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### Histochemical method of diagnosis of mixed pasteurellosis and ascaridosis bird disease

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For the first time was developed an effective and specific histochemical method for the diagnosis of mixed pasteurellosis and ascaridosis diseases of birds, which can be used in conjunction with bacteriological, serological and helminthocoprosopic studies, and in cases where it is impossible to diagnose serologic methods. The proposed method of histochemical diagnostics can be used for the detection of pasterellocarriers during mixed pasteurellosis and ascaridosis disease of birds. In the birds of the experimental group (clinically diseased bird), the pasteurial antigen in the histological preparations of the organs tissues after the histochemical study looked like local and diffuse clusters of oval and deep form of intense purple color and local clusters of oblong-shaped larvae with the presence of a head and a tail of blue and eggs of light purple color. On histological sections of tissues and organs of the control group (clinically healthy bird), such changes were absent. For investigation of the organs of the experimental group (clinically diseased poultry), we have established the following areas of accumulation of pasteurial antigen and larvae and eggs of ascarids: in the heart – observed postponement of the antigen *Pasteurella multocida* in the form of strips throughout the area of the histological cut along the length of the muscle fibers and the loss of their delineation and fragmentation; in the liver – observed the diffuse location of the *Pasteurella multocida* antigen throughout the histological section in the lumen of the blood vessels and around them, the Disse spaces, between the hepatocytes; histochemically detected reduction of AsAT – by 84.3% and bilirubin – by 74.5%; in the muscular stomach – there was a diffuse postponement of the pasteurial antigen throughout the histologic section, but the largest accumulation was observed around the fibers of smooth muscle tissue of dark purple; in the spleen – the presence of *Pasteurella multocida* antigen throughout the histological section around lymphoid clusters, blood vessel walls, and intensive deposition of the antigen were observed around the splenic artery in the form of a deep, intense violet color; in the duodenum – observed diffuse deposition of the *Pasteurella multocida* antigen across the entire histologic area around the blood vessels, lymphatic follicles, villi of the mucous membrane, between the enterocytes, the mucosal muscle folds, the intramural ganglion nodules in the form of the deep form; Intestinal wall, blood vessels, licorice glands, larvae of ascarid species of *Ascaridia galli* species were observed blue, around granular grains around the dead larvae; Histochemically, decreased activity of alkaline phosphatase – by 70% and mucin – by 82.4%; in the kidneys – locally observed the presence of a *Pasteurella multocida* pasteur antigen around the intercellular veins, a capsule of the nephron, a circular oval-shaped distal tubule, a dark violet color; Histochemically, creatinine decreased by 45.4%.

**Key words:** birds, mixed, diagnostics, histochemical method, pasteurella, ascaridia.

#### Introduction

Concentration of birds in a limited area naturally led to the emergence of new relationships between micro and macroorganism. As a result of this, there were mixed bird diseases, in which the pathogenesis, clinical signs, pathologic-anatomical and pathogistological changes sharply changed, which made it difficult to diagnostics and differential diagnostic. Today, the mixed course of diseases is

often observed. There are many new or atypical forms of the disease, due to so-called local microbialism, under which one should understand the set of conditions that promote the penetration of microbes in the body of the bird, their conservation, reproduction, development and variability (Berezovskyi, 2012; Plys, 2017; Plys, 2018).

Pasteurellosis and ascaridosis disease is a sharp contagious disease of farm birds, wild migratory and synanthropic birds and humans caused of causative agent pas-

teurellosis *Pasteurella multocida* and causative agent of ascaridosis *Ascaridia galli*, characterized by septicemia, hemorrhagic diathesis, endocarditis, necrotic lung disease, catarrhal hemorrhagic inflammation of the thin and thick parts of the intestine and high lethality (Plys, 2017).

The components of this mix are symbionts of different taxonomic groups, namely bacteria and helminth. For veterinary and humanistic medicine are essential the resulting and total effects of certain components of parasitosis, which manifests itself through the peculiarities of the course, clinical symptoms and pathological and anatomical changes of mixed or mixed bird diseases, the specifics of their diagnosis, treatment and prophylaxis (Zon et al., 2009).

Establishing the diagnosis of a mixed pasteurellosis and ascaridosis is difficult (Plys, 2017; Plys, 2018).

With Histological methods determine the chemical nature of cells, their individual components and the intercellular substance of tissues of various organs of animal organisms in normal, in pathology, in different functional states, and in the age aspect. The cyto- and histochemical methods of research can be much faster than histological, fix the age and functional changes in tissues and cells, which is extremely important for the objective interpretation of morphological changes, as well as the analysis of the differences of normal microstructures from pathologically altered (Prudnikov et al., 2004; Horalsky et al., 2016).

Histochemical methods are based on the use of specific chemical reactions, resulting in the formation of synthesis insoluble products, which are localized in certain places of the cell or intercellular substance and are clearly visible under the microscope and differ from other cellular components. These methods reveal amino acids, proteins, nucleic acids, carbohydrates, lipids, enzyme activity, etc. in certain tissue structures. The reaction products are quantitatively analyzed. In this case, various methods of morphometry, cytospectrophotometry, cytospectrofluorometry with the following mathematical processing of material are used (Ryibakov et al., 2003; Pototskyi et al., 2007; Shutchenko, 2007; Horalsky et al., 2016).

However, there are no reports on the histochemical method of diagnostic of pasteurellosis and ascaridosis birds diseases in domestic and foreign literature, which led us to carry out research of this work (Plys, 2017).

The purpose of our work was to develop an effective and specific histochemical method for the diagnostic of mixed pasteurellosis and ascaridosis diseases of the birds.

### Material and methods

The research was carried out on the basis of the farm "P" and private enterprise "P-1" of Dnipropetrovsk region and Dnipropetrovsk State Regional Laboratory of veterinary medicine in the department of histology.

On the basis of the principle of pairs of analogues, two groups of birds of each species (chickens, turkeys, geese, ducks, pigeons, parrots) were formed, control (clinically healthy bird) (n = 10) and experimental (clinically diseased bird) (n = 10) in each group.

The material for research was the pathological and biological material of control and experimental bird groups.

The diagnosis of microscopic pasteurello-ascariasis poultry disease was established in a complex manner, taking into account anamnestic and epizootological data, clinical signs, pathological and anatomical changes in the autopsy of dead birds and laboratory studies: bacteriological, helminthocoprosopic and histochemical.

For histological examination, the organs were fixed in a 10% neutral formalin aqueous solution for 48 hours, later dehydrated in ethanol of increasing concentration. After fixation of the sample material through chloroform was poured into paraffin and by using sanny microtomu, were obtained cut in a thickness of 7–10 microns and stained with hematoxylin and eosin according to the standard procedure. The obtained histological preparations were studied by light microscopy methods. During the histomorphometric evaluation of the manufactured preparations, were laid as a basis methodological recommendations for the histomorphological evaluation of the organs of the bird in the norm and pathology.

The following organs were subjected to the study: heart, liver, muscular stomach, spleen, duodenum, kidney.

Experimental studies in the poultry have been conducted taking into account the basic principles of bioethics.

The research results were processed using the *Microsoft Excel* suite of applications.

### Results and discussion

In the birds of the experimental group (clinically diseased bird), the pasteurial antigen in the histological preparations of the organs tissues after the histochemical study looked like local and diffuse clusters of oval and deep form of intense purple color and local clusters of oblong-shaped larvae with the presence of a head and a tail of blue and eggs of light purple color. On histological sections of tissues and organs of the control group (clinically healthy bird), such changes were absent.

For investigation of the organs of the experimental group (clinically diseased poultry), we have established the following areas of accumulation of pasteurial antigen and larvae and eggs of ascarids:

- in the heart – observed postponement of the antigen *Pasteurella multocida* in the form of strips throughout the area of the histological cut along the length of the muscle fibers and the loss of their delineation and fragmentation;
- in the liver – observed the diffuse location of the *Pasteurella multocida* antigen throughout the histological section in the lumen of the blood vessels and around them, the Disse spaces, between the hepatocytes; histochemically detected reduction of AsAT – by 84.3% and bilirubin – by 74.5%;
- in the muscular stomach – there was a diffuse postponement of the pasteurial antigen throughout the histologic section, but the largest accumulation was observed around the fibers of smooth muscle tissue of dark purple;
- in the spleen – the presence of *Pasteurella multocida* antigen throughout the histological section around lymphoid clusters, blood vessel walls, and intensive deposition of the antigen were observed around the splenic artery in the form of a deep, intense violet color;
- in the duodenum – observed diffuse deposition of the *Pasteurella multocida* antigen across the entire histo-

logic area around the blood vessels, lymphatic follicles, villi of the mucous membrane, between the enterocytes, the mucosal muscle folds, the intramural ganglion nodules in the form of the deep form; Intestinal wall, blood vessels, licorice glands, larvae of ascarid species of *Ascaridia galli* species were observed blue, around granular grains around the dead larvae; Histochemically, decreased activity of alkaline phosphatase – by 70% and mucin – by 82.4%;

– in the kidneys – locally observed the presence of a *Pasteurella multocida* pasteure antigen around the intercellular veins, a capsule of the nephron, a circular oval-shaped distal tubule, a dark violet color; Histochemically, creatinine decreased by 45.4%.

For histochemical studies of the material selected from the control groups (clinically healthy bird) pathological changes were absent and the indicators were within the limits of the physiological norm.

### Conclusions

1. For the first time an effective and specific histochemical method for the diagnostics of pasteurellosis and ascaridosis birds diseases has been developed, which can be used in conjunction with bacteriological, serological and helminthocoprosopic investigations and in cases where it is impossible to diagnose serologic methods.

2. The proposed method of histochemical diagnostic can be used for the detection of pasteurelo-ascaridium carriers during mixed pasteurellosis and ascaridosis disease of birds.

3. Characteristic histochemical changes in experimental groups (clinically diseased poultry) are in the liver decrease in AsAT – by 84.3% and bilirubin – by 74.5%; In the duodenum, the activity of alkaline phosphatase decreased by 70% and mucin decreased by 82.4% and creatinine decreased by 45.4%.

4. The most pronounced and intense changes were observed in the heart, liver, spleen, duodenum and kidneys.

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