Experimental group	Referent group	Experimental group	Referent group
1	0,06±0,001	0,052±0,001	0,026±0,001	0,027±0,001	0,018±0,0005	0,020±0,001
3	0,089±0,001*	0,060±0,001	0,02±0,0007*	0,029±0,0006	0,024±0,001	0,020±0,0005
5	0,086±0,001*	0,062±0,001	0,025±0,001*	0,030±0,001	0,036±0,002	0,030±0,001
7	0,1±0,0005*	0,080±0,002	0,03±0,006*	0,033±0,0005	0,034±0,001*	0,032±0,002
10	0,14±0,001*	0,10±0,001	0,042±0,001*	0,031±0,002	0,068±0,003*	0,036±0,001
14	0,12±0,003*	0,11±0,003	0,039±0,00*4	0,033±0,0005	0,072±0,002*	0,047±0,002
21	0,13±0,002*	0,11±0,002	0,044±0,001*	0,034±0,001	0,084±0,003*	0,04±0,003

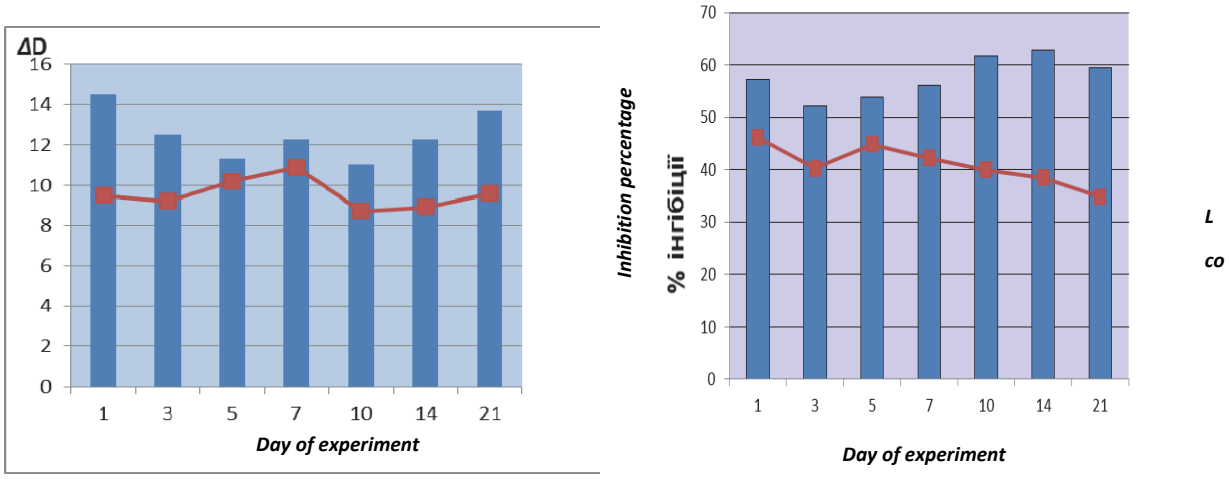
***Note:* *–** *the difference if statistically plausible in relation to control indices ($p \leq 0,05$).*

Also, on early stages of infection in poultry with LPAI, production of Ig G is depressed, and it is mostly expressed on third day, when the concentration of this immunoglobulin in experimental chickens was decreased by 31,8%. However, we observed rapid accumulation of Ig G during the period from 7 to 10 days, when it reached 35,3% in relation to control group, and the level of Ig G was at the average rate of 20,0% higher than control indices by the end of experiment. Active accumulation of Ig A starts from 10th day after infection, lasts till the end of experiment, and its concentration is at the average rate of 50 % ($p \leq 0,05$) higher than control indices.

During the study of oxidant-antioxidant homeostatic system it has been found that LPAI infection is accompanied with increasing of POL in tracheal mucous membrane. In particular, the level of lipoperoxidation final product, MDA (image 1a), maximally exceeded indices of referent group the day after infection (by

57,8%), and the accumulation of MDA slightly decreased during the period from 3rd to 10th day of the experiment. Active activity of POL was observed on 14-21 day, and on 21st day of experiment the level of MDA was 42,7% higher ($p \leq 0,05$). The same dynamics was detected in the producing of DC.

As it can be seen at image 1b, starting from 3rd day of the experiment, the suppression of total AOA also occurs in trachea of experimental chickens, as shown by the inhibition percentage. AOA was slightly decreasing throughout the whole experiment: the plausible difference on 21st day was 70,2%.



a) b)

Image 1. The dynamics of oxidant-antioxidant homeostasis markers in tracheal mucosal membrane with low pathogenic avian influenza: a) malonic dialdehyde; b) antioxidant activity of lipids.

Also, fluctuate changes of catalyze activity in infected chickens were observed throughout the experiment. Thus, these indices reached the highest level on 5th day of the experiment (compensative increasing of activity was 88,0 % in relation to indices of poultry from referent group), on 10th day they decreased down control level, and on 21st day the enzyme activity was 38,2 % lower in comparison with control ($p \leq 0,05$). The obtained data show that the total antioxidant pool is descend in tracheal mucosal membrane with low pathogenic avian influenza.

Conclusions and perspectives for further research

During the first 21 days of experimental low pathogenic avian influenza, the complex pathogenetic and compensatory changes in the system of unspecific mucosal immunity occur, and they are characterized by increasing of M and A immunoglobulins' induction and suppression of Ig G production at the first stage of infection. The rapid activization of lipids' peroxidation, decreasing of catalyze activity and total antioxidant pool are also observed.

The obtained results have common biological and practical value, and they can be used as the basis for the development of control strategies against avian influenza with using of specific preventive means and directional immunomodulators.

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