ORIGINAL ARTICLE

Experimental Toxemia of Chickens Contaminated with *Yersinia enterocolitica* Bacteria

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Abstract

Aim: The aim of this study is to study pathological processes dynamics at experimental infestation of chickens with *Yersinia pseudotuberculosis* bacteria. **Materials and Methods:** Bacteria were cultured on the Hottinger medium. The presence of toxins was assessed using "dilatation of the intestine" and "vascular permeability" tests. For the first test, the investigated liquid with bacteria was administered intranasally, the results were evaluated by comparing the weight difference of lungs, while for the second test the investigated liquid was administered per rectum determining after 4 h the ratio of the weight of the small intestine with the contents to body weight. For morphological studies, histological sections were stained with hematoxylin and eosin. For representative information, preparations were examined under optical microscopy "H604 TrinocularUnico" (USA), stereoscopic microscopy "BIOMED MS–1 Stereo" (Russia), and scanning electron microscopy "Hitachi-800" with scanning add-on device (Japan). **Results:** At the formation of biofilms consisting of *Y. pseudotuberculosis* bacteria, we observed the formation of extracellular matrix, which covered the closed structures of various sizes consisting of bacterial cells, followed by subsequent formation of clusters. At the experimental toxemia of chickens, we identified in general exudative-infiltrative processes, the proliferation of antigen-primed lymphocyte, macrophage infiltration of the sinuses of the lymphoid organs, and increasing number of T-lymphocytes. At violation of the porosity in blood vessels of organs of the cardiovascular, respiratory, digestive, and excretory systems, we noted signs of hemocirculation disorders, extensive swelling, liberation of formed elements of blood and the fibrinogenation, dystrophic and necrotic processes, as well as numerous caseous necrotic foci infiltrated by leukocytes, which were revealed in the parenchymatous organs. **Conclusion:** When culturing *Y. pseudotuberculosis* bacteria, we observed the intercellular matrix and the formation of biofilms. At experimental toxemia of chickens, the pathological process dynamics was characterized by exudative and infiltrative processes, general vascular reaction, toxic myocardial dystrophy, atrophy of bursa fabricii, dystrophic, and necrotic processes in the Garder glands, Meckel's diverticulum, serous-fibrinous aerosacculitis, broken wind, signs of enterosorption, and hepatorenal syndrome.

Key words: Bacteria, chickens, granulomas, toxemia, *Yersinia*

INTRODUCTION

The *Yersinia pseudotuberculosis* bacteria, during contamination of susceptible types of birds, implements the virulence factors, including those associated with the synthesis of exotoxins, secreted into the environment, and endotoxins, which are part of cell wall representing complex of polysaccharides [7,8]. Thermostable and thermolabile lethal exotoxins

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of *Y. pseudotuberculosis* bacteria possess immunogenic and allergenic properties; heat-stable endotoxins relative to the enterotoxins are involved in the pathogenesis of secretory diarrhea that is associated with the activation of adenylyl cyclase system.[1,2] When simulating infection process on laboratory models (mice, guinea pigs, and rabbits), we have noted the colonization of the ileocecal part of the intestine with *Y. pseudotuberculosis* bacteria, dissemination of the bacteria into liver and spleen, apoptosis-inducing effect on neutrophils and macrophages, and the development of generalized infection.[3-5] The relevance of the present research topic is conditioned by several factors. Thus, as a rule, laboratory models are not unified when assessing virulence factors during infection of susceptible species. Besides, the effect of toxins on the initiation, development, and outcome of the infectious process in the pathology of birds is not studied enough.

The aim of this work is to study the pathological process dynamics in chickens when experimentally contaminated with *Y. pseudotuberculosis* bacteria.

Research goals include the following:

- To study the morphology of the *Y. pseudotuberculosis* bacteria under optical and scanning electron microscopy;
- To determine the presence of toxins of the *Y. pseudotuberculosis* germ culture with the use of "dilatation of the intestine" and "vascular permeability" tests;
- To study the morphology of the chickens tissues and organs when experimentally contaminated with the *Y. pseudotuberculosis* bacteria.

MATERIALS AND METHODS

Germ cultures of *Y. pseudotuberculosis* No. 290 were cultivated on the Hottinger medium at 22°C for 24 h.

Preparations of bacteria were preserved for 3 min in ethanol, then in 3-5 drops of aqueous solution of crystal violet at a dilution of 1:2000, vapors of 25.0% solution of glutaric aldehyde – for 30-40 min, and 1.0% aqueous solution of osmic acid (O_SO_4) – for 1-2 min.[6]

When detecting the availability of toxins, germ culture was centrifuged over 30 min at 6000 rpm. The supernate in a volume of 0.2 cm^3 was injected in 3-day-old chicks of "White Leghorn" breed (sterile Hottinger liquid medium was used as a control). The presence of toxins was assessed using two tests – "dilatation of the intestine" and "vascular permeability." When applying the "dilatation of intestine" test, liquid was administered intranasally, while a solution of "evans blue" dye, not penetrating through the intact endothelium of blood vessels, was injected intravenously after 24 h. The results were evaluated by comparing the difference in weight of lungs (mg). "Vascular permeability" test was assessed per rectum, at that, the ratio of the mass of the small intestine with the contents to the body weight (g) was determined after 4 h.[2,7]

For morphological studies, histological sections were stained with hematoxylin and eosin. For representative information, preparations were examined under optical microscopy "H604 Trinocular Unico" (USA); stereoscopic microscopy "BIOMED MS–1 Stereo" (Russia), and scanning electron microscopy "Hitachi-800" with scanning console (Japan).

RESEARCH RESULTS AND DISCUSSION

When studying morphology, we revealed that the *Y. pseudotuberculosis* bacteria with S-shaped colonies d ≤1.0 mm had a rounded shape, light, with smooth edges, a slightly raised intensely colored core, radial striations at the periphery; the cells were arranged orderly, tightly adjoining to each other. In Gram-staining smear preparations, we detected Gram-negative bacteria, mainly single, coccoid, or ovoid in shape. When forming extracellular matrix, we observed the formation of closed structures of various sizes, consisting of bacterial cells with closed intercellular matrix, with the subsequent formation of clusters and biofilms [Figure 1].

Evaluating the results showing the availability of toxins, we revealed that the average coefficient of intestine expansion (K ≥ 0.60) changes within the range 0.081 ± 0.005 to −0.106 ± 0.008, while the permeability coefficient of lungs blood vessels (K ≥ 1.07) falls within the limits of 1.08 ± 0.11 to 1.33 ± 0.12.

The intoxication has led to macrophage reactions, perivascular tissue edema, and disseminated thrombosis of the vessels. Antigenic stimulation usually was accompanied by signs of accidental transformation of the thymus gland, such as circulatory disorders, reduction of lobules, formation of cysts and hyperplasia of the spleen, enlarged sinuses of red pulp, the devastation of the white pulp, perivascular edema and plasmatic impregnation of tissues, and proliferation of endothelial cells. Besides, we revealed a proliferation of lymphocytes in the periarterial lymphoid accumulations. Due to the phylogenetic development of birds, the structural features of tissues and organs of the immune system were manifested by atrophy of bursa fabricii, dystrophic and necrotic processes in the Garter glands, and Meckel's diverticulum of chickens.

Violation of the porosity of blood vessels in the organs of the cardiovascular, respiratory, digestive, and excretory systems has led to signs of blood circulation disorders, extensive swelling, the release of blood cells and the loss of fibrinogen, dystrophic, and necrotic processes, as well as numerous caseous necrotic foci infiltrated by leukocytes that was revealed in the parenchymatous organs.

Petechial hemorrhage was detected in the membranes of the heart, the pericardial sac was distended and filled with a serous - fibrinous exudate; the epicardium and adjacent areas of the myocardium were dull, reddened, thickened due to impregnation with serous-fibrinous exudate and infiltration by pseudoeosinophils, lymphocytes, histiocytes, and fibroblasts. Blood vessels of endocardium were enlarged and filled with densely distributed erythrocytes and pseudo-eosinophils. In case of the development of the toxic degeneration signs, we observed dilated cardiomyopathy, a stretching of the heart cavities, the cavity contained fibrin and erythrocytes, as well as focal necrotic changes and cardiomyocytes were detected.

A foamy exudate was detected in the trachea; pneumatic bags were thick, dull, with imposed films of fibrin, vessels filled with blood. There were signs of acute lobular emphysema, i.e., enlargement of the alveoli cavity, thinning of the parenchyma; respiratory capillaries were injected with blood, enlarged, knotty thickened in spots, intruded into the lumen of the alveoli. The apneumatic alveolar shells with bloodless capillaries due to the pressure of air accumulated in the alveoli were detected, small arteries and veins were dilated and filled with blood. We observed extension and overflow of inter-alveolar capillaries and veins of interlobular connective tissue of the lungs, edema of the connective tissue, swelling, and thickening of collagen fibers around blood vessels and bronchi.

Signs of enterosorption were manifested by irregular and sudden swelling of the stomach and intestine, accumulation of serous-hemorrhagic exudate in the lumen of the gastrointestinal tract, hydropic degeneration of enterocytes, hyperemia and lymphoplasmacytic diffusive and focal infiltration of the loose fibrous connective tissue of the villi of the intestinal mucosa, and diapedesis of red blood cells. With the development of signs of hepatotoxic syndrome, we revealed a vascular congestion of portal tracts and central veins, the expansion and thickening of the biliary passage, the polymorphism of hepatocytes, accumulation in sinusoidal capillaries of the granules of hemosiderin, macrophage, and lymphoid cells, infiltration with mononuclear leukocytes and pseudoepinephrine [Figure 2].

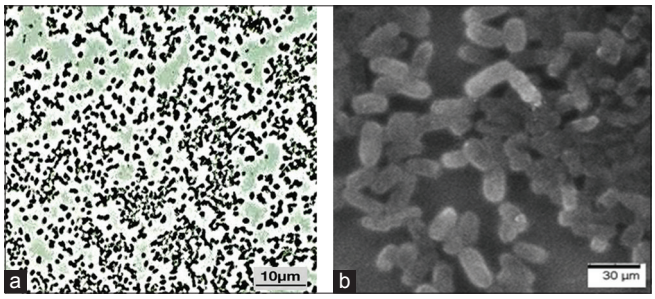

Figure 1: Formation of bacteria biofilms: (a) Optical microscopy, ×200; (b) scanning electron microscopy, ×3000

At nephrotoxic syndrome, we observed diffusive interstitial nephritis, characterized by proliferation of connective tissue cells with a predominance of polyblasts and epithelioid cells, with the signs of congestive hemorrhagic infarction and necrosis of the epithelial cells of the urinary ducts of the kidney with leukocyte infiltration, toxic changes in the vascular glomeruli, and the proximal convoluted tubule. Arterial glomeruli were enlarged, tightly adhered to the nephron capsules, the gaps did not differ due to disintegration of epithelial cells.

At the chickens experimental toxemia, we revealed generally the predominance of exudative-infiltrative processes, the proliferation of sensitized lymphocytes, macrophage infiltration of the sinuses of the lymphoid organs, and the increase in the number of T-lymphocytes. Violation of the porosity of the blood vessels of serous membranes of organs lead to an impairment of hemocirculation, extensive serous edema, release of the formed elements of blood and the loss of fibrinogen, as well as dystrophic and necrotic processes in the Garder glands, Meckel's diverticulum, serofibrinous aerosacculitis, atelectasis and pulmonary edema, signs of enterosorption, and the hepatorenal syndrome.

DISCUSSION

Implementation of *Yersinia* virulence factors is mediated by adhesion properties through fimbrial structures (fimbriae and pili), and afimbrial adhesins (invasines) involved in interaction with receptors of epithelial cells.[2,7,8] The

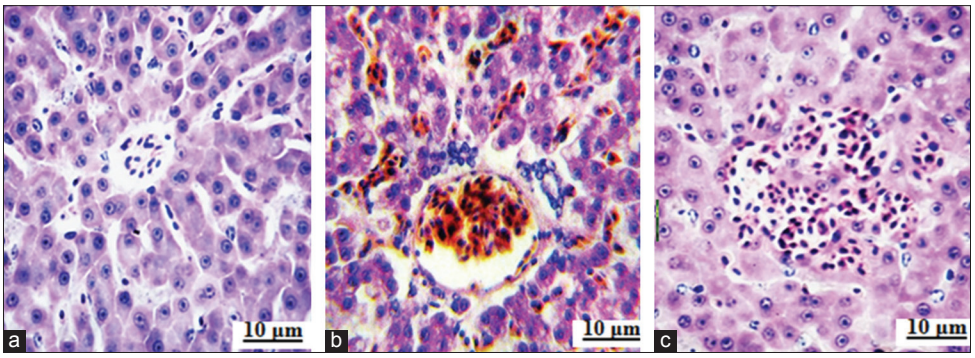

Figure 2: Chicken liver infected with yersiniae: (a) Control; (b and c) experiments; stained by hematoxylin and eosin, ×100

ability to form biofilms due to the synthesis of adhesins, for example, intercellular adhesion polysaccharide (polysaccharide intercellular adhesin), inhibits phagocytosis, and activates bacteria colonization processes.[6,9,10] Interaction of prokaryotes and eukaryotes cells due to the proteins of the outer membrane of *Yersinia* (*Yersinia* outer proteins) leads to the inhibition of phagocytosis, triggers apoptosis of macrophages with the subsequent elimination from the inflammatory tissue that creates favorable conditions for bacteria persistence in the body.[11,12]

The *Y. pseudotuberculosis* bacteria are recovered at the pathology of birds of the families of Ovsyanikov *Emberizidae*, pipits *Motacillidae*, sparrow *Passeridae,* accentor *Prunellidae*, and parrots *Psittacidae*.[13-17] The prevalence of strains containing the F-plasmid of virulence was up to 8.0% of the total number of isolates of *Y. pseudotuberculosis*, recovered at the availability of multiple foci of necrosis in the heart, spleen, lungs, kidneys, intestine, and liver in birds of the thrush *Turdidae* family.[18] At commercial stock keeping of birds of the pheasant *Phasianidae* family, antibodies to *Y. pseudotuberculosis* bacteria were detected in 13.6-32.2% of cases.[19] The *Y. pseudotuberculosis* bacteria were isolated from feces samples of dunghill-hens (*Gallinae*), housed in the backyards of Finland, as well as objects in the external environment.[20] The risk of bacterial contamination increases with the content of more than 10,000 heads of birds older than 18 months within limited spaces. At that, the correlation has been established between inflammatory changes in the gastrointestinal tract due to invasive properties of the bacteria, and hematological parameters, characterized by a decrease in the number of leukocytes, an increase in the number of monocytes and pseudoeosinophils.[12] In large poultry farms, bacterial infections caused by *Yersinia* usually occur in association with respiratory mycoplasmosis and virosis that greatly complicates the differential exclusion of diseases in farm animals, as well as reduces the effectiveness of anti-infective preparations and the effect of preventive measures.[2,21] At associative progression of mycoplasmosis and bacterial infections, the changes are localized in the heart, lungs, pneumatic bags, liver, spleen, and intestine in the form of a serosal, serosal-fibrinous, and fibrinous inflammation; migration of leukocytes and monocytes into the gastrointestinal tract leads to inflammation and damage of the intestinal mucosa, merging and flattening of the villi at viral infections, such as in particular chickens infectious anemia. Besides, it leads to inversion of the jejunum and part of the duodenum that is manifested through venous infarction, periportal focal coagulative necrosis of the liver and granulomatous inflammation of the cecal tonsils.[6,22-24]

The infectious process is accompanied by inflammatory changes in the blind appendages of the intestine that is causing immunosuppression, and in case of mucosal membrane break, promotes the penetration of bacterial toxins and vasoactive factors into the systemic circulatory system (toxemia) and the formation of multiple foci of necrosis in the liver.[25] Identification of pathogens is complicated due to common antigenic structure and genomic sequence similarity. In this respect, promising is the method of duplex polymerase chain reaction-based detection of polymorphic sites of genes of isolates.[26] Differential signs of respiratory mycoplasmosis are aerosacculitis, clouding, and thickening of the thoracic and abdominal pneumatic sacs. In the absence of complications by secondary microflora, there may be no signs of injury of parenchymal organs.[27]

Infiltration of the intestinal mucosa by pseudoeosinophils, polymorphonuclear leukocytes, and macrophages leads to inflammatory affection and limits the invasive properties of microorganisms similarly as at salmonellosis.[24,28-30] At bacterial contamination of birds, pathogenic microorganisms were isolated from the intestinal contents –74.0%, the development of the infectious process was accompanied by the dissemination of bacteria into the lungs –29.0%, liver, and the cecum –50.0%.[31] Dissemination of the *Y. pseudotuberculosis* bacteria in the liver and spleen of mammals and birds leads to formation of granulomas with mild lymphocytic infiltration, as well as amorphous eosinophilic masses with the necrosis areal in the core surrounded by pseudoeosinophils with central area of necrosis.[6,13,15,16,32,33]

CONCLUSION

At the formation of *Y. pseudotuberculosis* bacteria biofilms, we observed the formation of extracellular matrix which covers the closed structures of various sizes, consisting of bacterial cells, with subsequent formation of clusters. The experimental toxemia of chickens generally revealed exudative-infiltrative processes, the proliferation of sensitized lymphocytes, macrophagal infiltration of the sinuses of the lymphoid organs, and increase in the number of T-lymphocytes. Violation of the porosity of the blood vessels of serous membranes of organs is accompanied by violation of hemocirculation, extensive serous edema, release of blood cells and loss of fibrinogen, development of dystrophic and necrotic processes in the Garder glands, Meckel's diverticulum, serofibrinous aerosacculitis, atelectasis and pulmonary edema, signs of enterosorption, and hepatorenal syndrome.

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