UDC of 619:616-07:576.85

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Diagnostics of metapneumoviral infection of auk by means of domestic test-systems

Aim. To work out a IFA for control of Metapneumatic Infection (MPVI) of bird. Methods. For creation of test-system for determination of antibodies to Metapneumatic infection of bird the methods of accumulation of virus were applied on the culture of cages and cleaning of virus with the use of stage-by-stage. Results. Home Test systems of IFA is worked out for determination of specific antibodies to Metapneumatic infection in the serums of blood of chickens. Technology of raising of diagnosticum is worked out in one breeding of serum of blood that improves exactness of analysis. Conclusions. At the State experimental station of the poultry farming of NAAS first in Ukraine home тест-системy of IFA is created for determination of specific antibodies to Metapneumatic infection in the serums of blood of chickens. A diagnosticum is the best expressmethod of exposure of infection, by means of that it is possible to control tension of immunity for the instiled bird, and also mediated to determine the presence of disease at the uninstiled herds of chickens

Keywords: метапневмовірус, Test system of IFA, antibodies, serum of blood of chickens.

Entry. Annually all anymore to Ukraine leave a high-performance bird from abroad. In this process is both positive parties and negative. A negative moment is spreading disease, that before was not on our territory. To such illnesses the infection of bird belongs also. More careful research in relation to this infection began after 2000 (in our laboratory — from 2009, first in Ukraine). As morbidity and death rate after Metapneumatic infection among a bird on poultry factories can present 10-90%, there was a requirement in correct diagnostics of disease and in differentiation of him from other diseases [2, 4].

At the left imported cross-country races to the bird Metapneumatic infection began to discover in many regions of country. This disease is characterized by inflammation of overhead standard (nasal bosoms, sines and trachea). There is a disease for chickens, turkey-cocks and chickens-broilers. Methods of transmission of virus — pin.

About distribution of this disease it is known in the whole world, him found out practically on all continents [1-3]. 4 virus, that cause a disease, is presently found: 2 from them (But also B) — in Europe, Asia and South America; 1 (D) — only in France; C is distinguished only in North America.

Control after distribution of this disease in Ukraine did not conduct before. It is set our researches, that on territory of Ukraine happens mainly In.

In many countries of the world for diagnostics ofuse different reactions: PHIF, PH, IFA and method. For their help it is possible to set a diagnosis on MPVI, but estimating tension of immunity after the inoculation of bird is impossible. It is also possible to draw on molecular-genetic researches, however a final diagnosis needs to be put complex with the use of virologic and serum researches [7, 8].

For that, to conduct monitoring on tension of immunity to this disease, necessary more accessible, simple in the use, more sensible serum reactions that can be used both for the study of epizootic situation in economies in relation to MPVI and to control of immunity after the inoculation of bird [4-6, 9, 10]. Such reactions are a reaction of indirect hemagglutinationand analysis (IFA). Abroad there were worked out Test systems of IFA for the exposure of antibodies to MPVI, but they practically inaccessible for the veterinary laboratories of Ukraine, as a cost them within the limits of 20-25 thousand hrn. For period from 2009 on 2015 in our laboratory Test systems was worked out for determination of antibodies to MPVI for chickens and turkey-cocks. Advantage of this system is in her universality (research can be

conducted on two types of bird) and simplicity of the use. Also we are work out Test systems of ІФА for determination of antibodies to MPVI for chickens and turkey-cocks. Advantage of this is higher exactness comparatively with РНГА and price less in 4 times comparatively with foreign analogues.

In future these diagnosticums can become on the armament of many veterinary laboratories. With their help it will be possible to control both distribution of this disease in Ukraine and tension of immunity for the instiled bird. Drawing on home sets (not only to MPVI but also to other diseases for a bird) will give an opportunity to have a healthy bird in the economies of Ukraine and economize plenty of money during the purchase of foreign preparations.

An aim of researches is development of IFA for control of MPVI of bird.

Materials and methods. The diagnostic value of Test systems of IFA was checked up comparatively with American Test systems of IFA by the productions of firm «IDEXX» (THE USA).

Works of MPVI for IFA of diagnostics carried out the method of his reproduction on the culture of cages of Vero.

For a IFA the turned out antigen was cleared and concentrated on the chart that included the preparatory stage worked out by us — freezing and unfreezing of virus, his previous cleaning is low-speed centrifugation, concentration of virus with the use of (PEG-6000) and finishing cleaning of virus through the layer of saccharose. Methodology of cleaning of virus is adapted taking into account his properties (to firmness to physical influence, size of viral particles, specific closeness).

The Hyperimmune serum of blood was got the method of hyperimmunization of 30-daily chickens on the chart (a patent is got) worked out by us. A normal serum was got from blood of intact chickens by age a 90 twenty-four hours by the method of the total draining of blood. The diagnostic serums of blood kept in the frozen (for the temperatures of -20oC) and lyophilized state. Sensitization of plane-tables, bringing of the investigated serums on plane-tables and antispecificconducted after the generally accepted methodologies [9].

Results of researches. Development of Test systems consists of selection of the optimal working breeding of serum and leadingout of formula with the aim of determination of titles of antibodies in one breeding. For this purpose 100 tests of the field serums of blood of chickens were investigational with the different level of antibodies to МПВІ. The level of antibodies was determined by the method of indirect of ІФА successive breeding of serum from 1:100 to 1:12800.

Optimal correlations of components, that use for raising reactions (to the antigen) and certainly them working breeding by the method of «chess titration», are also exhaust:

to the antigen (cleared MPVI), that inflicted on a plane-table — 1:500;

antispecific against Ig G chickens of -1:4000.

For determination of the optimal breeding of serums and leadingout of formula of accounts of titles of antibodies conducted the mathematical analysis of the got results the method of one breeding. Calculated the value of S/P (correlation of absorbancy of the investigated serum and absorbancy of positive control, with deduction of optical index of negative control) : S/P100, S/P200, S/P400, S/P800 — in breeding of 1:100, 1:200, 1:400, 1:800 (results processed with the use of the computer program Microsoft Excel).

For every breeding determined the coefficient of correlation with the value of titles, successive breeding (a value of lg of T is to lg of S/P) got a method. The coefficient of correlation presented for breeding: 1:100 — 90,59%; 1:200 — 92; 1:400 — 92,16; 1:800 — 88,90%. Breeding of serum of 1:400 small the greatest coefficient of correlation and it is taken for working.

Accordingly with the values of optical of the investigated serums, by means of the computer program Microsoft Excel a gauge curve is built and shown out equalization of linear regression for the account of logarithmic value of titles of antibodies in serums (rice. 1).

The formula of titles of antibodies conducts account in the serums of blood of chickens during their testing in one breeding: Lg T=3,7981+0,8524·lg (S/P400), that simplifies an analysis.

For the objective estimation of immune answer a positively-negative threshold (PNT) is set. 40 negative serums of blood are investigational from the chickens got for growing in terms. Serums are tested by the set of IFA worked out by us for determination of antibodies by the method of the successive breeding. As positive and negative control is taken the control serums got from intact chickens. A count is conducted for testing in one breeding and it is calculated PNT. On the basis of the got results after before by the shown out formula of lg of T=of 3,7981+0,8524·lg (S/P) certainly PNT is negative serums from 0 to 850, positive — from 850 and higher.

After introduction of data after a formula for a calculation in the program Microsoft Excel it is possible to determine the titles of antibodies in the investigated serums in one breeding of serum.

The comparative analysis of results of testing of serums is conducted in тест-системі of IFA, worked with the IFA system of firm «IDEXX». At the same time compared 60 serums of blood of chickens to different activity (30 serums of blood from chickens not against MPVI, and 30 serums of blood of the chickens instiled against MPVI).

For the uninstiled bird the titles of antibodies in ІФА MPVI (ДДСП) presented from 24 to 79 (after PNT 850), in IFA MPVI («IDEXX») — from 37 to 390 (after PNT 396). For the instiled bird — from 3017 to 9756 (in IFA MPVI, ДДСП), and from 674 to 2578 (IFA MPVI, «IDEXX»). As sets have different PNT, the titles of antibodies differ after absolute values.

Thus, 100% is traced -ва similarity of results after these diagnosticums, id est serums of blood of chickens that does not have antibodies to this disease it is not discovered in both sets, and vice versa, serums with the presence of протективних titles of antibodies to this disease find out both used Test systems.

In 2014 the interlaboratory test of «Set of components was conducted for determination of antibodies to the infection of bird in the serums of blood of chickens by a method». Testing of blood is carried out serums with the different titles of antibodies on indexes: specificity, sensitiveness and producibility (table).

Results of verification of specificity, sensitiveness and producibility of тест-системи of IFA

Specificity of Test systems for correlation of negatively reactive and really negative serums presented 100%. Sensitiveness of Test systems — 100% (correlation of positively reactive serums and really positive serums). There is a producibility of Test systems that was determined after the percent of running approach from the mean value of absorbancy of standards of one serum presented 0,5-3,3% for a positive serum (3 serums are in 5-ти repetitions) and 3,7-9,6% — for a negative serum (3 serums are in 5-ти repetitions), id est within the limits of norm. Hyperimmune serums to ІБК, ІББ, РЕО, НХ, ССЯ did not have titles during testing (rice. 2).

Taking into account the above-mentioned given, it is possible to assert that home тест-система is worked out for determination of antibodies to МПВІ of chickens specificity cheaper foreign analogues in 4 times has a high sensitiveness, that is why is accessible for the use in the laboratories of veterinary medicine of Ukraine.

Conclusions

Due to worked out тест-системі of IFA for determination of specific antibodies to MPVI of bird it is possible to conduct the monitoring of this disease and control of immunity for the instiled bird at Ukraine. A reaction is quantitative, to the outage in the use, conducted in one breeding of serum.

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