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EFFICACY OF THE ETIS-2 COMPLEX PREPARATION IN THE TREATMENT OF SALMONELLOSIS PATHOGEN DISEASE.

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Summary
The article presents the results of testing the antibacterial efficacy of the drug ETIS-2 against the pathogen *Salmonella Enteritidis* in the body of 9 sheep.

Keywords: ETIS-2 complex preparation, antibiotics, panacea, toxicity, therapy, vaccination, strain, microorganism, GPA, serovar, LD50, anemia, salmonellosis, shiga toxin.

Relevance of the Topic.

Currently, salmonellosis is a significant issue not only in our country but also worldwide in the fields of veterinary medicine and human health. Often, the pathogens *Salmonella Enteritidis, Salmonella Typhimurium,* and *Salmonella Dublin* are found among agricultural animals, causing harm to livestock. These very *Salmonella* serovars are known to cause foodborne epidemics in both animals and humans [1].

The study of the effectiveness of various antibiotics in relation to *Salmonella* pathogens and the identification of the effectiveness of the ETIS-2 complex preparation in treating several types of bacterial diseases, including salmonellosis, is essential.

As an excipient and adjuvant, vitamin-rich plant oil (Trivit or Tetravit) is used in the preparation. Considering the effect of the medicinal components of the complex preparation on various microorganisms, the efficacy of the preparation in treating various infectious and non-infectious diseases has been studied in laboratory and production conditions. This method of treatment for bacterial etiology diseases in livestock is being applied in the republic's livestock farms [2].

The ETIS-2 complex preparation was developed by scientists at the Veterinary Research Institute's Tuberculosis Laboratory. The combination of components in the "ETIS-2" complex preparation creates an advantage over other bacteriostatics. Such a combination produces a synergistic (enhancement of one drug's effect by another) and prolongation (extension of the drug's duration of action) effect. The preparation can be used on all types of animals, starting from 10 days old, regardless of their physiological condition [2].

Currently, salmonellosis is a significant issue not only in our country but also worldwide in the fields of veterinary medicine and human health. Often, the pathogens *Salmonella Enteritidis, Salmonella Typhimurium,* and *Salmonella Dublin* are found among agricultural animals, causing harm to livestock. These very *Salmonella* serovars are known to cause foodborne epidemics in both animals and humans.

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Sulfonamide antibiotics of various groups are used for the prevention and treatment of salmonellosis. The study of the sensitivity of different *Salmonella* species, as well as other bacterial types, to various antibiotics and ETIS-2 preparations in aqueous and oily concentrated solutions is essential.

In our country's livestock farms, several infectious diseases are frequently recorded among cattle. One of these diseases is salmonellosis in newborn animals, which occupies a significant place among calves [3].

The study of the etiology of salmonellosis began with Bollinger's observations in 1876, focusing on the following:

The relationship between septicopyemic diseases in domestic animals and the occurrence of the disease in humans;

Those who consumed their meat [6].

In 1885, American veterinary doctors Salmon and Smith reported the isolation of the pathogen from the organs of diseased pigs. The disease later took the name *B. cholerae suis* [8]. A short time later, in 1888, during a gastroenteritis epidemic in Thuringia, German scientist Gertner isolated the same microbe from beef and found that it was the cause of death in a person who had eaten the meat. The isolated microbe was named *B. enteritidis* [8].

In 1890, Leffler in Breslau determined that ***B. typhimurium*** was the cause of mass mortality in rats. Other researchers, including Leffler himself, later identified ***B. typhimurium*** as the cause of gastroenteritis in humans with acute clinical symptoms.

In 1898, French scientist Layner suggested naming this group of pathogens *Salmonella* and the diseases they cause in honor of the veterinarian Salmon, who discovered them [6, 8].

The pathogen of salmonellosis is currently classified under the *Enterobacteriaceae* family and the *Salmonella* genus. It contains over 2,200 serotypes. The *Salmonella* genus includes only one species, which is divided into seven small species, distinguishable by DNA hybridization or biochemical characteristics [9].

Each small species is divided into serovars based on the specificity of the O and H antigens of the strains. The O-antigen is somatic, thermostable, and associated with the body of the microbial cell. The H-antigen is thermolabile and associated with the flagella. Based on the common somatic O-antigen, *Salmonella* is divided into 5 main groups:

The groups are designated with uppercase Latin letters: A, B, C, D, and E. Within each group, *Salmonella* strains are distinguished by the H-antigen. Additionally, *Salmonella* contains a surface capsule K-antigen. Some *Salmonella* serotypes also have other antigens, such as the Vi-antigen or "virulence" antigen (one of the components of the O-antigen) and the M-antigen (mucosal).

Morphologically, all bacteria of the *Salmonella* genus are similar in appearance. They are rod-shaped, with a length of 2 µm and a width of 0.5 µm.

They are 4 µm in length and 0.5 µm in width. They have flagella. They stain well with aniline dyes and are gram-negative. *Salmonella* grows well on simple nutrient media at a temperature of 37°C and a pH of 7,2–7,4. They can survive in the external environment for a long time: up to 2 weeks in water, up to 4 months in meat and sausage, up to 6 months in frozen meat, up to 20 days in milk, and up to 1 year in cheese. In food products, they not only remain viable but also proliferate. They are highly heat-resistant, so they will only die after 2.5 hours of boiling 400g of meat.

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At low temperatures, *Salmonella* can maintain its viability for over 100 days, and freezing enhances the survival time of microorganisms in products. When *Salmonella* dies, endotoxins are released. Most *Salmonella* species are pathogenic to both humans and animals, but the most significant pathogens in humans are *S.enteritidis*, *S.typhimurium*, *S.panama*, *S.infantis*, *S.mission*, *S.newport*, *S.derby*, and *S.london*. In the late 20th century, *S.typhimurium* was the leading cause of salmonellosis, but now *S.enteritidis* accounts for 76-78% of salmonellosis cases in humans [4, 5].

Epidemiology Salmonellosis is found in all regions of the world. It is one of the most common zoonoses in developed countries. The main reservoirs of *Salmonella* in nature and the source of infection for humans are numerous domestic and wild animals, especially birds (particularly waterfowl). They play a significant role as sources of infection.

Gastrointestinal forms of salmonellosis should be differentiated from foodborne toxic infections, escherichiosis, the gastroenterocolitic form of shigellosis, and mushroom poisoning [6, 8, 9, 10].

Materials and Research Methods

The scientific research on the effect of the ETIS-2 preparation against salmonellosis was conducted in the Microbiology and "Study of Young Cattle Diseases" laboratories' vivarium on 6 lambs. For this, a one-day culture of *Salmonella Enteritidis* strain grown on meat peptone agar (GPA) was prepared into a suspension using a physiological solution. The pathogenicity of the *Salmonella Enteritidis* strain obtained from the Microorganism Collection was tested in the Tuberculosis Laboratory on 9 pigs. For this, the McFarland Standard International unit, developed in France, was used for comparison. The suspension was diluted to an LD_{50} $BaSO_4$ ratio of $1.92*10^{-4}$ mol/L and was inoculated into 6 lambs to determine the pathogen's pathogenicity (Table 1).

In this experiment, 3 lambs in Group 1 were injected with 2 ml of *Salmonella Enteritidis* strain at a dose of LD_{50} $2.5x10^9$ CFU (LD_{50}) into their abdominal cavity. Similarly, 3 lambs in Group 2, as a control group, were also injected with 2 ml of *Salmonella Enteritidis* strain at a dose of LD_{50} $2.5x10^9$ CFU (LD50) into their abdominal cavity (Table 1).

Before and after inoculation, the general condition, physiological and clinical status (body temperature, heart rate, and respiration rate), as well as hematological parameters of the experimental sheep were monitored.

The purpose of our research is to study the effect of the ETIS-2 complex preparation on *Salmonella Enteritidis* pathogens, compare the effectiveness of the preparation in treating the disease, and analyze the hematological changes in the treated sheep.

For the treatment of the sheep in Group 1 (infected with salmonellosis), after the clinical signs of the disease appeared following inoculation, the ETIS-2 complex preparation was administered subcutaneously at a dose of 5.0 ml per 100 kg of body weight. The treatment consisted of administering 3 ml per sheep subcutaneously once a day for 7 days. No preparation was administered to the control group (Table 2).

After the infection, blood samples were taken from the jugular veins of the sheep in the experimental, control, and intact groups at 3, 6, and 9 days. Hematological parameters were measured using the photometric method. For this, 0.2 ml of blood from the experimental, control, and intact group sheep were placed in test tubes and analyzed using the MINDRAY

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BC-20, MINDRAY BA-88A, and MINDRAY BS 30s hematological analyzers (manufactured in China).

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Experiment to Determine the Effect of ETIS-2 Preparation Against Salmonellosis

1-table

Name of groups	Number of Sheep	Strain Name	Infection		Treatment				Result
			dose	method	drug	dose	method	duration	
Experiment I	3	*Salmonella Enteritidis*	2.5x 10^9 CFU	Abdominal cavity	ETIS-2	3 ml/sheep	Subcutaneous injection (left neck, chest, right neck)	7 days	Alive and recovered
Control II	3	*Salmonella Enteritidis*	2.5x 10^9 CFU	Abdominal cavity	0	0	0	0	Alive and sick

Results of an experiment to determine the effect of the drug ETIS-2 against salmonellosis in sheep

2-table

№	Name of the pathogen	Infection method and dose	Method	Number-ber of sheep	Dose of ETIS-2, 100 kg/ 5 ml	Number of ETIS-2 injections, subcutaneous					Result
						Day 1	Day 2	Day 3	Day 4	Day 5	
1	*Salmonella Enteritidis (experimental group)*	LD_{50}2,5x1 0^9 KXB	Abdomen	3	2-3 ml	I	II	III	IV	V	100% Treated
2	*Salmonella Enteritidis (control group)*	LD_{50}2,5x1 0^9 KXB	Abdomen	3	-	control					Sickened
3	***Intact control (healthy) group***	-	-	-	-	-					healthy

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The aim of this research was to investigate the effect of the ETIS-2 complex preparation on *Salmonella Enteritidis* pathogens, compare its efficacy in treating the disease, and analyze the hematological changes in the treated sheep.

In the first experimental group (sheep infected with salmonellosis), 36 hours after infection, initial clinical symptoms of the disease were observed: an increase in body temperature (+40,3°C), lethargy, and decreased appetite. In the following days, these symptoms worsened, and by the 3rd-4th day of the experiment, two sheep in this group showed signs of diarrhea.

After the appearance of clinical signs of the disease in the experimental group, the sheep were treated with the ETIS-2 complex preparation, administering 3 ml per sheep once a day subcutaneously for 7 days.

Research Results

In the conducted research, initial clinical signs of the disease were observed in the sheep of the first experimental group and the second control group 36 hours after infection. In the experimental animals, symptoms such as an increase in body temperature (+41,6°C), lethargy, and loss of appetite were identified. In the following days, these clinical signs intensified, and by the 3rd-4th day of the experiment, diarrhea was observed in the sheep.

After the appearance of clinical signs in the experimental sheep, treatment was initiated with the ETIS-2 complex preparation, administering 3 ml per sheep subcutaneously once a day for 7 days (see Table 2). On the 3rd day of treatment, the body temperature of the sick sheep returned to normal levels (+38,4°C), appetite was restored, and diarrhea ceased. During the 6th-7th days of treatment and in the subsequent period, no further signs of the disease were observed in the experimental sheep.

In the control group, 36 hours after infection, the initial signs of the disease appeared. Clinical signs such as an increase in body temperature (+40,9°C), accelerated heart rate, difficulty in breathing, general lethargy, decreased rumination, and loss of appetite were observed. In the following days, these signs intensified, and by the 3rd-4th day of the experiment, two sheep in the group experienced diarrhea and blood clots in the feces. The Salmonella Enteritidis strain demonstrated typical virulence characteristics in the control group sheep. The typical clinical signs of salmonellosis appeared fully in these animals.

In the III intact group, no disease signs were observed, and these sheep were not treated with the ETIS-2 preparation (see Table 2).

According to hematological investigations, in the first experimental group of animals, after infection with Salmonella and the appearance of clinical signs, the hemoglobin level was 6,4±0,24 x10 g/L, erythrocyte count was 7,21±0,34 $x10^{12}$ g/L, leukocyte count was 15,9±0,70 $x10^{9}$/L, and lymphocyte count was 81,4±3,99% $x10^{9}$/L. The results showed a decrease in hemoglobin and

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Table 3

Hematological Test Results of Blood Samples from Sheep Infected with Salmonella:

№	Group Name and Inventory Numbers	Hemoglobin (x10 g/L)			Erythrocytes ($x10^{12}$ g/L)			Leukocytes ($x10^{9}$/L)			Lymphocytes (% $x10^{9}$/L)			Hematocrit ($x10^{-2}$ L/L)		
Physiological norm		9-15			9-15			4,0-12,0			40-75 2x9			27-45		
Group I (Experimental Group)		***3rd Day***	***6th Day***	***9th Day***	***3rd Day***	***6th Day***	***9th Day***	***3rd Day***	***6th Day***	***9th Day***	***3rd Day***	***6th Day***	***9th Day***	***3rd Day***	***6th Day***	***9th Day***
1	Right ear painted	6,9±0,24	8,6±0,46	9,6±0,28	7,31±0,31	9,87±0,35	9,82±0,33	15,2±0,73	13,6±0,45	12,1±0,47	81,3±3,82	75,9±3,42	70,4±3,73	22,1±0,62	26,7±1,31	29,2±1,72
2	Left ear painted	6,6±0,18	8,7±0,41	9,1±0,17	7,14±0,34	9,62±0,16	9,76±0,51	16,5±0,56	12,8±0,73	11,4±0,33	80,5±3,46	74,8±2,02	68,3±1,50	23,4±0,87	27,2±0,79	30,1±1,11
3	Forehead painted	6,4±0,27	9,5±0,31	9,9±0,23	7,16±0,30	9,84±0,53	9,88±0,21	15,9±0,57	13,1±0,56	12,3±0,65	82,4±2,64	74,7±3,29	69,5±3,54	21,9±1,01	28,1±1,04	29,4±1,26
M±m		**6,4±0,24**	**8,9±0,41**	**9,5±0,28**	**7,21±0,34**	**9,78±0,36**	**9,82±0,41**	**15,9±0,70**	**13,2±0,58**	**11,9±0,47**	**81,4±3,99**	**75,1±3,53**	**69,4±2,22**	**22,5±1,06**	**27,3±1,07**	**29,6±1,24**
II - Control Group		***1 st Day***	***3rd Day***	***9th Day***	***1 st Day***	***3rd Day***	***9th Day***	***1 st Day***	***3rd Day***	***9th Day***	***1 st Day***	***3rd Day***	***9th Day***	***1 st Day***	***3rd Day***	***9th Day***
1	Right side of the neck painted	6,8±0,26	6,5±0,28	6,4±0,19	7,81±0,34	6,51±0,23	6,24±0,21	14,8±0,71	15,4±0,51	16,7±0,65	83,9±3,91	82,9±3,73	86,1±4,56	25,4±0,71	22,3±1,09	21,8±1,29
2	Left side of the	6,9±0,19	6,8±0,32	6,9±0,13	7,43±0,33	6,78±0,12	6,46±0,34	15,2±0,52	15,9±0,91	17,5±0,51	81,5±3,50	86,7±2,34	88,2±1,94	23,2±0,86	21,5±0,62	20,2±0,75

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	neck painted															
3	Chest painted	6,7±0,28	6,4±0,21	6,3±0,14	7,65±0,32	6,74±0,36	6,88±0,14	15,8±0,57	16,2±0,70	16,9±0,90	84,5±2,70	86,1±3,79	86,9±4,43	22,8±1,05	20,7±0,77	19,9±0,86
M±m		**6,8±0,22**	**6,6±0,21**	**6,5±0,32**	**7,63±0,28**	**6,68±0,13**	**6,53±0,30**	**15,3±0,69**	**15,8±0,59**	**17,1±0,65**	**83,1±3,74**	**85,2±4,01**	**87,1±3,66**	**23,8±0,83**	**21,5±0,82**	**20,6±0,95**
III - Intact Group		***3rd Day***	***6th Day***	***9th Day***	***3rd Day***	***6th Day***	***9th Day***	***3rd Day***	***6th Day***	***9th Day***	***3rd Day***	***6th Day***	***9th Day***	***3rd Day***	***6th Day***	***9th Day***
1	Right front leg painted	9,7±0,37	9,5±0,41	9,8±0,28	9,73±0,46	9,52±0,33	9,41±0,32	10,4±0,50	10,9±0,36	11,2±0,43	61,8±2,90	62,2±2,80	60,7±3,22	32,5±0,91	31,2±1,53	31,8±1,88
2	Left front leg painted	9,3±0,225	9,1±0,43	9,2±0,17	9,61±0,42	9,59±0,16	9,63±0,50	11,5±0,39	10,7±0,61	11,3±0,33	59,7±2,57	59,6±1,61	57,5±1,27	31,4±1,16	32,6±0,95	32,9±1,22
3	Left abdomen painted	9,4±0,39	9,2±0,30	9,3±0,21	9,58±0,40	9,37±0,51	9,42±0,20	10,9±0,39	10,5±0,45	11,4±0,60	61,8±1,98	58,5±2,57	59,8±3,05	29,8±1,37	31,3±1,16	30,7±1,32
M±m		**9,5±0,41**	**9,3±0,38**	**9,4±0,46**	**9,64±0,45**	**9,49±0,35**	**9,49±0,37**	**10,9±0,48**	**10,7±0,57**	**11,3±0,44**	**61,1±2,87**	**60,1±2,22**	**59,3±2,49**	**31,2±1,19**	**31,7±1,24**	**31,8±1,24**

erythrocytes, and an increase in leukocytes and lymphocytes. The hematocrit level decreased to 22,5±1,06 x10^{-2}L/L on the 3rd day after infection. The sheep in the experimental group developed anemia.

According to hematological investigations, in the first experimental group of animals, after infection with Salmonella and the appearance of clinical signs, the hemoglobin level was 6,4±0,24 x10 g/L, erythrocyte count was 7,21±0,34 x10^{12} g/L, leukocyte count was 15,9±0,70 x10^{9}/L, and lymphocyte count was 81,4±3,99% x10^{9}/L. The results showed a decrease in hemoglobin and erythrocytes, and an increase in leukocytes and lymphocytes. The hematocrit level decreased to 22,5±1,06 x10^{-2}L/L on the 3rd day

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After infection. The sheep in the experimental group developed anemia.

According to the hematological results (see Table 3), on the 9th day of the experiment, the hemoglobin level in the experimental group was 9,5±0,28 x10 g/L, erythrocytes were 9,82±0,41 $x10^{12}$ g/L, leukocytes were 11,9±0,47 $x10^{9}$/L, and lymphocytes increased to 69,4±2,22% $x10^{9}$/L. The hematocrit level was 29,6±1,24 $x10^{-2}$L/L on the 9th day. These results indicate that anemia persisted in the experimental sheep after infection for 3 days.

In the second control group, after infection with Salmonella and the appearance of clinical signs, on the 3rd day, the hemoglobin level was 6,8±0,22 x10 g/L, erythrocytes were 7,63±0,28 $x10^{12}$ g/L, leukocytes were 15,3±0,69 $x10^{9}$/L (decreased), and the lymphocyte count was 83,1±3,74% $x10^{9}$/L. The hematocrit level decreased to 23,8±0,83 $x10^{-2}$L/L on the 3rd day of infection. The results indicate that anemia developed in the control sheep.

In the III intact group, on the 3rd day of the experiment, the hemoglobin level was 9,5 x10 g/L, erythrocytes were 9,64±0,45 $x10^{12}$ g/L, leukocytes were 10,9±0,48 $x10^{9}$/L, lymphocytes were 61,1±2,87% $x10^{9}$/L, and hematocrit was 31,2±1,19 $x10^{-2}$L/L. During the experiment, no significant changes were observed in their levels. On the 9th day of the experiment, the sheep in the third intact group had the following values: hemoglobin level of 9,5±0,46 x10g/L, red blood cells (erythrocytes) at 9,49±0,37 $x10^{12}$g/L, white blood cells (leukocytes) at 10,6±0,40 $x10^{9}$/L, lymphocytes at 59,1±2,48 %$x10^{9}$/L, and hematocrit at 30,2±1,39 $x10^{-2}$L/L. No significant changes in these values were observed throughout the experiment.

Therefore, based on the results of the study, in sheep treated with the ETIS-2 complex, after being infected with Salmonella pathogens, clinical signs of the disease started to appear. The results showed a decrease in hemoglobin, erythrocytes, and hematocrit levels, while lymphocyte and leukocyte counts increased. This suggests that the disease had an impact on the hematological parameters of the sheep, but no significant changes were observed in the intact group, which did not receive treatment.

These indicators revealed signs of anemia in the sheep's bodies. The ETIS-2 complex preparation demonstrated a high therapeutic effect in the treatment of sheep salmonellosis. After being infected with the disease-causing agent and showing clinical signs, the sheep treated with ETIS-2 fully recovered, with a 100% recovery rate.

In the hematological analysis of sheep treated with ETIS-2, after being infected with Salmonella pathogens and showing clinical signs of the disease, a decrease in hemoglobin, erythrocytes, and leukocytes, an increase in lymphocytes, and a reduction in hematocrit were observed. These indicators confirmed the presence of anemia in the sheep's bodies.

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Thus, it can be concluded that when ETIS-2 is used to treat salmonellosis, there is no need for the use of other types of antibiotics or treatments.

After the sheep were infected with the Salmonella Enteritidis strain and the disease fully manifested, the treatment with ETIS-2 (3 ml per sheep, administered subcutaneously once a day for 7 days) resulted in the normalization of body temperature on the 3rd day of treatment (+38,4°C), restoration of appetite, and cessation of diarrhea. No further signs of illness were observed in the sheep on days 6-7 of the treatment and in the subsequent period.

Blood analysis in animals is a crucial diagnostic tool. The blood-producing organs are highly sensitive to various physiological, especially pathological, factors, and their effects are clearly reflected in blood parameters. Examining hematological parameters with laboratory instruments reflects the health status of the animal, as well as the impact of various pathologies or treatment procedures.

Conclusions:

1. **Hematological Findings:** In the sheep treated with the ETIS-2 complex preparation, after being infected with the Salmonella pathogens, clinical signs of the disease appeared. Hemoglobin, erythrocytes, leukocytes, and lymphocytes increased, while hematocrit decreased. These indicators suggest the presence of anemia in the sheep's bodies.
2. **Hematological Changes Over Time:** According to hematological tests, on the 9th day of the experiment, the sheep in the experimental group showed an increase in hemoglobin (9.5±0.28 x10g/L), erythrocytes (9,82±0,41 $x10^{12}$g/L), leukocytes (11,9±0,47 $x10^{9}$/L), and lymphocytes (69,4±2,22% $x10^{9}$/L), while the hematocrit value decreased to 29,6±1,24 $x10^{-2}$L/L. The results indicate that anemia was observed within 3 days after infection.
3. **Treatment Effectiveness:** After the experimental sheep were infected with the Salmonella Enteritidis strain, the disease showed complete clinical signs. Following the administration of ETIS-2 complex preparation (3 ml per sheep subcutaneously, once a day for 7 days), by the 3rd day of treatment, the sheep's body temperature normalized (+38,4°C), appetite was restored, and diarrhea ceased. No further signs of illness were observed in the sheep on days 6-7 of the treatment and in the subsequent period.
4. **Therapeutic Implication:** The use of the ETIS-2 complex preparation in the treatment of salmonellosis eliminates the need for the use of other antibiotics or treatment methods.

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