PATHOPHOLOGICAL CHANGES IN CHICKS INFECTED WITH SALMONELLA PULLOROM GALLINARIUM

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Abstract: The article provides information on the age-related dynamics of broiler chicken infection with S. pullorom gallinarium. Also, information about pathomorphological changes in the organism of chicks is given.

Key words: pullorosis, infection, leukocyte, basophil, eosinophil, atrophy, dystrophy, thrombosis, infiltration, colony-forming unit, damaging dose, lethal dose, nutrient medium.

Relevance of the topic: Poultry disease is one of the diseases that are causing enough damage to the poultry industry, which occurs especially in the early life of chicks. In many cases, when the pullorosis of chickens damages poultry farms, it is possible to interpret the results of scientific research more from pasteurellosis, colibacteriosis and streptococcosis from secondary diseases. In the early stages of life, chickens are susceptible to diseases like other young organisms, including the mixed form of infections, especially the age-related forms of pullorosis, which are severe in some stages, have been studied in more foreign literature sources.

The increase in the epidemiological importance of poultry and poultry products, the inextricability of this process, the changes in the sanitary-epidemiological service in our republic, the epidemiological control system over existing salmonellosis and other secondary diseases require its reconstruction. The analysis of the literature data shows that until now, in poultry farms of our Republic, the weight of chickens from chickens among all infectious diseases is 26-40 percent. Pathomorphological diagnosis of chickens infected with avian pullorosis is one of the urgent problems facing specialists. In addition, the dynamics of infection of broilers with S. pullorum gallinarium according to age has not been studied in detail.

Object and methods of research: in the study of hematological processes in pullorosis of chicks in the laboratory of hematology and biochemistry of the Central Hospital of Samarkand region (before and after the experiment results), pathomorphological in the body of birds infected with the causative agent of the disease (S. pullorom gallinarium) changes detection studies were carried out in the laboratories of microbiology, pathomorphology and research of diseases of young animals of Veterinary ITI. The Panchenkov method was used to analyze erythrocytes from the blood samples of chicks infected with different concentrations of S. pullorum gallinarium for one week (until death in some groups) and the Sali hemometer was used to determine hemoglobin.

In order to study the histogram of pullorosis in laboratory conditions, samples were taken from the organs of infected and forcibly slaughtered chickens for pathomorphological research. For this purpose, to examine the samples in a small histological manner, initially using the biopsy method, pieces are taken from the following organs from sick chickens; samples were taken from trachea, lymph node, liver, lungs, heart, spleen and kidneys, and pathological changes were examined by histological method. Pathological samples were taken from the internal organs of all examined birds for bacteriological examination, and inoculations were planted on different nutrient media (Levin, Ploskiriev agar, salmonella shigella and 5% blood agar). Sections were taken from the blocks with the help of a microtome, a micropreparation was prepared on a glass slide, stained with hematoxylin and eosin, and subjected to microscopy. Microscopy revealed pathogistological changes in internal organs of birds. A 10x0.25 lens of a Carl Zeiss microscope was used for this [8]. For histological examination of pathological samples (slices) taken from internal organs and tissues, a histopreparation was prepared by the paraffin method as follows (50-100 milliliters were transferred in dark glass bottles)

I. Fixation

1. The obtained pathological samples (pieces) were stored in 10-12 percent formalin solution for 24 hours.

2. It was stored for 24 hours in a solution of equal ratio (1:1) of ethyl alcohol and formalin at 960C;

3. These fragments were kept in absolute alcohol at 96-1000C for 12-24 hours.

II. Dehydration

1. The obtained pathological samples (pieces) were kept in 960C alcohol solution for 24 hours for dehydration;

2. The next day, it was kept in an alcohol solution at 960C for another 24 hours;

III. Putting paraffin

1. For 6-12 hours, it was placed in a solution of equal proportions of alcohol and chloroform at 960C;

2. Kept in pure chloroform solution for 6-12 hours. At the end of storage, it was observed that the color of the pieces became clear;

3. In order for the paraffin to absorb better at once, the pieces were placed in a solution of equal volume of melted paraffin and chloroform and left for 2-3 hours in a thermostat with a temperature of +35 +400C. Sometimes such solutions were stored frozen when not in use;

4. Then the slices were placed in melted paraffin kept in a thermostat at +54 +550C. In this case, the fragments were kept in the melted paraffin in the first container for 1.5-2.5 hours, then they were placed in the second container using heated tweezers and kept for 0.5-1.5 hours, paying attention to the size and thickness of the fragments;

5. The pieces were placed in a jar with glycerin placed on the bottom and heated to +60+700C using a gas burner, and melted clean paraffin was placed on top until it was covered with a thickness of 0.5 cm;

6. The paraffin container with the fragments was cooled in a large container filled with cold water. In this case, the cooling of paraffin was carried out based on its melting moving from the bottom to the top;

7. After hardening, the paraffin was cut from the edges, this paraffin was definitely in a homogeneous state, if it was determined that there were limited flowing

areas in the paraffin (crushing, rubbing when broken), it was re-laid with a new portion of paraffin;

8. Blocks were cut from solidified paraffin, leaving a paraffin layer at least 2 mm thick around the pieces. In this case, each piece was made separately;

9. The obtained blocks were glued with a spatula so that the edges of the heated blocks do not come out of the board.

Histosections were prepared from the blocks using a microtome, and a micropreparation was prepared on a glass slide, stained with hematoxylin and eosin, and subjected to microscopy. Microscopy revealed pathogistological changes in internal organs of chicks.

Results and their analysis: When blood samples were taken from the underwing vein of infected birds on days 1-5 after the experiment, following aseptic and antiseptic rules, the number of erythrocytes was 29.7%, the number of leukocytes and platelets was 12.45 and 6.72%, and hemoglobin and it was determined that there were changes in the blood parameters of chickens in the II comparative control group to 21.6 percent (Table 1).

Table 1

Hematological changes in chicks infected with S. pullorom pathogen

Checkout time	Erythrocyte, million/µl	Leukocyte, thousand/µl	Leukoformula					
			E	B	M	L	Neutrophils	
							rod nucleated	articular core
Norm	3,18±0,14	25,18±1,5	2,8	2,2	4,4	56,6	4,4±0,31	40,4±3,23
Experimental group I 0.5 ml 05 billion m.h. n=10								
1- day	3,24±0,18	31,45±1,48	3,1	1,76	3,9	58,2	4,6±0,27	35,5±2,31
2- day	3,19±0,17	30,29±1,62	3,2	1,8	4,0	58,0	4,2±0,24	34,5±2,26
3- day	3,16±0,22	30,20±2,28	2,6	1,7	4,7	59,4	4,1±0,38	40,4±2,34

4-day	3,32±0,26	31,28±2,04	2,2	1,7	4,4	59,2	4,3±0,34	41,0±2,61
5-day	3,20±0,28	29,74±2,18	2,9	1,75	4,2	61,4	4,0±0,26	43,2±2,64
II experimental group 0.25 ml 05 billion m.h. n=10								
1-day	3,24±0,17	29,61±1,54	3,1	1,76	3,9	55,2	4,4±0,24	35,5±2,31
2-day	3,12±0,13	29,83±1,91	3,2	1,8	4,0	53,0	4,3±0,26	36,5±2,56
3-day	3,24±0,14	31,20±2,25	3,6	1,6	4,3	52,4	4,1±0,33	42,4±2,74
4-day	3,21±0,17	31,28±2,4	3,1	1,8	4,1	51,2	4,2±0,3	41,4±2,61
5-day	3,25±0,21	29,94±2,31	3,0	1,7	4,0	50,4	4,1±0,25	43,4±2,84
Control group III 0.5 ml of 0.9 percent physiological solution n=10								
1-day	3,20±0,19	25,21±1,52	3,1	2,2	3,9	55,2	4,6±0,27	35,5±2,31
2-day	3,21±0,18	24,33±1,86	3,2	1,8	4,0	53,4	4,2±0,24	34,5±2,26
3-day	3,34±0,18	22,26±2,04	2,6	1,9	4,2	54,1	4,1±0,38	40,4±2,34
4-day	3,31±0,19	23,28±2,07	2,2	1,8	4,1	54,2	4,3±0,34	41,0±2,61
5-day	3,35±0,24	24,74±2,01	2,9	2,1	4,3	51,6	4,0±0,26	43,2±2,64

Note: xxx-P<0.01;, xxxx- P<0.001.

The number of basophils in the blood smear was not significantly different from the number of basophils in the blood of healthy chickens of the comparative control group.

The main changes were observed in the remaining types of leukocytes. Of course, it is difficult for any young organism to adapt to this pathological process.

The number of eosinophils increased by 16.9%, pseudoeosinophils by 34.8%, and the number of monocytes by 19.42%, while the number of lymphocytes decreased by 11.86%.

Thus, the age-related damage dynamics of the morphological parameters of the blood of chicks fed on milk, i.e., the amount of erythrocytes and hemoglobin, decrease, and the number of leukocytes and platelets increase, was determined in the research.

In the leukocyte formula, the number of eosinophils, pseudo-eosinophils and monocytes increased sharply by 21.16%, and the number of lymphocytes decreased, without changing the number of basophils.

According to the results of histological research; when the internal organs of chicks were examined pathomorphologically, most of the main changes occurred in parenchymatous organs. A strong development of hemodynamic and dystrophic processes was observed in them, especially in the first 1-5 days of chicks.

Cardiovascular vessels are dilated, vascular wall cells are swollen, endothelium is displaced, there are a lot of histiocyte, lymphoid and leukocyte cell clusters around some vessels, muscles are divided into fibers, some fibers have undergone granular dystrophy.

Hemorrhagic necrotizing pneumonia developed strongly in the lungs. The cavities of most alveoli are filled with erythrocytes. Interalveolar capillary nets are expanded and filled with blood, as a result of which the walls are thickened, connective tissue fibers are swollen. As a result of these changes, it was found that a large part of the lung parenchyma was affected by atelectasis. It was observed in comparative experiments that the interstitial tissue was swollen in all sections of the lungs.

Changes in the larynx and larynx were expressed in the form of catarrhal or severe fibrinosis - hemorrhagic and desquamative inflammations. Because desquamation of the respiratory epithelium was strongly aggravated in the first experimental group of birds, the private layer of the mucous membranes was completely opened and sharply swollen, as well as infiltrated with a large number of pseudo-eosinophilic leukocytes. Lymphoid cells are collected along some vessels. It was found that the mucous membranes of some birds were partially necrosed and swollen.

A necrotic mass consisting of fibrin, fragments of respiratory epithelium, pseudoeosinophils, lymphocytes, and erythrocytes was detected in the cavity of the larynx and larynx.

Pathohistological changes in the spleen are expressed by the fullness of the vessels, a little thickening of the trabeculae, and the uncertainty of the appearance of

the fibers. The border of the red pulp is enlarged. Small hemorrhages and lymphoid collections are visible in some places. These changes are the effect of the general pathogistological process taking place in the body.

Histological changes in lymph nodes are not the same in all nodes. Noticeable changes are in nodes between the portal, intestinal mesentery, and lung wall, where serous edema, serous-hemorrhagic lymphadenitis, and extravasates of various sizes have developed. In addition to hemorrhages in the lymph nodes located near the parts of the lungs with severe pathological processes, the sinuses are filled with lymphocyte and leukocyte collections.

The pathogistological changes in the kidneys are mainly general pathological processes, hemodynamic changes and granular, and in some places, fatty dystrophy of the epithelium of the renal tubules are often detected. As a result of the expansion of the capillary nets of the kidney balls, only erythrocyte clusters were visible under the microscope. It was found that the capsules around the balls were enlarged, filled with purulent and fibrinous exudate. As a result of the enlargement of the epithelia, the circular and straight tubes have no boundaries. Epithelial cores have undergone rhexis and lysis. Morphological changes occurred in the kidneys, and irreversible processes occurred in this part, like other organs.

General diagnosis of S. pullorum gallinarium was carried out on the basis of bacteriological research in the microbiology laboratory of the Veterinary Institute of Veterinary Medicine only by isolating the causative agent, identifying its type and serotypes, and confirming its pathogenicity by biotesting. Determining the pathogenicity of a microbial culture by conducting a biological test is important in determining the effectiveness of biological drugs, immune sera and therapeutic agents. Therefore, the virulence indicators of S. pullorum gallinarium, the main causative agent of this disease, by conducting acute experimental experiments in poultry pullorose, will make part of our experiments to study LD50 and LD100.

These experimental experiments were conducted on broiler chickens in the field of meat in 5 (five) groups at the Microbiology Laboratory of VITI. Since it is clearly

impossible for poultry pullorosis to occur acutely in the first ten days of the chicks' life, the age of the chicks in the experiments was determined as 2-8 days. The results of determination of LD50 and LD100 indicators of S. pullorom gallinarium in broiler chickens are given in Table 2 below.

Our experiments on the results of determining the virulence indicators of S. pullorum gallinarium, the main causative agent of salmonella in broiler chickens, were performed in 4 experimental and 1 control groups. 10 chickens in 4 experimental groups fed 2-8 days of meat were infected with S. pullorum gallinarium according to the experimental diagram, and 12 chickens in the 5th control group were left as uninfected control and were given the same volume of saline solution was sent.

Table 2

Results of determination of LD50 and LD100 indicators of S. pullorom gallinarium in broiler chickens.

Groups	The number of infected S. pullorum gallinarium cells (1ml/piece) KHQB	Number of infected chicks	Number of dead and alive chicks		Scientist %
			Dead	Alive	
1- experience	$750x10^6$	10	10	0	100
2-experience	$650x10^6$	10	8	2	80
3-experience	$500x10^6$	10	5	5	50
4-experience	$350x10^6$	10	3	7	30
5- control	Phys. solution	12	0	12	0

In order to clarify the results of the experiment, the chicks in the experimental group were observed for 10 days, and the dead and survivors were recorded in the relevant journals. 100% and 50% chick lethality in the experiment was determined by the method of Reed and Mench, based on the number of chicks that died and survived at the end of the experiment.

According to the data of Table 2, by the end of the experiment, not one out of 10 chicks survived in the chickens infected with 750 million pieces of S. pullorom gallinarium in the 1st experiment group. In the 2nd experiment group infected with 650 million microbe cells (KHQB), 8 chicks died and 2 chicks survived. Of the chickens infected with 500 million microbial bodies in the 3rd experiment group, 5 died, and the remaining 5 survived. 3 out of 10 chicks in the 4th experiment group infected with 350 million microbial cells died, the remaining 7 survived. None of the chicks in the control group died before the end of the experiment and they are healthy.

According to the results of the research, chicks infected with a large number of bacterial cells are unable to protect themselves from pathogens based on their biological laws, and quickly become infected with experimental salmonellosis without showing any clinical symptoms. it was noted that he died. When the dead chicks were dissected and examined, pathological anatomical changes characteristic of salmonellosis were clearly visible. However, according to the results of bacteriological tests, S. pullorum gallinarium was re-isolated from the pathological samples of dead chickens. In other chicks of this group, the disease passed in an acute form, and in the end death was observed in them as well.

24-36 hours after infection, when dead chicks were examined clinically and pathologically, obvious clinical and pathomorphological changes characteristic of pullorosis were observed. The chicks that did not die during the experiment were lagging behind in growth and development compared to the control group, and became prone to external environmental factors and susceptible to non-infectious diseases.

500 million in experience. 500 million due to the fact that 5 out of 10 infected chickens in the 3rd experiment group, where the S. pullorum gallinarium microbe was injected into the body, died by 50%. amount of salmonella was LD50 and 750 mln. This amount was determined to be the LD100, as all 100% died in the immunized group.

Conclusions:

1) Pathomorphological changes in pullorosis infection of chicks are mainly general dystrophic processes, especially hemodynamic and dystrophic changes were detected in 2-8 days.

2) Morphological indicators of blood in chicks' blood, i.e., erythrocytes and hemoglobin decreased by 29.7 and 21.6 percent, respectively, and the number of leukocytes and thrombocytes increased by 12.45 and 6.72 percent, according to private studies.

3) 500 mln. LD50 of S. pullorum gallinarium in the experimental group, 750 mln. LD100 was found in 2-8-day-old chicks in the group injected with the microbe.

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