Aspects of Salmonellosis pathogenesis using chicken models

Lenchenko E.M., Vatnikov Yu.A., Kulikov E.V., Lozovoy D.A., Gavrilov V.A., Gnezdilova L.A., Zimina V.N., Kuznetsov V.I., Annikov V.V., Medvedev I.N., Petryaeva A.V., Glagoleva T.I.

ABSTRACT

Salmonellosis (also called “a disease of civilization”) is widespread across the globe, including the Russian Federation. Hence, measures to lower the morbidity of human beings, animals, and to limit propagation of infectious causative agents are needed. We studied colonization resistance and Salmonella typhimurium №5715 dissemination into chickens’ tissues and organs. The duration of the experiment was 30 days. We identified microorganisms to study colonization resistance of bowels and salmonella dissemination into chickens’ tissues and organs. We used the microscope “H604T Trinocular Unico” for morphological studies. We noticed acute and subacute course of salmonellosis, extensive vessel hemorrhages, cyanosis, hyperemia, numerous dotted and spotted hemorrhages in chickens’ tissues and organs. Pathological signs developed were accidental transformation of thymus, bursa of Fabricius’ atrophy, hyperplasia of the spleen, catarhal-hemorrhagic pericarditis, air sacculosis, enteritis, perihepatitis, stagnant hemorrhagic infarct of kidneys. Common symptoms of infection were observed, such as exudation and infiltration, increase of plasma clumps, macrophage reactions, perivascular edema, and disseminated thrombosis. The study confirms that salmonellosis is a dangerous disease that can affect many organs and requires etiologic and pathogenetic treatment.

Keywords: salmonellosis, diagnostics, etiological structure, epizootic situation, chickens.

INTRODUCTION

In the course of ontogenesis, a living being can develop various pathologies. Environmental factors contribute much to the development of noninfectious and infectious pathologies. It influences many important vital processes negatively as it weakens a living being and raises the risk of death. According to statistical data developed countries in recent years, more than 60.0% of diseases in humans and animals are caused by bacteria. Salmonellosis is one of the most widespread pathogenic bacteria, its prevalence reaches 35.0%.

Salmonellosis is a disease of civilization”) is widespread across the globe, including the Russian Federation. Hence, measures to lower the morbidity of human beings, animals, and to limit propagation of infectious causative agents are needed. Integration of countries into world economic areas and the increase of export potential call forth the need to harmonize legal base, provison of safety and quality level of import and export. Epizootic monitoring of infectious pathology should be done by applying analytical methods. Therefore, identification of epizootic strains is connected with the study of Salmonella pathogenesis into birds’ tissues and organs. Of great interest is the evaluation of the pathogenic potential of one of the most common strains Salmonella typhimurium №5715. It was supposed to reveal the features of the pathogenesis of its development in the body that could lay the foundations for the subsequent search for variants of pathogenetic correction.

The aim of this research is to study the pathogenic aspects of the infectious process at lowering colonization resistance and dissemination of Salmonellina into tissues and organs using chicken models.

MATERIALS AND METHODS

The study was conducted in strict accordance with the ethical principles established by the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes (adopted in Strasbourg on March, 18th, 1986, and confirmed in Strasbourg on June, 15th, 2006). Laboratory tests were performed by a specialist with 20 years of veterinary training. The study was conducted on the basis of the Department of Veterinary Medicine of the Russian University
of Peoples’ Friendship. Salmonella typhimurium №5715 was cultivated on Hottinger’s agar at 37°C for 24 hours. The bacterial suspension was made in 3-5 ml of isotonic dilution (0.9% NaCl) according to the optical standard of turbidity “McFarland, 0.5”. Chickens of the breed “Australorp blue” were used in this experiment age 5, 10 days.

The birds, with similar age, sex, live weight, and conditions of feeding and keeping, were subdivided into control and experimental groups. The control group was given 0.5 cm³ of 0.9% NaCl dilution intranasally, whereas the experimental group was infected intranasally with Salmonella typhimurium in the dose of 0.5 cm³ in the concentration of 1 billion/cm³. The experiment lasted for 30 days.

In order to study colonization resistance and salmonella dissemination into chickens’ tissues and organs, microorganisms were identified by morphological, tinctural, cultural, and enzymatic features.

Histological sections were imbued with haematoxylin and eosin for morphological studies.

Optical microscopy studies were conducted with casual selection method using field microscope “H604T Trinocular Unico” (USA). Upon Gram staining, salmonella was identified as a Gram-negative bacteria with length 0.5-1.4 mcm and width 0.2-0.4 mcm. It was determined as an aerobic bacteria, which broke up glucose by oxidation with acid without gas formation, with the help of Hugh-Leifson’s “+” oxidative-enzymatic test.

The results were processed by Student’s criterion (t). Statistical processing of received information was made with the help of a program package “Statistics for Windows v. 6.0”, “Microsoft Excel”. Differences in data were considered reliable in case of p<0.05.

RESULTS

The morbidity rate (ratio of the number of individuals with the disease to the number of infected ones) was 100.0%, while the lethality rate (ratio of the number of fallen individuals to the number of diseased ones) was 46.0%. Acute and subacute clinical courses were observed. All the 5-days chickens and one 10-days chicken had acute clinical course, whereas subacute clinical course occurred only in two 10-days chickens.

The acute clinical course was observed during 24-72 hours after infection. Clinical signs were characterized by central nervous system damage, such as convulsion, pareses, paralysis of extremities, and depression. Strained breathing with moist rails occurred at body temperature 43.4-43.8°C (Does this symptom occur in all chickens? If not, please specify). The most common clinical feature of subacute illness was common flaccidity 5-6 days after infection. Whitish-gray foamy mucus mixed with blood was observed in the nasal foramina and beak. The birds were sitting with wings down, the feathers disheveled, and body temperature was 43.1-43.3°C. Death was caused by increasing weakness, adynamy, disturbance of cordial activity, dehydration.

Salmonella dissemination was characterized by extensive hemorrhages of vessels, cyanosis, hyperemia, numerous dotted and spotted hemorrhages in chicken’s tissues and organs. Pathological processes developed in the models were exudative-infiltrative signs, plasma clumps, macrophage reactions, perivascular edema of tissues, and disseminated thrombosis in tissues and organs of the immune, cardiovascular, respiratory, digestive and excretory systems.

Immune System Changes

Pathological processes in the immune system observed were changes in the lymphoid tissue, macrophage reactions, hyperplasia and plasmocytic transformation of lymphocytes, and increased vascular permeability in the microcirculation. Accidental thymus transformation was also seen, characterized by hemocirculation disturbance and decrease of lobules. Cortical and cerebral substances borders of this thymus were not detected. Integrity damages in bursa of Fabricius were observed of cortico-medullary epithelial layer and atrophied lymphoid follicles, which contained cellular detritus. In the spleen, the red pulp sinuses were dilated; the cells of endothelium were in the state of proliferation, pulp destruction; perivascular edema, plasmatic impregnation of tissues, and periarterial lymphoid clumps were detected.

Cardiac Changes

Extensive serous edema and porosity disturbance in blood vessels of internal organs’ serous coats were seen. A yield of erythrocytes and prolapse of fibrinogen took place; symptoms of catarrhal-hemorrhagic pericarditis developed. Exudative pericarditis developed. The presence of massive necrosis of cardiac histiocytes, hemorrhages and stagnant hyperemia, lymphoid-cellular infiltration of areolar tissue, perivascular edema of tissues, disseminated thrombosis, proliferation of sensitized lymphocytes were observed.

Respiratory System Changes

Numerous bacterial embolisms in blood vessels of respiratory system’s mucous membranes and serocatarrhal pneumonia were detected. Air sacs
contained fibrinogenous exudates. As the infection progressed, cheesy exudates formed; fibrin sediments appeared on pleura; the tissue of lungs became brown with brownish bruises on incision. Symptoms of acute catarrhal or fibrinogenous inflammation, lobular pneumonia occurred. The lungs increased in size; affected sections were dark-red colored. Bloody-foamy exudates were observed on the surface of the incision. Viscous mucus discharge was seen in bronchial tubes. Signs of acute lobular emphysema were observed; capillaries and veins of the lungs’ interlobular fibrous areolar tissue were widened and overfilled by blood. Serous exudates, which filled alveoli, were seen as a homogeneous mass; they were also detected in the interstitial peribronchial and perivascular connective tissue. Numerous necrotic foci were found and were infiltrated by leucocytes. Lumens of bronchial tubes, bronchioli and alveoli were filled by transudate in which we detected epithelial cells, erythrocytes, lymphocytes, pseudoeosinophils (Figure 1).

Gastrointestinal Tract and Hepatic Changes
Dystrophic-necrotic processes and extensive hemorrhages were found along the gastrointestinal tract and in all digestive glands. Serous coats of organs were covered with fibrinogenous exudates. Signs of hemorrhagic enteritis and perihepatitis accompanied by porosity disturbance of blood vessels, yield of regular blood elements and generation of fibrinogen, development of dotted hemorrhages on serous and mucous membranes of organs occurred. Abundant fibrin deposit on the surface of liver, which looked as a jelly-like mass, was often observed, indicating the development of inflammation in the liver capsule. The organ’s pulp was of argillaceous color and had loose consistency when incised. The gallbladder was often stretched and filled with dark-green contents. Under the liver capsule, dotted hemorrhages were found; the organ was increased in volume, often regenerated, and had loose consistency.

Kidney Changes
Nuclei of the epithelial cells in kidneys decreased in size. Microabscesses and the presence of bacteria in the lumen of tubules, and in the parenchyma of cortical and cerebral layers kidneys were noticed. Stagnant hemorrhagic infarct and necrosis of tubules’ epithelium, inflammatory hyperemia of vessels, leucocyte infiltrations of necrotic sections were observed. Tubules and capsules of kidneys’ glomeruli were filled with leucocytes; the lumens were not detected resulted by the epithelial cells’ disintegration. Glomeruli and adhered capsules were increased; their vessels were overfilled with blood.

Colony-forming units/g of chickens’ bowels contents was 5.95±0.19 – 7.07±0.21 units/g. The ratio of enterobacteria quantity in bowels’ contents of sick chickens (experiment) to clinically healthy chickens (control) was determined; the index of colonization was 0.84%.

Isolated cultures of microorganisms were oxidase-positive and catalase-positive. These microorganisms reduced nitrates and nitrites, had proteolytic activity (liquefied gelatin and coagulated blood serum, hydrolyzed casein), and coagulated litmus milk. The test resulted negative for maltose fermentation, and indole and hydrogen sulfide formation.

DISCUSSION
It is known that pathogenic bacteria which cause clinically evident diseases of animals (including birds), are adapted to concrete species.19,20 The percentage of positive fecal test in birds which inhabit the territory near pig-breeding farms is 48.0-51.8%.17 The etiologies of salmonellosis in birds are S. typhimurium 11.7 – 23.4 %.21 Salmonella enterica subspecies enterica serovar Gallinarum biovar Gallinarum, which causes a systemic disease, leads to high lethality in birds. The absence of filaments activates the cellular immune response.22 Infiltration of the bowels’ mucous membrane by pseudoeosinophils, polymorphonuclear leucocytes and macrophages leads to inflammatory damages, which correlate with the changes
of hematological indices. It also limits microorganisms’ invasion. However, macrophage reaction isn’t expressed in bodies of susceptible species. Thus, the spread of infection worsens the condition of the macroorganism and increases the risk of its death.23

Salmonella and other enterobacteria’s virulence is mediated by the presence of adhesive and invasive features, resistance to phagocytosis, and production of thermolabile and thermostable toxins.24,25 Pathogenic strains are able to show their adhesion abilities due to fimbrial structures (saws) and the ability to form afibrylial adhesions with receptors on the surface of epithelial cells. As a result of such contact, the activation of the metabolism of bacterial cells takes place and conditions are created for their multiplication.24,26 Invasion of microorganisms occurs actively by binding with receptors of epithelial cells and passively through phagocytosis.19 Salmonella produces toxins which give rise to toxigenic features: thermostable and α-hemolysins which damage membranes; toxins which interact with membrane receptors and have catalytic activity; injections which penetrate into cell-target with activation in the cytoplasm of eukaryote.14,27

CONCLUSION
Distribution of salmonella in tissues and organs of the macroorganism in experimental salmonellosis is accompanied by acute and subacute flow. Accidental transformation of the thymus, atrophy of the Fabricia bursa, spleen hyperplasia, catarrhal-hemorrhagic pericarditis, aerosackitis, enteritis, perihepatitis, congestive hemorrhagic infarction of the kidneys develops. This process is characterized by dystrophic and necrotic phenomena, hemorrhages throughout the gastrointestinal tract and in the glands of external secretion. The serous membranes of the organs were often covered with fibrinous exudate. Characteristic development of signs of hemorrhagic enteritis, perihepatitis and violation of permeability of blood vessels. As the pathological process intensified, the epithelial cells of the kidneys experienced dystrophic changes, and in the cortical and cerebral layers, microabscesses appear. The conducted research is able to develop views on the pathological picture in the human body as a result of infection Salmonella typhimurium №5715. This information can help to find points of therapeutic effect for more effective pathogenetic therapy.

CONFLICT OF INTEREST
No Conflict of interest to declare.

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