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Diseases of the poultry reproductive organs (Case study: Altai Region, Russia)

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ABSTRACT

We carried out a complex research on the determination of pathogenesis and etiology of diseases of egg-forming organs in laying hens in conditions of industrial breeding. We considered pathologies of the reproductive system of laying hens within the framework of three main directions: prevention of reproductive system diseases, mechanisms of organism defense and pathomorphogenesis of reproductive system pathologies, etiological factors, and potential biomarkers of reproductive system pathology. Based on the characteristics of localization of the pathological focus with the clinical and anatomical findings and physiological processes in the genital organs of hens, we have described the folliculitis of the ovary, magnuit, metritis, and vaginitis of the oviduct as an independent nosological unit. Analysis of biochemical changes in the blood of clinically diseased hens, postmortem autopsy data and the results of morphological and histological studies, which allowed us to describe new diseases folliculitis of the ovary; magnuitis, metritis and vaginitis in the oviduct independent of the disease. Specialists in industrial poultry farming can use the results obtained for the timely prevention and therapy of egg-forming organ diseases in laying hens. The waste of laying hens from egg formation organ pathology on poultry farms in the region increased by 30% in all diseases of infectious etiology. Dysfunction of the chicken's genital organs is mainly caused by disorders of feeding, housing, and veterinary care, with a significant intensification of production aimed at increasing egg production.

Keywords: Salpingitis, Oophoritis, Folliculitis of the ovary, Metritis, Vaginitis.
Article type: Research Article.

INTRODUCTION

The poultry industry plays a vital role in meeting the daily protein requirements of the human population through the consumption of meat and eggs (Attia *et al.* 2022). The availability of protein of animal origin for humans is mainly due to the intensification of poultry production aimed at improving the breeding and performance of the flock (Fedotov *et al.* 2018). Due to breeding achievements over the decades, approximately twice as high egg production of laying hens has been achieved (Dudde *et al.* 2018; Höhne *et al.* 2023). A side effect of these advances has been hen pathologies, including reproductive disorders. Excessive calcium demand during frequent egg laying leads to osteopenia and oviduct atony, as calcium is required for muscle contraction (Greenacre 2015). In laying hens, the risk of ovarian cancer increases with age, along with a decrease in egg production (Palani *et al.* 2015; Paris *et al.* 2021). In addition, genetic factors may influence the development of pathology of reproductive organs of laying hens, rearing conditions, aging, and infectious diseases also affect the reproductive function of laying hens. Among the common reproductive disorders in laying hens, internal laying and egg-related syndrome are well known to reduce egg production and, in severe cases, are fatal (Palani *et al.* 2014, 2015). Reproductive organ pathologies can account for 20 to 50% of all diseases of non-communicable etiology in individual farms, causing great economic losses to the poultry industry due to reduced productivity, mortality,

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and culling of laying hens (Fedotov *et al.* 2018). Infection with highly pathogenic avian influenza virus (HPAIV) causes a wide range of clinical manifestations in poultry, including a marked decrease in egg production and isolation of HPAIV from eggs laid by infected hens. In the experiment, adult hens were intranasally inoculated with three strains of HPAIV. All three strains caused reproductive tract lesions 36-72 hours after inoculation. Positive immunostaining was observed in all segments of the reproductive tract, occurring predominantly in stromal cells and superficial germinal epithelium of the ovary, in mucosal epithelial cells, and less frequently in glandular epithelium throughout the oviduct, as well as in vascular endothelium (Sá e Silva *et al.* 2013). During the experimental period, it was found that during oral administration of *Salmonella heidelberg* and *S. enteritidis* to laying hens, these microorganisms colonized the intestinal tract and invaded the liver, spleen, ovaries, and oviducts of inoculated hens. *S. heidelberg,* and *S. enteritidis* were isolated from the internal liquid in contamination of eggs, whereas the introduction of *S. enteritidis* and *S. typhimurium* did not result in egg infection (Balan *et al.* 2016). Colonization of the reproductive tract by *S. enteritidis* is evidenced by the results of several studies (De Buck *et al.* 2004; Mizumoto *et al.* 2005; Bohez *et al.* 2007; Coward *et al.* 2013). *S. typhimurium* DT104 strains had a low probability of egg infection (Okamura *et al.* 2010). Infection of laying hens caused by *S. pullorum* reduces reproductive function and causes pathology of ovaries and fallopian tubes (Niu *et al.* 2023).

Contents of eggs laid by infected hens (Gast *et al.* 2004). In a similar study, the following data were obtained: colonization of ovaries and oviducts during oral infection of laying hens with different *Salmonella* serovars was significantly higher with *S. heidelberg* compared to *S. enteritidis* and *S. typhimurium*. Moreover, infection with *S. heidelberg* resulted in the infection of eggs, whereas the introduction of *S. enteritidis* and *S. typhimurium* did not result in egg infection (Balan *et al.* 2016). Colonization of the reproductive tract by *S. enteritidis* is evidenced by the results of several research (De Buck *et al.* 2004; Mizumoto *et al.* 2005; Bohez *et al.* 2007; Coward *et al.* 2013). *S. typhimurium* DT104 strains had a low probability of egg infection (Okamura *et al.* 2010). Infection of laying hens caused by *S. pullorum* reduces reproductive function and causes pathology of ovaries and fallopian tubes (Niu *et al.* 2023). The role of *Gallibacterium anatis* biovar *haemolytica* in the infection of the reproductive tract and respiratory system of laying hens has been established (Zloch 2018; Niu *et al.* 2023). Studies analyzing *G. a.* biovar *haemolytica* have identified multidrug resistance phenotypes (Karwańska *et al.* 2023). Antibiotic resistance testing of *G. anatis* showed that isolates were sensitive to enrofloxacin, florfenicol, and gentamicin but resistant to ampicillin, erythromycin, oxytetracycline, and sulfamethoxazole-trimethoprim (Nassik *et al.* 2021). Studies have emphasized the important role of *Escherichia coli* in the reproductive system pathologies of laying hens (Zloch *et al.* 2018). A group of researchers isolated *E. coli* carrying *bla CTX-M-1* and *qnrS1* genes from the reproductive organs of parental broilers and internal contents of hatching eggs. The isolates showed the same multidrug resistance profile, including resistance to ampicillin, ticarcillin, piperacillin, cefazollin, cephalothin, cefotaxime, nalidixic acid, tetracycline, and sulfonamides (Benameur *et al.* 2018). In modern commercial poultry production, with the application of new technological schemes aimed at increasing egg production, the body burden of birds is increasing. Minor violations of housing, feeding, and veterinary care can lead to a decrease in nonspecific resistance of poultry and the development of pathological processes in the reproductive organs (Fedotov & Igoshin 2004; Fedotov & Davydov 2005). Notably, in diagnosing egg formation organ diseases in poultry, the pathology is generally considered, without a differentiated approach to different segments of the oviduct. In particular, world literature has not described the causes of the disease of the vagina, uterus, and protein part of the oviduct. Each part in the formation of the eggs performs a specific function. Therefore, the etiological factors that cause pathological processes in ovaries and oviducts differ. Based on the identification of these reasons, preventive and curative measures can be held (Fedotov & Bessarabov 2006). Nowadays, professionals in the poultry industry have many questions about diagnosing and treating organ diseases in chicken egg formation that are still open. Hence, there is a need for an integrated scientific solution to several theoretical, methodological, and practical issues of diagnosis, treatment, and prevention of chickens' reproductive organ diseases that will contribute to improving the efficiency of veterinary measures in farms with industrial poultry keeping.

MATERIALS AND METHODS

The task of our research was to study the spread of poultry reproductive organ diseases in industrial poultry enterprises in the Altai region and to identify the main causes of pathologies. For experiments under production

conditions, we used laying hens at the age of 150- 400 days (breed Leghorn cross "Hajseks white") in the period of freedom from dangerous infectious diseases. The research on isolation and identification of conditionally pathogenic microorganisms in poultry was carried out based on the recommendations of the All Russian Research Veterinary Institute of Poultry Breeding (ARVIPB). The microbiological tests were carried out at the Russian State Agrarian University Moscow Timiryazev Agricultural Academy, Department of Veterinary Medicine. Modified Postgate's B (MPB), MPA and Columbia Blood Agar (CA) containing 5-10% defibrinated horse blood were used as nutrient media for primary cultures.

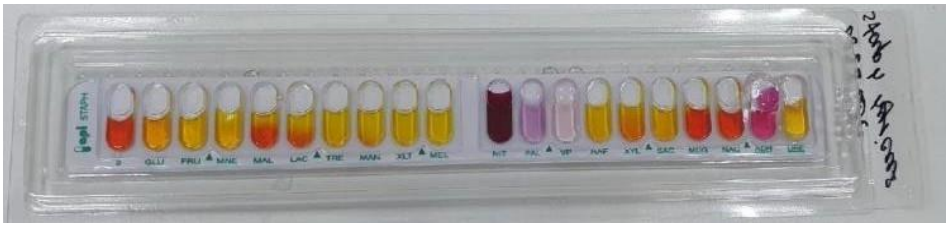
The cultures were incubated under aerobic conditions at 37 °C for 24 hours in a TC-80MU4.2 temperature control unit. The growth characteristics of the isolated microorganisms in solid and liquid nutrient media were used to determine their cultural features and properties. When evaluating colonies grown on solid nutrient media, the shape, size, consistency, features of their edge, relief, structure, transparency, luster, and pigment formation were considered. The presence/absence of surface membrane, wall ring, sediment formation, its nature, and medium turbidity were considered in liquid nutrient cultures. Morphological (shape, size of bacteria, and their mutual arrangement) and tinctorial properties (color features) of the isolated microorganisms were studied in smears - preparations stained according to Gram, Ziehl-Neelsen on a light microscope. To determine the presence of spores, we used the Schaeffer-Fulton staining method and capsules, which are the Olt or Mihin method. The mobility of microorganisms was determined in "hanging drop" preparations. When isolating pure cultures of microorganisms and determining their species affiliation, we used selective and differential diagnostic nutrient media: *Listeria* Selective Medium (LSA), Modified Postgate's B (MPB) containing 6.5% NaCl, Endo, and Sabouraud. To clarify the species identity and some pathogenicity factors of microorganisms, culture and biochemical tests were performed, and API systems (Bruker Daltonik Ink., USA) and MALDI- TOF (Bruker Daltonik Ink., France) were used (Fig. 1). A major evaluation of nonspecific resistance of chickens was carried out with the help of microtests of determining bactericidal activity of blood cells according to the content lysosomal-cationic proteins in granulocytes and quantitative content of the main class of immunoglobulins (Ig G). The experimental data were processed using biometric methods and Microsoft Office Excel computer product "Data Analysis" package.

RESULTS

Guided by the research plan, we conducted an analysis of the poultry state industry in the Altai region, whose goal was the creation of a statistical basis for carrying out production experiments. All the Altai poultry farms work on an industrial basis, and despite the high concentration of livestock, they are safe from acute and chronic infectious diseases. Chicken perishing and culling are mainly due to internal non-communicable diseases. Thanks to the smart work of experts and the administration of the poultry farms, mortality decreased by 37% by the year 2022 compared to 1999. When we conducted a statistical analysis of postmortem diagnoses of the fallen chickens, we drew attention to the increase in the perishing of chickens with diseases of the reproductive organs. So, at the "Molodezhnaya" poultry farm of the Pervomaisky region, the perishing of chickens in 2014 were: hepatitis 8.3%, alimentary dystrophy 12.2%, pathology of the digestive system 13.8%, asphyxia 2.4%, yolk peritonitis 9.9%, and pathology of eggs formation organs 24.3%. The percentages of chicken perishing in 1996 were 7.6, 16.4, 19.0, 10.2, 6.0 and 8.3 respectively. In connection with violations in the technology of chicken keeping and feeding with intensification of production, aimed at increasing egg production, the number of poultry diseases of reproductive organs was increased. The perishing of the laying hens from this disease reached 30% of all infectious etiology diseases on the region's poultry farms. In this regard, there is a need for early diagnosis of egg formation organ pathology, considering the localization of the pathological focus on immediate action for treatment and prevention. Thus, it is necessary to conduct differential diagnosis with consideration of the structure and functioning of the genital organs of chickens.

The unilateral poultry oviduct is divided into five parts: a funnel, a protein part, an isthmus, a uterus, and a vagina. Each region of the oviduct performs a number of functions, and through their successive interaction, eggs are formed. Empirically, we have proved that certain etiological factors can lead to the pathology of individual sections of the oviduct, and further inflammatory processes can fully cover the oviduct and the ovary in the form of ovariosalpingitis or egg yolk peritonitis. In the protein part of the oviduct, the egg stays about 2.5-3 hours during this period. Due to the mucus of the tubular and unicellular glands, the ovum coats the thick protein layer first, then a watery protein layer, and again a thick protein layer. Under the influence of unfavorable factors, egg formation and the emergence of the inflammatory process in the protein division of the oviduct are possible

failures. The disease manifests itself outwardly weak: oviposition is missing, and there is pallor of the comb and weight loss. On postmortem examination, we observed hyperemia of the mucous membrane of the protein part with large amounts of mucus. In the lumen, we found clots of cheesy masses of a yellowish or greenish color. Sometimes, we found immature eggs enclosed in a fibrous capsule in the protein part of the oviduct. According to our observations, pathology of the protein part of the oviduct is closely connected with the violation of protein metabolism with a deficit of vitamins A and D, and it is accompanied by microbial contamination of the oviduct, where the predominant types are staphylococci and *Escherichia*. The most common by *E. Coli* were 01, 02, and 078. In turn, the discovered *Staphylococcus* had quite diverse biochemical properties, and their isolates were distributed as follows: *St. aureus, St. epidermidis*, and *St. saprophyticus* (Figs. 1-3).



A

B

Fig. 1. (A) API test-systems for *S. aureus*; Figure (B) API test-systems for *E. Coli* (Figure compiled by the authors).

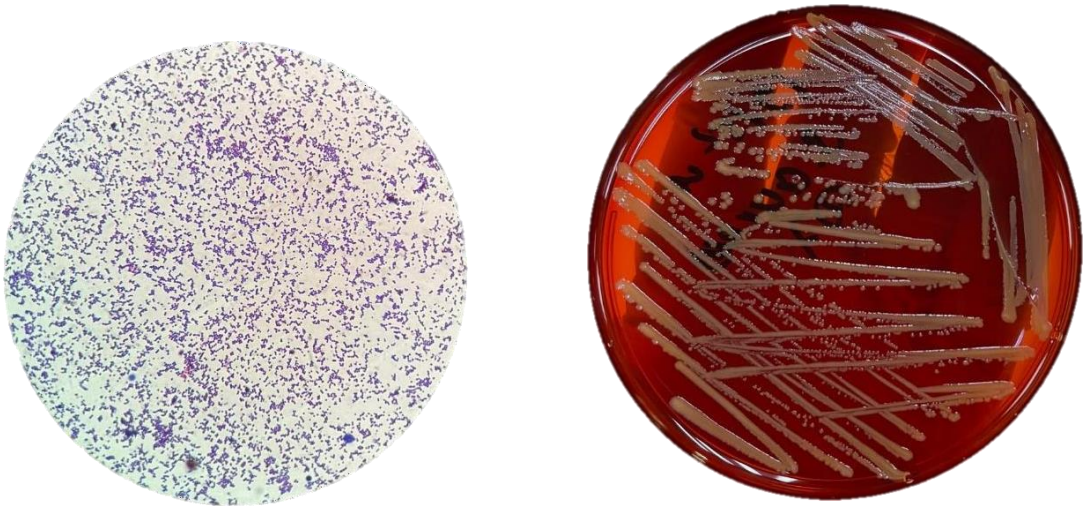


Fig. 2. Tinctorial properties of *S. aureus.* (Figure compiled by the authors).

The research shows a wide variability of staphylococci isolated from chickens with pathology of the reproductive organs. In the uterus of the oviduct, the egg is in for 19-21 hours. During this time, the shell is formed. Crystals of calcium salts are slowly deposited in the spaces between the fibers of the frame, giving rise to the formation of the spongy layer.

When the egg reaches the uterus middle part, the frame formation is almost finished, and the shell substance is delayed for the first three hours after the eggs fall into the uterus. Metritis of the oviduct of laying hens, according

to our findings, arose from the insufficiency and poor absorption of calcium salts. Calcium salts were fed in limestone, and they were not always of good quality and had insufficient solubility. Clinical manifestations of oviduct metritis were accompanied by a prolonged presence of chickens in a laying egg pose. The feathers became dull, and you could freely palpate the egg with the cloaca. In some cases, we revealed the casting of the eggs, and we also watched the eggs without the shell or with a rough surface. During the inspection of internal organs from dead chickens, we found that the endometrium was not wet

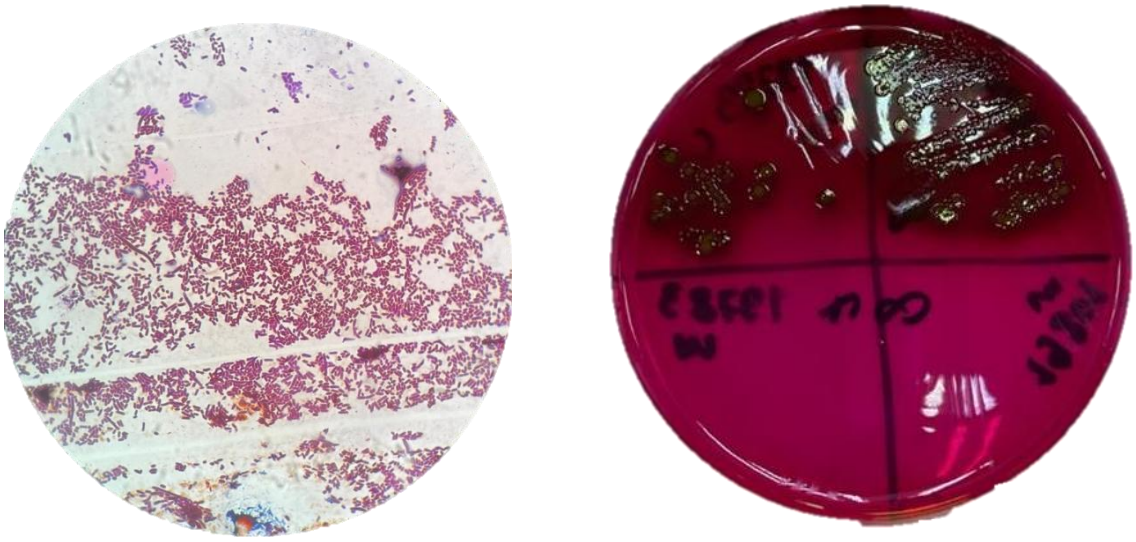


Fig. 3. Tinctorial properties of *E. colli* (Figure compiled by the authors).

In the lumen of the oviduct, some formed eggs were found without shells or with rough surface shells and the tight mucosa of the uterus. According to our observations, the factor that contributes to the inflammation of the uterus of the oviduct may be a violation of both mineral and protein feeding. On poultry farms, more calcium and vitamin D are sometimes added to the diet for protein deficiency to stimulate oviposition. We included bacteriological studies in a comprehensive diagnosis of metritis oviduct of laying hens. It should be noted that the conditional-pathogenic microflora were sown only with the cadaveric material and presented in a relatively wide range: *Pasteurella haemolytica*, *P. gallinurum*, *Staphylococcus aureus*, *Streptococcus faecalis* and *E. coli*.

Given conducting biochemical studies on the blood serum of chickens with clinical signs of metritis oviduct, we drew attention to the decrease in the level of ionized calcium. The uterus of chickens directly uses ionized blood calcium to form the shell, the concentration of which varies in the egg formation cycle. The ionized calcium level in the laying hens' blood is significantly higher than in hens with low egg production. When we analyzed the nature of changes in the content of ionized calcium in the blood, we focused the research on the linkages between the dynamics of the level of ionized calcium in the blood and the intensity of the pathological process in the oviduct. During the experiment to determine the concentration of ionized calcium in hens' blood, we found that in clinically healthy chickens aged 150-250 days, the content of ionized calcium in the blood did not change significantly and was in the range of 1.61 to 1.63 mmol L^{-1}.

These figures, by size, correspond to the physiological norm for this group of chickens. The concentration of total calcium in plasma also remained at the level typical for pullets, suggesting that hens have embarked on an intensive period of puberty, accompanied by rapid growth of follicles in chickens. At the same time, sick chickens' ovaries found a large number of white follicles and only a few small yellow oocytes. The concentration of ionized and total calcium in the blood of these chickens was on the bottom border of the physiological norm, reaching the level typical for young laying hens only 250 days ($p < 0.001$). Obviously, a significant increase in calcium concentration in the blood in this period was due to physiological processes occurring in the arduous process of folliculogenesis. By the beginning of oviposition, the concentration of all metabolites in the blood of healthy chickens continued to remain at a high level ($p < 0.05$-0.001). The above data were obtained on a group of chickens, where the blood samples were taken for analysis at poultry slaughter. For a more accurate and detailed analysis of the dynamics of ionized and total calcium in the blood of hens in the intensive period of folliculogenesis in the parallel experiment, an experimental group of chickens at the age of 250-350 days in which we have recorded the clinical signs of inflammation of the oviduct.

The content of the total and ionized calcium in the blood of chickens with clinical signs of inflammation of the oviduct is almost two times lower compared to their levels in healthy chickens ($p < 0.001$). The total blood calcium concentrations in the sick chickens at 300 - 350 days were reduced by 34 % while ionized by 58 %. The correlation coefficients between the ionized calcium concentration in blood as well as total and bound calcium contents were 0.71 and 0.66 respectively ($p < 0.001$), reflecting a fairly significant connection. Thus, the main etiological factor for the metritis in oviduct of chickens can be considered disorders of mineral metabolism, primarily the failure total and ionized calcium, and normal or excessive content of inorganic phosphorus. The vagina of the oviduct is a matter of seconds, however, in this part the surface of the shell is covered with a thin membrane over the shell, which chemical composition is close to the same one under the shell. In the future, due to the contraction of the muscles of the vagina, the egg is released from the body of the hen. We basically found vaginitis of laying hens in an acute form. The leading cause of vaginitis in the process of observation on large poultry farms have been fodder injuries and mechanical damage. Feed injuries are related to excessive amounts of indigestible fiber (especially the husks of barley and oats) in the diet, and mechanical damage was caused by the pecking of the cloaca while laying eggs. At the external examination of the hens, there was marked anxiety, frequent subsidence, and partial or complete protrusion of the vagina through the cloaca. Palpation is detectable in the oviduct, evident of the egg presence in the shell. At autopsy, the mucous membrane of the vaginal part of the oviduct was hyperemic, swollen, and dry. Visual inspection and analysis of the internal organs status of slaughtered and fallen chickens with the clinic vaginitis allowed us to conclude that anatomophysiological alterations occurred in the vaginal part of the oviduct. This part is responsible for the output-shaped eggs from reproductive organs into the cloaca, and accordingly, in the case of adverse causes, undergoes significant morphological and physiological alterations indicative of postmortem examinations.

At autopsy, slaughtered for diagnostic purposes, signs of inflammation of the vagina of laying hens were noted by pathological alterations on the mucous membrane of the vagina. Given that the slaughtered of the cloaca equally applies to the rectum, we examined the mucosa of the vagina, cloaca, and rectum in a comparative perspective. Research on the rectum discovered swelling of the mucous membranes, hyperemia, abundant mucous secretion, and, in some cases, the remnants of the indigestible husks of grain crops. Husks of oats and barley have a reasonably thick spine, which can injure the sheath of the rectum, causing destructive changes in adjacent tissues. Alterations in microcirculation and tissue structure of the vagina, cloaca, and rectum indicate mechanical injury of organs by undigested awns of solid barley. In the study of inflammation of the ovary in laying hens, we drew attention to the development of the pathological process in the follicles. Follicle normal does not only support but also greater exchange function. In the growth phase of the oocyte, the size of the follicle increases from 9 to 35 mm in hens of breed white Leghorn. The presence of eggs in any part of the oviduct on the ovary surface is detected by the two or three yellow follicles with an average diameter of 32-37 mm, attached to the body by a stalk. Unlike mammals, birds' follicles are anatomically less to do with the ovary. We observed an acute inflammation of the follicle in the absence of pathological changes in the ovary. According to our observations, the defeat of the follicles delays their development and destroys the epithelium. The inflammatory process may spread to adjacent tissue, causing disorder of blood circulation, exudation, and infiltration. When we opened sick hens, we found deformed follicles, the contents of which were diluted or condensed. Petechial hemorrhages were noted in the membranes of the follicles.

At the same time, normally preserved follicles were observed near pathological. Clinical manifestation of folliculitis of the ovary in laying hens stopped egg production. During all periods of observation, the ovaries of hens with pathological signs of folliculitis were contaminated with *Staphylococcus, Escherichia coli, Streptococcus,* and *Proteus*. The maximum number of contaminated birds with conventionally pathogenic bacteria was established in the period of active egg production. The microflora of the sick chicken's follicle was isolated mainly in the form of associations, while more microorganisms with a high degree of virulence, such as *St. aureus* and hemolytic strains of *Escherichia*, were isolated (Fig. 3). Conditionally pathogenic microorganisms in clinically healthy chickens were not sown. If there is folliculitis ovarian, the nature of the structure alterations of follicles indicates a pronounced cytotoxic effect of conditionally pathogenic microorganisms. As a rule, contamination of the internal reproductive organs reduces the natural resistance of chickens. Evaluation of nonspecific resistance of sick hens was carried out comprehensively using micro tests. Blood sampling was conducted on birds of different ages to obtain an objective picture. The research of blood granulocytes of healthy chickens aged from 150 to 210 days shows a slight increase in the content of lysosomal-cationic proteins. By the

end of oviposition (310-400 days), the level of lysosomal-cationic proteins in granulocytes of the blood of laying hens significantly decreased but exceeded the level of LKB from sick poultry ($p < 0.001$ and $p < 0.001$). In hens with clinical signs of inflammation of the ovary during the first phase of egg production lower level lysosomal-cationic proteins were remained, compared with the control (1,77 ± 0.024 and 1.88 ± 0.017 against 2.16 ± 0.024 and 2.08 ± 0.012).

The content of immunoglobulin (Ig) G in the control group differs from that of chickens with signs. These findings are particularly evident when the hen is at the peak of egg production. The IgG level of healthy chickens during this period varies from 9.72 ± 0.19 to 8.48 ± 0.12. The IgG level in the blood of sick chickens was below the limits of physiological norms throughout the study. By analyzing biochemical alterations in the blood of clinically sick hens, postmortem autopsy results, and morphological studies results, it can be assumed that the folliculitis nature of pathological changes in the structure of the ovarian follicles indicates a pronounced cytotoxic effect of the pathogen. We found increased lymphocytic infiltration and follicular technical shells of follicles, increasing the number of blood cells and tissue basophils. The ovary from hens with pathological signs of salpingohoritis was contaminated with *Staphylococcus, Escherichia coli, Streptococcus*, and *Proteus* during all observation periods the maximum number of chickens contaminated with opportunistic bacteria set in the period of active egg production. Microflora of the follicle of the sick hen's ovary were isolated mainly in the form of associations, while more isolated microorganisms with a high degree of virulence, such as *St. aureus* and hemolytic strains of *Escherichia*. So, from the ovarian follicles of hens at the age of 150-250 days, staphylococci were allocated more frequently than other microorganisms in 56 cases (63.64%), including *E. coli* in 14 (15.92%); *E. coli* and *Streptococcus* in 8 (9.09%); with *Streptococcus* in 1 (1.14%); *Proteus* 7 (7.95%); with the *Proteus* and *E. coli* 4 (4.55%) and in 22 (25.0%) they were isolated in the monocultures form. *E. coli* (42.05%) is characterized by a high degree of sowing; this was especially noticeable in association with staphylococci (15.92%). The number of chickens to which the contents of the follicles were allocated of the association of *E. coli* with *Streptococcus* was 3 (3.41%) of the head. In 2 (2.27%) cases, *E. coli* was isolated with *Proteus* and 8 (9.09%) with staphylococci and streptococci. In 10 (11.36%) cases, *E. coli* was isolated in monocultures. In most cases, streptococci and *Proteus* from the content of the follicles in the form of monocultures were isolated only from the cadaveric material of adult laying hens. staphylococci and *E. coli* were sown from the cadavers and from slaughtered chickens. When a microbiological diagnosis of the reproductive organs of clinically healthy poultry, staphylococci, enterococci, etc. were sown. The ovarian follicles were of various sizes. The contents of the follicle had a yellow color and moderately dense consistency. Ovarian disease in birds has polymicrobial etiology; its leading role belongs to conditional pathogenic microorganisms, predominantly in associations with facultative anaerobic and anaerobic microflora. Microbiological research has demonstrated that poultry can detect the spread of strains of staphylococci of the following types: *St. aureus* (72.7%), *St. saprophyticus* (13.6%), and *St. epidermidis* (13.6%). For further typing, *St. aureus* revealed different biological options. So, the chickens with the clinical sign of salpingohoritis had the following biovars: hominis, gallinae, and A/B. The result of serological typing of 78 enteropathogenic cultures, 87% related to serovars: 01, 02, 078, 09, 055, OIII, and OI4I. The serotype 02, we took 65.5% cultures, 078-13.3%, 01-11.6%. For serovars 09, OIII, OI4I related to only one culture of *E. coli*, for serovar 055, to 2 cultures. Thus, the most common and virulent for laboratory animals was a culture of *E. coli* serovars 01, 02, 078. The research identified three groups of streptococci b-hemolytic streptococci (*Str. hemolyticus*) cause hemolysis, a- verdant streptococci (*Str. viridans*) form around colonies of the green zone hemimetamorphic, newemailusecase y-streptococci (*Str. anhemolyticus*) do not modify red blood cells and cause hemolysis blood agar. From a 32-year-studied culture of streptococci isolated in the dynamics of the disease from sick chickens, pathogenic for white mice was 14, which is 39.64%. Studying the morphological and cultural-biochemical properties of bacteria of the genus *Proteus* isolated from the affected follicle, we spent typing. *Proteus*, corrupting glucose (K+G+), and sucrose were attributed to *Proteus mirabilis*, and corrupting glucose (K+G+), sucrose, and maltose - *Proteus vulgaris*. Isolated from chickens in the dynamics of folliculitis microbes of *Proteus* group were characterized by a high degree of pathogenicity: of the 27-year isolated culture 14 (57.33%) were pathogenic for white mice.

Summarizing the obtained results of microbiological research, we note that staphylococci were allocated from the affected ovary, which was mainly presented: *St. aureus, St. saprophyticus* and *St. epidermidis*, and equally of *E. coli* in the form of serovars 01, 02, 078: less allocated streptococci and *Proteus vulgaris*.

DISCUSSION

We have observed that laying hens have an increased risk of reproductive organ abnormalities with age, which can be fatal. This finding aligns with observations by Palani *et al.* (2014, 2015).

We found that *Staphylococcus aureus* was isolated from ovarian follicles of hens aged 150-250 days more frequently than other microorganisms in 56 cases (63.64%). In the case of *E. coli*, it was in 14 (15.92%); with *E. coli* and *Streptococcus* in 8 (9.09%), *Streptococcus* in 1 (1.14%); *Proteus* in 7 (7.95%), *Proteus* and *E. coli* in 4 (4.55%), and in 22 (25.0%) they were isolated as monocultures. *E. coli* (42.05% of cases) was also characterized by high isolation, primarily associated with staphylococci (15.92%).

The number of birds in which associations of *E. coli* with *Streptococcus* were isolated from follicle contents was 3 (3.41%) heads. In 2 (2.27%) cases, *E. coli* was isolated with *Proteus* and another 8 (9.09%) with *Staphylococcus* and *Streptococcus*. In 10 (11.36%) cases, *E. coli* was isolated as monocultures. Ovarian diseases in birds have a polymicrobial aetiology, the leading role which belongs to opportunistic microorganisms, mainly in associations of facultative- anaerobic and anaerobic microflora, which is again in accordance with the studies of Bohez *et al.* (2007), De Buck *et al.* (2004), Mizumoto *et al.* (2005) and Coward *et al.* (2013).Some authors (Dudde *et al.* 2018) consider the pathology of reproductive organs of hens as a whole without a differentiated approach to different parts of the oviduct. In particular, the world literature does not describe the agents that cause the disease of the vagina, uterus, and the protein part of the oviduct. Each department performs a specific function during egg formation, so the etiological factors causing pathological processes in the ovaries and oviducts differ: Based on the identification of these causes, preventive and health-improving measures were adopted; based on the peculiarities of localisation of the pathological focus, taking into account clinical and anatomical data and physiological processes in the genital organs of birds, we described folliculitis of the ovary; magnuitis, metritis and vaginitis of the oviduct as independent nosological units.

CONCLUSION

Waste of laying hens from egg formation organs pathology on poultry farms of the region increased and reached 30% percent of all diseases of infectious etiology. Dysfunction of the chicken's genital organs is mainly caused by disorders of feeding, housing, and veterinary care, with a significant intensification of production aimed at increasing egg production. We consider pathologies of the reproductive system of laying hens within the framework of three main directions: prevention of reproductive system diseases, mechanisms of organism defense and pathomorphogenesis of reproductive system pathologies, etiological factors, and potential biomarkers of reproductive system pathology. Based on the characteristics of localization of the pathological focus witch the clinical and anatomical findings and physiological processes in the genital organs of hens, we have described the folliculitis of the ovary; magnuit, metritis and vaginitis of the oviduct as an independent nosological units.

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