Results of a study to determine the lethal doses (LD100 and LD50) of S. typhimurium in experiments on laying chickens

A.Kh. Khatamov¹, H.S. Salimov²
¹Student of PhD, ²Doctor of Veterinary Sciences, Professor of Sam.I.V.M

Abstract

In laboratory experiments carried out on 12 day old chickens, the 100% lethal dose (LD100) was established in the amount of 600 million microbial bodies - S. typhimurium. Thus LD50 (killing 50% of chickens) amounted to 400 million Salmonella. The duration of the incubation period in the hyperacute course of experimental salmonellosis of chickens (5 heads) was 6 to 10 hours. Clinical signs and pathological changes characteristic of this disease were not observed. They died 8-12 hours after infection. The incubation period in acute course lasted from 10 hours to 14 hours. In two sick chickens, clinical signs of the disease began to appear after 16 hours, in four more - after 20-24 hours after infection. As a result of bacteriological examination of pathological material (parenchymal organs, tubular bone) of chickens with salmonellosis, cultures of S. typhimurium with all cultural, morphological, biochemical and tinctorial properties were re-isolated.

Key words. Poultry farming, S. enteritides, S. typhimurium, Laying chickens, Toxicoinfection, LD100, LD50, Nutrient medium, Microbial bodies.

Introduction

Poultry farming is one of the most important branches of domestic animal husbandry, designed to meet the growing needs of the population in high-value and dietary products - poultry meat and eggs. In recent years, Uzbekistan has seen a stable development of the poultry industry, increasing the number of poultry. According to statistics, at the end of 2019, the number of poultry in the Republic reached 83.774 million heads, and the production of eggs was more than 8 billion, which is much more than in previous years. However, infectious diseases are a significant obstacle to the successful development of poultry farming, including salmonellosis, which is caused by S. enteritides and S. typhimurium.

Salmonellosis is widespread in all branches of industrial and non-industrial poultry farming. The greatest damage from illness and salmonella is observed in poultry farming of chickens, ducks, geese and blue-breeding. Outbreaks have become more frequent at breeding and keeping sites for pheasants, turkeys, quails and other birds.

Salmonellosis causes significant economic damage to the poultry industry, which consists of mortality, decreased meat productivity, egg production, and the cost of medical and preventive and recreational activities. In chickens, salmonellosis is acute, causing the death of up to 70-80% of young animals in some farms. Sick chickens and chickens that have recovered for a long time remain bacterial carriers and excretors of the pathogen. They serve not only as a source of the causative agent of infection for birds, animals, rodents, but often the cause of mass toxic infections in humans. Salmonella-seeded eggs and poultry meat are the main causes of foodborne toxic infections in humans. According to numerous scientists, toxic infections and salmonella etiology are common in almost all countries of the world, which is primarily due to the increase in the infection of domestic animals and birds with Salmonella (1-6).

All this predetermined the conduct of research in experiments on the laying chicken direction to determine the lethal doses of S. typhimurium.

Material and research methods. The pathogenic properties of the isolated bacteria Salmonella typhimurium were studied on 36 chickens of 12 days of age. They were divided into 6 groups of 6 heads each. Of these, the first 4 groups were experimental, and the 5th and 6th groups served as control. The first group of chickens was infected by introducing a bacterial suspension into the abdominal cavity at a dose of 800 million microbial bodies. The second, third and fourth groups of chickens were also infected with S. typhimurium in doses of 600, 400 and 200 million microbial bodies, respectively. The chickens of the 5th
group were injected with 0.5 ml of sterile saline into the abdominal cavity, and the chickens of the 6th group were not injected with anything; they also served as a control as the 5th group.

Generally accepted methods were used for clinical and pathological studies. In a clinical study, the general physiological state was determined: appetite, activity, condition of the outer integument, mucous membranes, body temperature, respiration and pulse.

The dead chickens from the experimental groups were subjected to pathological anatomical, and their internal organs were subjected to bacteriological studies. At the same time, inoculations were done on MPB, MPKA, Endo and Ploskarev media, 2-3 tubes from each sample of dead chickens and incubated in a thermostat at 370-380°C, pH 7, 4-7.5. Simultaneously with the inoculation of samples on nutrient media, smears-prints were made from each sample of the internal organs of the dead chickens and they were stained according to Gram or Romanovsky-Giemsa. The morphology of bacteria was studied under a microscope. To identify the re-isolated bacteria, Bergi (1980) bacterial determinant was used.

When studying the biochemical properties of the Salmonella culture in the inoculum, the formation of indole, hydrogen sulfide, gelatin dilution, urea breakdown, milk coagulation, growth on Simmons citrate medium, as well as reactions with methylroth were determined.

Research results. The scheme of the experiment on the study of lethal doses of S. typhimurium in laying chickens are presented in Table 1.

<table>
<thead>
<tr>
<th>Group names</th>
<th>Number of chicks</th>
<th>Doses of administration (in million.)</th>
<th>Exploration time (in hours)</th>
<th>Total</th>
<th>Exploration results</th>
<th>Exploratory results</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>24</td>
<td>48</td>
<td>72</td>
<td>96</td>
</tr>
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<td>Experienced</td>
<td>6</td>
<td>800</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>2</td>
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<tr>
<td>Experienced</td>
<td>6</td>
<td>600</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Experienced</td>
<td>6</td>
<td>400</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Experienced</td>
<td>6</td>
<td>200</td>
<td>1</td>
<td>5</td>
<td>-</td>
<td>5</td>
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<tr>
<td>Control</td>
<td>6</td>
<td>Phys/s.</td>
<td>-</td>
<td>6</td>
<td>-</td>
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<tr>
<td>Control</td>
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</tbody>
</table>

From the data in the table, it can be seen that in the first group of experimental chickens infected with S. typhimurium at a dose of 800 million microbial bodies into the abdominal cavity, 3 heads died a day after injection, after 2, 3, 4 days, one head remained alive. The same result was obtained in the second group of chickens infected with a dose of 600 million microbial bodies, but with the difference that, firstly, two heads died from salmonellosis a day later, and not three as in the first group, and secondly, after 2, 3, 4 and 5 days, one head. Consequently, in the second group, all infected chickens also died, but after 5 days, i.e. one day later. At the same time, in both groups, a 100 percent mortality was revealed (lethal doses of LD100), but since the second group consumed comparatively smaller doses of Salmonella, we consider that LD100 is equal to 600 million microbial cells.

In the third group of chickens infected at a dose of 400 million microbial bodies, two heads died one day after injection, two days later one head, after 3, 4 and 5 days and the next 15 days, 3 heads from this group remained alive, as well as control chickens. (5th 6th group) groups. Consequently, in our experiments it was found that when infected with Salmonella (S. typhimurium) at a dose of 400 million microbial bodies into the abdominal cavity, exactly 50% of the chickens fell ill and died. Thus, in our experiments, Salmonella in a dose of 400 million microbial cells kills 50% of the chickens, which means that this dose is a lethal dose - LD50. In the fourth group of chickens infected with a dose of 200 million microbial bodies one day after the introduction of a pala one head, three days later also one head, after 4 and 5 days and the next 15 days 4 heads from this group remained alive. only two heads out of six, accounting for 33.3%. The remaining alive
4 heads and control chickens were monitored for another 15 days.

The duration of the incubation period in the hyperacute course of experimental salmonellosis of chickens (5 heads) was 6 to 10 hours. Clinical signs and pathological changes characteristic of this disease were not observed in them. They died 8-12 hours after infection. The incubation period in the acute course lasted from 10 hours to 14 hours. In sick chickens, clinical signs of the disease began to appear 16 hours later in two, in two more after 20 hours, in three more, 24 hours after infection. At the same time, at the onset of the disease, the infected chickens developed an increase in temperature to 42.5-43.0°C, depression, drowsiness (photo 1) and intestinal upset. The stool was thin, slimy, often white, sometimes brownish-green with an admixture of blood. In almost all cases, the fluff around the anus stuck together with liquid stool, clogging the cloaca. In sick chickens, a decrease in appetite, ruffled plumage, and increased respiration were noted; they became lethargic, often squeaked, with lowered wings, with half-open eyes. The main and typical symptom in the acute course of salmonellosis in chickens is the presence of diarrhea. The feces are initially liquid, with gas bubbles, around the cloaca are almost always stained with liquid feces (photo 2).

In chickens that died from experimental acute salmonellosis, the main characteristic pathoanatomical changes in the small and large parts of the intestine were found. In the lumen of the small intestine, accumulations of mucus and gases were detected. Mucous membrane was swollen, hyperemic; in some areas, small punctate hemorrhages were noted.

Photo 1: The chicks come together and their wings are down.

Photo 2: Lethargy, drowsiness, disheveledness, diarrhea, manifested by contamination of the perimeter of the cloaca with feces.

In the large part of the intestine, the mucous membrane was covered with a plaque, in some places there were erosions. The accumulation of caseous masses in the cecum was often noted. In most cases, the spleen was enlarged, swollen; an increased blood filling of the pulp can be detected on the incision. The liver is enlarged, brownish-brown in color, with a greenish tinge. Under the capsule and in the thickness of the liver parenchyma, small foci of necrosis of a light gray and whitish color with a yellow tint are often found. Such necrotic nodules were found in the heart muscle and lungs. The mucous membrane of the gallbladder is also swollen and hyperemic.

Photo 3. Enlargement of the liver with foci of gray-white necrosis.
When making a diagnosis in their studies, the main emphasis was placed on the bacteriological method based on the isolation and identification of the pathogen, which, in our opinion, is the most reliable and basic in salmonellosis. Clinical and pathological changes characteristic of salmonellosis in our studies served for making a preliminary diagnosis. At the same time, studies were carried out to isolate pathogen, its cultivation on differential media, studied tinctorial properties, pathogenicity. Re-isolated Salmonella cultures were identified with monoreceptor diagnostic sera.

Fresh corpses of chickens served as pathological material for bacteriological research. Inoculations from the blood of the heart, liver, bile and bone marrow (more of the tibia and femur) of the corpses were carried out on MPB, MPA, Endo medium, which were incubated in a thermostat for 18-20 hours at a temperature of 36-38°C. Ovary, oviducts, testes, heart, and kidneys were also used as samples for diagnostic cultivation. Since in diseases caused by pathogenic salmonella colonization of the intestine occurs, intestinal tissue samples were taken with the contents for the isolation of salmonella. Differential diagnostic, elective (Endo and Levin), and selective (Ploskireva and bismuth-sulfite agar) media were also used for cultivation.

Identification of the isolated pathogen, which has a typical growth for Salmonella on Endo, Levin, Ploskirev and other differential media, was carried out using microscopy of smears stained according to Gram, and in a drop agglutination reaction on glass with monoreceptor agglutinating salmonella O- and H-sera. Serotyping of Salmonella is economical, gives comparable results across laboratories, and provides important epidemiological information.

Serotyping is carried out in accordance with the Kauffmann-White Salmonella antigenic formula. The pathogenicity of the isolated culture was confirmed by the degree of virulence by setting up a bioassay on laboratory animals (white mice, chickens).

As a result of bacteriological examination of pathological material (parenchymal organs, tubular bone) of chickens with salmonellosis, S. typhimurium cultures with all cultural-morphological, biochemical and immunological properties were re-isolated.

CONCLUSIONS.
1. In laboratory experiments conducted on 12 day old chickens, 100% - the nallet dose (LD100) was established in the amount of 600 million microbial bodies - S. typhimurium. Thus LD50 (killing 50% of chickens) amounted to 400 million Salmonella.
2. With the experimental infection of 12 day old chickens at a dose of 800 and 600 million Salmonella into the abdominal cavity, a 100% mortality was established, but when 600 million microbial cells are infected, the disease lasts longer, all chickens die slowly and later.
3. The duration of the incubation period in the hyperacute course of experimental salmonellosis of chickens (5 heads) was 6 to 10 hours. Clinical signs and pathological changes characteristic of this disease were not observed. They died 8-12 hours after infection.
4. The incubation period in acute course lasted from 10 hours to 14 hours. In two sick chickens, clinical signs of the disease began to appear after 16 hours, in four more - after 20-24 hours after infection.
5. In chickens that died from acute salmonellosis, the main characteristic pathological changes in the small and large parts of the intestine were established. At the same time, accumulations of mucus and gases are detected in the lumen of the small intestine. The mucous membrane of the small intestine is swollen, hyperemic, in some areas small punctate hemorrhages are noted. In the large part of the intestine, the mucous membrane was covered with plaque, in some places there were erosion in it.
6. As a result of bacteriological examination of pathological material (parenchymal organs, tubular bone) of chickens with salmonellosis, cultures of S. typhimurium with all cultural, morphological, biochemical and tinctorial properties were re-isolated.
References