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CLINICAL, PATHOMORPHOLOGICAL, AND BACTERIOLOGICAL DIFFERENTIAL DIAGNOSIS OF COLIBACILLOSIS AND SALMONELLOSIS IN SHEEP

Veterinary Scientific Research Institute Laboratory Head, DSc, proffessor in Veterinary Sciences – **B.A. Elmurodov,**

Senior Scientific Researcher, PhD in Veterinary Sciences – **I.Yu. Sultanova**

Laboratory Head, PhD in Veterinary Sciences Navruzov N.I., Senior researcher PhD in Veterinary Sciences **Murodov X.U.**

Senior researcher PhD in Veterinary Sciences **Sheraliyeva I.D.**

Aktamov Umidjon - Junior researcher at the Veterinary Scientific Research Institute

Abstract: This article discusses the simultaneous occurrence of colibacillosis and salmonellosis in sheep raised on sheep -breeding farms, their clinical signs, pathological and pathomorphological changes, and the results obtained during laboratory research. It also outlines rapid treatment and prevention measures for colibacillosis and salmonellosis in sheep. In addition, the differential diagnosis of the causative agent *E. coli* and *S. enteritidis* is presented.

Keywords: fibrinous necrotic foci, hemorrhagic bleeding, leukopenia, GNB (Gram-negative bacilli), GPC (Gram-positive cocci), Endo, Levine, bismuth sulfite, Salmonella-Shigella selective culture media, antibiotic sensitivity.

Relevance of the Topic: Medical and veterinary specialists recommend sheep meat for people suffering from allergies, hypertension, gastrointestinal

disorders, diabetes, gallbladder and liver diseases. This is due to the high biological value of sheep meat, its tenderness, and its low fat and cholesterol content, making it a suitable dietary product. Given these qualities, sheep meat can be widely consumed and used in various forms. Therefore, in sheep breeding farms and private livestock holdings, veterinary specialists must develop preventive measures to control the spread of infectious diseases in sheep. For this reason, studying the combined occurrence of salmonellosis and colibacillosis in sheep is currently one of the most relevant tasks. The co-occurrence of salmonellosis and colibacillosis is most commonly observed in young sheep from the age of 20 days up to 2.5 months. It primarily manifests as inflammation of the gastrointestinal tract (septicemia). Infected young sheep typically show signs such as yellowish-blue diarrhea, abdominal bloating due to gas accumulation in the intestines, weakness, refusal to eat, lethargy, sleepiness, and a rise in body temperature up to 40–41°C. If left untreated, the disease can lead to death within 3–7 days.

When salmonellosis and colibacillosis occur simultaneously in sheep, the main pathological changes are observed in the digestive organs, particularly the gastrointestinal system. In some cases, depending on the progression, the disease can also affect the excretory and respiratory organs. This combined disease is most frequently observed during the weaning period when young sheep transition from milk to solid feed.

The simultaneous spread of salmonellosis and colibacillosis in sheep remains a pressing issue due to the significant economic losses they cause to farms within the sheep breeding sector. Although the individual progression of salmonellosis and colibacillosis has been comprehensively studied by scientists, recent years have witnessed a growing concern

regarding the mixed occurrence of these diseases—not only in sheep but also in other animal species.

Level of Understanding of the Problem: Salmonellosis is an infectious disease that primarily affects sheep aged 10 days to 3 months, as well as pregnant females, with peak incidence typically occurring during the summer and autumn seasons. Clinically, affected animals display symptoms such as loss of appetite, lethargy, drowsiness, and diarrhea. Post-mortem examinations often reveal inflammation of the gastrointestinal tract, hemorrhages in the large intestines, fibrinous-serous gastritis, and splenomegaly with necrotic lesions [2,9,11]. Colibacillosis, on the other hand, is characterized by high mortality rates in sheep aged 5 to 9 weeks. Clinical manifestations include excessive water intake, fever, yellowish-gray diarrhea, rapid weight loss due to dehydration, emaciation, mucosal inflammation, and redness in the anal region [1,5,8]. Both salmonellosis and colibacillosis have been extensively studied as individual diseases in cattle, small ruminants, and fur-bearing animals, with effective vaccines developed for their prevention. Moreover, several studies have examined the mixed occurrence of colibacillosis and salmonellosis in large and small livestock, as well as poultry, leading to the creation of polyvalent vaccines [3,7]. The mixed form of colibacillosis and salmonellosis in sheep aged 20 days to 2.5 months—resulting in mortality rates as high as 65%—is currently one of the most urgent issues faced by sheep farms and private breeders in Uzbekistan [4,6,10].

Objective of the Research: With the rapid growth of sheep breeding in Uzbekistan in recent years, there is an increasing need for a comprehensive investigation into the infectious diseases affecting these animals—particularly

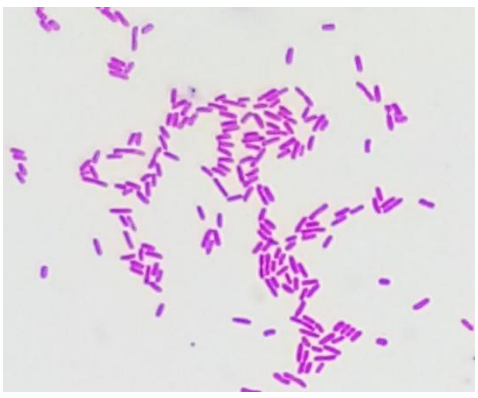
those with mixed etiologies. Special attention must be paid to their pathological and histological manifestations in order to develop effective diagnostic and preventive strategies. The aim of this study is to investigate the polyetiology of one of the mixed infectious diseases in sheep—salmonellosis and colibacillosis occurring simultaneously. The research focuses on treatment, prevention, pathological anatomy, and pathomorphology, as well as the development of modern methods for controlling these diseases. It is also considered essential to determine which specific species within the *Enterobacteriaceae* family—particularly *Escherichia coli* and *Salmonella*—are responsible for the disease when they occur jointly or separately in sheep.

Materials and Methods. The scientific research was conducted in laboratories specializing in the study of diseases in young animals and the pathomorphology of the National Institute for Infectious Diseases (NIIV). A total of 8 sheep, aged 1 to 2 months, were used in the study, employing clinical, pathological, and histological methods for analysis. Sheep in the experimental group (n=4) were inoculated with mixed cultures of *E. coli* and *S. enteritidis* at a dose of 0.5 (0.25) ml of culture suspension, corresponding to 1.5 billion (0.75 billion) microbial cells of the pathogens, which equates to the LD50. The second group of animals served as the control group and was not subjected to infection.

Results. The necropsy of 12 sheep, aged 1.5–2 months, brought from the farms revealed significant pathological changes primarily in the digestive system, with slight alterations observed in the respiratory system. In the stomach, undigested food was surrounded by a white, viscous mass. Upon examination, catarrhal inflammation of the gastric mucosa, pinpoint hemorrhages in the gastric sphincters, and rapid gas accumulation in the

intestines were observed. The feces were coated in a slimy mass. In the small intestine, the feces were foamy, yellowish-brown in color, with the presence of slimy fibrinous material. The odor of the feces was foul. Inflammatory changes in the intestinal mucosa were evident, with pinpoint hemorrhages and enlargement of the mesenteric lymph nodes, accompanied by hemorrhaging. Fibrinous nodules of varying sizes, resembling millet or rice grains, were found in the cecum. In the lungs, hemorrhagic congestion was present, with foamy exudate observed in the trachea, along with pinpoint hemorrhages. In other parenchymal organs, direct pathological changes were also noted. The heart had a yellowish exudate accumulation in the epicardium, with pericarditis and infarction in the left ventricle. The liver appeared enlarged, showing signs of dystrophic changes, with a softened consistency. Hemorrhagic foci were found in the kidneys, and the urinary bladder was filled with urine. The anal region showed signs of redness and inflammation. After the pathological examination of these sheep, tissue samples were inoculated onto a solid medium (GPB) and incubated at 37°C in a thermostat. The characteristics of the obtained cultures were analyzed. In the GPB medium, a white precipitate of 1 mm was observed at the bottom of the test tube, and uniform turbidity was noted. Upon mixing, the precipitate easily dissolved into the medium. A smear was prepared from the culture and stained using the Gram method. Under the microscope, gram-negative, rod-shaped bacteria of *Salmonella* (short rods, 0.5–2 µm) and *E. coli* (length 1–3 µm, width 0.5–0.7 µm) were identified (Figure 1). The culture was then re-inoculated onto GPB, and selective media including Endo and Blood Agar were used for further analysis. On Endo medium, pink colonies were formed. Re-inoculation onto GPB followed by plating on Blood Agar resulted in the formation of colonies with a hemolytic zone at the edges. After re-inoculation

onto GPB and isolating a pure culture under the microscope, the sample was transferred to Blood Agar via diffusion from GPB. The plate was incubated at 37°C for 40 minutes. Antibiotic discs were placed on the plate, with a 2 cm gap between them, and the plate was kept in the thermostat for 16 hours. The antibiotics tested included gentamicin (4%), cotrimazole (25), Vetillozin (50 mg), oxytetracycline (100), brovasol, oxiprol, ceftioclin, enrofloxacin (50), and ciprofloxacin (30). The results showed that enrofloxacin produced the largest inhibition zone with a diameter of 25 mm, followed by cotrimazole and oxytetracycline with diameters of 22 mm, gentamicin with 18 mm, brovasol, oxiprol, and Vetillozin with 16 mm, and ceftioclin with 12 mm (Figure 2). Given that *Salmonella* and *E. coli* mixed infections in sheep showed high sensitivity to enrofloxacin, this antibiotic was recommended for treatment. Additionally, it was advised to add 1g of Macropyrim per 10 kg to the feed. Furthermore, adherence to zoo-hygienic rules was recommended, such as washing drinking containers weekly with a 2% active chlorine solution, removing moldy and rotten food, maintaining the room temperature above 35°C, and monitoring the sheep' body temperature to ensure it remains between 38.5°C and 39.5°C.



Additionally, after studying the characteristics of the isolated pathogens, experimental infections were carried out in sheep under laboratory conditions. For this, the following procedures were applied: In Group 1, sheep were infected with 1.0 billion (LD100 lethal dose) microbial cells of *E. coli*, administered at a dose of 2,0 ml into the abdominal cavity. In Group 2, sheep were infected with 1.0 billion (LD100) microbial cells of *S. enteritidis*, administered at a dose of 2,0 ml into the abdominal cavity. In Group 3, sheep were infected with a combined dose of both pathogens, totaling 2.0 billion (LD100) microbial cells, administered at 2,0 ml into the abdominal cavity. Group 4 served as a control group, where sheep received 2,0 ml of physiological saline without any pathogens (Table 1).

Scheme of Infection with *S. enteritidis* and *E.coli* in the Experiment

Group	Number of Sheep	Type and Quantity of Pathogens (billion), LD (Lethal Dose)	Site of Infection and Dose (ml)
I-Experiment	4	*E. coli* 1.0 billion (LD100)	Abdominal cavity, 2,0 ml
II-Experiment	4	*S. enteritidis* 1.0 billion (LD100)	Abdominal cavity, 2,0 ml
III-Experiment	4	*E. coli* + *S. enteritidis* 2.0 billion (LD100)	Abdominal cavity, 2,0 ml
Control-IV	4	Physiological saline	Abdominal cavity, 2,0 ml

The infected sheep were monitored for one week and their body temperatures were measured. According to the results of the conducted

research, in Group I, sheep infected with *E. coli* showed a loss of appetite, bluish-yellow diarrhea, and an increase in body temperature, which reached +40.8°C ± 1°C. The heart rate was recorded at 27 beats per minute, and the respiratory rate increased to 25 breaths per minute. The animals died between the 5th and 8th days of the experiment (Table 2).**Table 2 Clinical Examination Results Before and After Infection with *E. coli* in Group I**

No	**Inv. No.**	**Before, After**	**Body Temperature (°C)**	**Heart Rate (beats/min)**	**Respiratory Rate (breaths/min)**
			Morning	Evening	Morning
1	00045	Before	39.5	39.6	25
		After	40.0	40.9	32
2	00034	Before	38.5	38.6	18
		After	40.1	40.8	35
3	00015	Before	39.2	39.3	20
		After	40.2	40.8	24
4	00012	Before	39.5	39.5	18
		After	40.1	40.7	27
Average	**Total Results**	40.1	40.8	40,0	25

In Group II (infected with *S. enteritidis***)**, death was observed within 3-5 days. In sheep infected with *Salmonella*, some showed signs of septic diarrhea, with body temperature rising to +41.5°C ± 1°C, heart rate increasing up to 28 beats per minute, and respiratory rate increasing up to 24 breaths per minute. The death occurred rapidly, within 3 days (Table 3).

Table 3

Clinical Examination Results Before and After Infection with *S. enteritidis* in Group II Sheep.

№	Inv. No.	Before and After Infection	Body Temperature (°C)	Heart Rate (beats/min)	Respiratory Rate (breaths/min)
			Morning	Evening	Morning
5	00065	Before	38.5	38.6	22
		After	41.2	41.2	18
6	00022	Before	38.2	38.8	24
		After	40.6	41.4	25
7	00044	Before	38.5	38.6	19
		After	41.5	41.6	24
8	00090	Before	39.0	39.3	28
		After	40.5	41.7	30

№	Inv. No.	Before and After Infection	Body Temperature (°C)	Heart Rate (beats/min)	Respiratory Rate (breaths/min)
Average		Before	38.5	38.8	28
		After	41.0	41.5	24

Under laboratory conditions, samples were taken from the parenchymal organs of all the sheep that died after being infected with the pathogens. Histological preparations were made by following all the steps in the process.

The pathological picture of the study of the corpses of dead sheep from the first group was characterized by myocardial infarction, inflammation of the pericardium, exudative and proliferative phenomena. In the spleen, follicular hyperplasia and splenitis were noted, and fibrinous-hemorrhagic changes and nodules at the entrance of the appendix, cecum, and colon were also observed. Dystrophic changes and necrotic lesions were detected in the liver, and inflammation was seen in the stomach, along with sphincter hemorrhages. At the same time, pieces of internal organs were taken from all dead animals, which were fixed in 12% formalin, processed histologically, and embedded in paraffin. The sections were then stained with hematoxylin-eosin and subjected to microscopy. A morphological study of sections from the parenchymal organs of sheep revealed serious changes. In the myocardium, increased fibrousness, edema, and hemorrhages were observed, along with hemostasis and hemosiderin microthrombi in the blood vessels, and deformation and lysis of myocytes. The venous vessels of the pulmonary tissue are full-blooded, and in the capillaries, processes such as hemostasis,

aggregation and agglutination of erythrocytes, alternative dystrophic and necrotic changes, necrosis, and desquamation of alveocytes are observed. Degeneration of vacuoles, desquamation of alveolar macrophages, and the presence of hemosiderin grains in their cytoplasm are also noted. Atelectasis is observed in most alveoli. In some parts of the histological section, emphysematous expansions are observed, in the lumen of which there are cellular detritus, lymphocytes, macrophages, hemosiderin grains, and plasma. Microscopy of histosections of the kidneys revealed that in the renal glomeruli, there is infiltration of lymphohistiocytic cells, filling of the lumens with serous exudate, necrobiosis, and necrosis of cells in some parts of the convoluted tubules, as well as infiltration of individual tubules with lymphocytes, fibroblasts, and fibrocytes. The Shumlyansky-Bowman capsules are edematous, and the blood vessels are full of blood. The collecting ducts of the medulla of the kidneys are infiltrated, and the interstitial connective tissue is edematous, indicating tubulointerstitial nephritis.

General lymphohistiocytic infiltration and hemosiderosis of the parenchyma, rhexis, and pyknosis of hepatocyte nuclei were observed during the study of histosections of the liver. At the same time, in some areas, swelling and infiltration of the walls of blood vessels were also observed. On the mucosa of the small and large intestines, desquamation of epithelial cells and fibrinous necrosis are noted. In the lumen of the cecum, there is an accumulation of desquamated lymphocytes perienterocytically. Intestinal enterocytes are hypertrophied, with multiple lymphocytes located between them (Figures 8-10).

Additionally, it was found that the villous cells are subject to necrosis and hydropic hyaline-droplet degeneration. Histological examination of sections

from the spleen revealed one of the types of protein dystrophy – hyalinosis. In this case, the protein fractions of hyaline are densely located in the form of translucent cartilage-like substances along the walls of blood vessels. Desquamation and necrotic changes developed in the lymphoid follicles. During pathological studies of animals in the second control group, changes in the internal organs were not detected. After histopathological examinations, the samples were stored in the museum, and all the sheep in the farm underwent treatment and preventive measures.

Conclusion: The incidence of colibacillosis and salmonellosis in sheep during the transition period from milk to solid food results in mortality rates reaching up to 65%.

2. Colibacillosis and salmonellosis are observed in sheep from 20 days old to 2.5 months old.

3. It was found that the antibiotic enrofloxacin is highly effective in treating mixed infections of colibacillosis and salmonellosis.

4. During the pathological anatomical changes in mixed infections of colibacillosis and salmonellosis, the formation of fibrin clots of the size of the jejunum and large intestine, as well as hemorrhagic, proliferative, exudative, and indurative changes in the gastrointestinal tract, indicate a strong systemic intoxication caused by mixed bacterial infections.

In general, the simultaneous mixed course of colibacillosis and salmonellosis in sheep infected with pathogens of these diseases, in contrast to the disease with these infections separately, is very different. In this case, the mixed infection is manifested by deeper and broader irreversible

pathological and morphological changes, which are most pronounced in the respiratory and digestive systems.

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