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EFFICACY OF ETIS-2 COMPLEX PREPARATION IN THE TREATMENT OF DISEASES CAUSED BY COLIBACTERIOSIS PATHOGENS

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Annotation
The article presents the results of a study conducted on nine sheep to test the antibacterial effect of the ETIS-2 complex preparation against Escherichia coli.

Keywords: ETIS-2 complex preparation, antibiotics, panacea, toxic, therapy, vaccination, microorganism, GPA, LD50, anemia, colibacillosis, Shiga toxin, enterohemorrhagic E. coli.

Relevance of the Topic:
Throughout history, infections have been one of the main causes of death for both humans and animals. Recently, over half a century ago, infectious diseases often played a dominant role in determining the fate of patients. As science has advanced globally, the battle against infections continues to evolve. Numerous studies dedicated to this problem began in the 19th century. However, medicines created later, based on substances like mercury, arsenic, silver, and similar compounds, were known for their high toxicity and low effectiveness. The first substances to have a harmful effect on microorganisms but remain safe for humans were discovered in the 1930s at the Pasteur Institute in France among synthetic dye derivatives. These substances, called “sulfonamides” (or “chemical drugs”), led to the emergence of a treatment process known as chemotherapy.
The issue of combating infectious diseases took a significant turn in 1929 when the English microbiologist A. Fleming discovered the first antibiotic, penicillin. This discovery, which was accidental yet one of the most remarkable of the 20th century,

marked the beginning of a new era in medicine — the era of antibiotics. Humanity learned to harness the phenomenon of bacterial antibiosis (antagonism), where, in the fight against bacteria, living organisms, like bacteria themselves, may act against each other. In this battle, the primary weapon is enzymes produced by certain bacterial species that have harmful effects on other species.

With this great discovery, a significant step was taken in medicine and veterinary practice to save the lives of millions of people. It seemed as if humanity had found a panacea that would forever rid the world of infectious diseases. Every year, new classes of antibiotics emerged, and by the 1970s, there was a sense that all major infectious diseases had already been conquered. Furthermore, in 1969, the president of the International Association of Surgeons, William Stewart, stated: “Given the achievements of antibiotic therapy and vaccination programs, it may soon be possible to close the book on infectious diseases”.

However, it soon became clear that celebrating victory over pathogens was premature, and the idea of human superiority over nature was an illusion. The question of who was in control — us over microbes or microbes over us — became increasingly contentious. In 1940, a strain of Escherichia coli was already discovered by E. Abraham and E. Chain, which was not sensitive to penicillin [1]. Moreover, by the late 1960s and early 1970s, just 20-25 years after the widespread use of antibiotics began, the "staphylococcal plague" emerged. Microorganisms, particularly staphylococci, adapted to the widely used penicillin, mutated, and strengthened their resistance. (© Yulish E. I., 2014 © "bola salomatligi", 2014 © Zaslavskiy A. yu., 2014)

The preparation enters the cell and actively begins to produce enzymes — beta-lactamases — that hydrolyze (destroy) antibacterial preparations. Today, over 350 different beta-lactamases, synthesized by both gram-positive and gram-negative microorganisms, have already been identified.

In the early 1970s, Streptomyces clavuligerus was cultured, leading to the discovery of the first strong beta-lactamase inhibitor — clavulanic acid [2]. In 1981, the first combined drug, amoxicillin and clavulanic acid — known as Amoksiklav, was introduced into clinical practice.

Escherichia coli is one of the leading pathogens in animal infectious diseases. For example, this pathogen is responsible for approximately 50-60% of poultry mortality [3].

One of the key factors in successfully combating colibacillosis, as well as other infectious diseases, is the proper treatment regimen. Due to the widespread occurrence of antibiotic-resistant strains of microorganisms, particularly within the Enterobacteriaceae family, the effectiveness of many antibacterial drugs and antibiotics has significantly decreased [4].

Currently, more than 150 different serovariants of Escherichia coli are known. It is established that in the intestines of healthy animals, there is a dynamic balance between beneficial and opportunistic microflora, characterized by numerous symbiotic and competitive interactions. This balance is associated with the selective pressure of the intestinal environment. Microbiocenosis is maintained in two main ways: through the adhesion of microorganisms to the intestinal mucosa and the high population growth of microbes, which significantly exceeds the rate at which intestinal contents are expelled due to peristaltic contractions. As a result, a complex microflora is formed in the intestines [5].

Shiga toxin-producing E. coli (STEC) is often found in the intestines of humans and warm-blooded animals. Most strains of E. coli are harmless. However, certain strains, such as enterohemorrhagic E. coli (STEC), can cause severe foodborne illnesses. This bacterium primarily infects humans through the consumption of contaminated foods, such as raw or undercooked ground meat, unpasteurized milk, contaminated raw vegetables, and sprouts [6].

Scientists from the Tuberculosis Laboratory of the Veterinary Research Institute have developed the ETIS-2 complex preparation. The unique combination of components in the ETIS-2 complex preparation provides advantages over other bacteriostatics. This combination results in a synergistic effect (where one drug enhances the action of another) and a prolongation effect (extending the duration of the drug's action). The preparation can be used for all types of animals from 10 days of age, regardless of their physiological condition [7].

Materials and Research Methods

The study of the effect of the ETIS-2 preparation against colibacillosis was conducted in the vivarium of the Microbiology and "Young Animals Disease Research" laboratories on six sheep. For this purpose, a suspension was prepared using the physiological saline method from a one-day culture of Escherichia coli strain, cultivated on meat-peptone agar (MPA) obtained from the Microorganism Collection of the Veterinary Research Institute (VRI).

Additionally, the pathogenicity of the Escherichia coli strain was tested in the Tuberculosis Laboratory on nine guinea pigs. A suspension was prepared based on McFarland Standard International (developed in France) for comparison with international units. The suspension was diluted to LD50 using BaSO4 at a ratio of 1.92×10^{-4} mol/L to ensure its safety for testing on six sheep to assess the pathogenicity of the strain (Table 1).

In the first group, three sheep were injected intraperitoneally with 2 ml of Escherichia coli strain at a dose of LD50 1.5×10^8 CFU (colony-forming units). In the control group (Group 2), three sheep were similarly injected with the same dose of LD50 1.5×10^8 CFU of the Escherichia coli O111 strain (Table 1).

Experiment to determine the effectiveness of the drug ETIS-2 against colibacillosis

Table 1

Name of groups	Number of Sheep	Strain Name	Infection		Treatment				Result
			dose	method	drug	dose	method	duration	
Experiment I	3	Escherichia coli O 111	$1,5x10^8$ KXB	Abdominal cavity	ETIS-2	3 ml/sheep	Subcutaneous injection	7 days	Alive and recovered
Control II	3	Escherichia coli O 111	$1,5x10^8$ KXB	Abdominal cavity	0	0	0	0	Alive and sick

The general condition, physiological and clinical parameters (body temperature, heart rate, and respiratory rate), and hematological indicators of the experimental sheep were monitored before and after exposure.

In the experiment, after the infection with colibacteriosis, the clinical signs of the disease appeared in the sheep of Group 1 (the infected group). After this, the sheep were treated with the ETIS-2 complex preparation. The treatment was carried out by subcutaneous injection with 5.0 ml of the ETIS-2 complex for each 100 kg of body weight.

The treatment regimen for the experimental group involved subcutaneous injections of 3 ml per sheep, once daily for 7 days. The control group received no treatment (as shown in Table 2).

The results of the experiment to determine the effect of the ETIS-2 preparation on sheep with colibacteriosis:

Table 2

№ Name of the pathogen Infection method and dose

Method

Number of sheep Dose of ETIS-2, 100 kg/ 5 ml Number of ETIS-2 injections, subcutaneous Result

№	Name of the pathogen	Infection method and dose	Method	Number 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	Colibacteriosis (experimental group)	$LD_{50}2,5x10^9$ KXB	Abdomen	3	2-3 ml	I	II	III	IV	V	100% Treated
2	Colibacteriosis (control group)	$LD_{50}2,5x10^9$ KXB	Abdomen	3	-	control					Sickened
3	Intact control (healthy group)	Physiological solution	Abdomen	3	-	-					healthy

Blood samples from the jugular vein of the sheep were taken on days 3, 6, and 9 after infection for hematological analysis. The samples were analyzed using a photometric method. From each sheep (including those in the experimental, control, and intact groups), 0,2 ml of blood was taken and analyzed using

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

In Group 1 (sheep experimentally infected with colibacteriosis), 36 hours after infection, the initial clinical signs of the disease were observed: elevated body temperature (+39,6°C), lethargy, and reduced appetite. Over the following days, these signs became more pronounced, and by the 3rd or 4th day of the experiment, two sheep in this group exhibited symptoms of diarrhea.

Once the clinical signs of the disease were evident in the experimental sheep, they were treated with the ETIS-2 complex preparation. The treatment involved subcutaneous injections of 3 ml per sheep once daily for 7 days.

Research Results

By the third day of treatment, the sick sheep showed significant improvements: their body temperature returned to normal (+38.5°C), heart rate stabilized at 71 beats per minute, appetite was restored, and diarrhea ceased. In the 6th-7th days of treatment and throughout the remainder of the experiment, no further signs of illness were observed in the experimental sheep.

In the second control group (sheep infected with E. coli), clinical signs of the disease were observed. The sheep exhibited typical clinical symptoms of the disease, such as increased body temperature (+39,7°C), lethargy, and decreased appetite. These signs worsened over the next few days, and by the 3rd or 4th day of the experiment, two sheep in this group developed diarrhea. No treatment was administered to these sheep, and hematological tests were conducted.

According to the hematological test results (Table 3), after the experimental sheep in Group 1 were infected with colibacteriosis and exhibited clinical signs of the disease, the following results were recorded: hemoglobin level was 6,7±0,25 x10 g/L, erythrocyte count was 7,16±0,34 $x10^{12}$ g/L, leukocyte count was 16,7±0,74 $x10^{9}$/L, lymphocyte count was 82,5±4,04% $x10^{9}$/L, and hematocrit value was 22,45±1,06 x10-2 L/L.

According to the obtained results, a decrease in hemoglobin, erythrocytes, and hematocrit levels, as well as an increase in leukocytes and lymphocytes, was observed. Clinical signs of anemia were noted in the experimental sheep.

Based on the hematological test results, on the 9th day of the experiment, the hemoglobin level in Group I sheep was 9,4±0,27 x10 g/L, erythrocytes were 9,81±0,41 x10^{12} g/L, leukocytes were 11,9±0,46 x10^{9}/L, and lymphocytes were 71,5±1,97 %x10^{9}/L. The hematocrit level decreased to 29,5±1,24 x10-2 L/L. These results indicate that, after 3 days post-infection, clinical signs of anemia were observed in the experimental sheep.

In the second control group (infected with E. coli), after clinical signs of the disease appeared, the hemoglobin level was 6,5±0,28 x10 g/L, erythrocytes were 7,53±0,35 x10^{12} L, leukocytes increased to 15,9±0,70 x10^{9} L, and lymphocytes increased to 82,4±3,87 %x10^{9}/L. The hematocrit level decreased to 23,4±0,75 x10-2 L/L on the third day after infection. These results also indicate the presence of anemia in the infected sheep.

On the 9th day of the experiment, in Group II, the hemoglobin level was 6,1±0,30 x10 g/L, erythrocytes were 6,29±0,25 x10^{12} g/L, leukocytes were 17,1±0,67 x10^{9}/L, and lymphocytes were 87,4±3,67 %x10^{9}/L. The hematocrit level decreased to 17,8±0,70 x10-2 L/L. These results further confirm the presence of anemia in the infected sheep.

In the intact control group (Group III), on the 9th day of the experiment, the hemoglobin level was 9,5±0,46 x10 g/L, erythrocytes were 9,94±0,46 x10^{12} g/L, leukocytes were 10,6±0,40 x10^{9}/L, lymphocytes were 59,1±2,48 %x10^{9}/L, and the hematocrit was 30,2±1,39 x10^{-2} L/L. No significant changes in these values were observed throughout the experiment.

Conclusion: Based on the hematological test results, it can be concluded that in the sheep treated with the ETIS-2 complex preparation for colibacteriosis, after infection with colibacteriosis, a decrease in hemoglobin, erythrocytes, and hematocrit levels, along with an increase in leukocytes and lymphocytes, was observed.

Hematological Test Results of Blood Samples from Sheep Infected with E. coli

Table 3

№	Group Name and Inventory Numbers	Hemoglobin (x10 g/L)			Erythrocytes (x10^{12} g/L)			Leukocytes (x10^{9}/L)			Lymphocytes (% x10^{9}/L)			Hematocrit (x10^{-3} L/L)		
Normal Values		9-15			9-15			4,0-12,0			40-75 2-9			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	6,6±0,25	8,6±0,46	9,1±0,26	7,11±0,31	9,93±0,35	9,89±0,34	16,3±0,78	12,7±0,42	11,2±0,44	83,7±3,93	72,7±3,27	60,3±3,20	23,4±0,66	27,8±1,36	28,3±1,66
2	Left ear painted	6,7±0,18	9,3±0,44	9,6±0,18	7,17±0,32	9,45±0,16	9,71±0,50	17,4±0,59	12,3±0,70	12,3±0,36	82,2±3,53	73,5±1,98	61,8±1,36	22,7±0,84	26,6±0,77	30,5±1,13
3	Forehead painted	6,9±0,29	9,4±0,31	9,4±0,22	7,19±0,30	7,24±0,50	9,84±0,21	16,5±0,59	11,8±0,51	12,1±0,64	81,6±2,61	71,1±3,13	62,3±3,18	21,3±0,98	26,7±0,99	29,7±1,28
M±m		**6,7±0,25**	**9,1±0,42**	**9,4±0,27**	**7,16±0,34**	**9,54±0,35**	**9,81±0,41**	**16,7±0,74**	**12,3±0,54**	**11,9±0,46**	**82,5±4,04**	**72,4±3,40**	**71,5±1,97**	**22,45±1,06**	**27,0±1,05**	**29,5±1,24**
II - Control 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 side of the neck painted	6,5±0,25	6,2±0,27	6,2±0,18	7,72±0,33	6,35±0,22	6,14±0,21	15,2±0,73	16,5±0,54	17,3±0,67	81,1±3,81	82,3±3,70	86,2±3,57	24,4±0,68	21,6±1,06	18,2±0,71
2	Left side of the neck painted	6,6±0,18	6,4±0,30	5,8±0,11	7,34±0,32	6,72±0,11	6,26±0,33	16,3±0,55	16,8±0,96	17,1±0,50	83,6±3,59	85,8±2,32	87,5±4,21	22,3±0,83	19,4±0,56	17,8±0,66
3	Chest painted	6,4±0,27	6,3±0,21	6,2±0,14	7,53±0,32	6,61±0,36	6,48±0,14	16,1±0,58	16,6±0,71	16,8±0,89	82,4±2,64	86,6±3,81	88,5±4,21	23,6±1,09	20,8±0,77	17,5±0,75
M±m		**6,5±0,28**	**6,3±0,26**	**6,1±0,30**	**7,53±0,35**	**6,56±0,24**	**6,29±0,25**	**15,9±0,70**	**16,6±0,88**	**17,1±0,67**	**82,4±3,87**	**84,9±3,14**	**87,4±3,67**	**23,4±0,75**	**20,6±0,80**	**17,8±0,70**
V - 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
4	Right front leg painted	9,8±0,37	9,5±0,41	9,9±0,29	9,62±0,41	9,37±0,33	9,22±0,31	11,4±0,55	10,6±0,35	10,3±0,40	59,9±2,82	58,6±2,64	59,3±3,14	29,5±0,83	31,8±1,56	30,6±1,81
6	Left front leg painted	9,4±0,25	9,6±0,45	9,2±0,17	9,31±0,41	9,58±0,16	10,37±0,54	11,3±0,38	11,5±0,66	10,2±0,30	57,6±2,48	60,3±1,63	59,4±1,31	32,6±1,21	31,4±0,91	30,7±1,14
7	Left abdomen painted	9,7±0,41	9,5±0,31	9,3±0,21	9,44±0,40	9,64±0,52	10,24±0,22	10,8±0,39	10,2±0,44	11,3±0,60	60,4±1,93	59,6±2,62	58,7±2,99	29,1±1,34	29,5±1,09	29,3±1,26
M±m		**9,6±0,32**	**9,5±0,31**	**9,5±0,46**	**9,46±0,35**	**9,53±0,23**	**9,94±0,46**	**11,2±0,50**	**10,8±0,40**	**10,6±0,40**	**59,3±2,67**	**59,5±2,79**	**59,1±2,48**	**30,4±1,06**	**30,9±1,17**	**30,2±1,39**

These indicators suggest the presence of anemia in the sheep's organism. When treated with the ETIS-2 complex preparation for colibacteriosis, there is no need to use other types of antibiotics or symptomatic drugs.

After the sheep were infected with the E. coli strain, and after the full clinical signs of the disease appeared, treatment was initiated using the ETIS-2 complex preparation. The preparation was administered subcutaneously at a dose of 3 ml per sheep once daily for 7 days. By the third day of treatment, the sick sheep's body temperature returned to normal (+38,3°C), appetite improved, and diarrhea stopped. In the following 6-7 days and throughout the remaining period of the experiment, no signs of the disease were observed in the treated sheep.

Blood analysis is an important diagnostic tool in veterinary medicine. Blood-producing organs are highly sensitive to various physiological and, especially, pathological factors, which is clearly reflected in the blood parameters.

Researching hematological parameters using laboratory instruments provides an insight into the animal's health status and can reflect various pathologies and the effects of treatments.

Conclusions:

1. In sheep treated with the ETIS-2 complex preparation, hematological tests revealed that after the animals in the experimental groups I and II were infected with colibacteriosis pathogens, the clinical signs of the disease began to appear, and it was found that the levels of hemoglobin, erythrocytes, leukocytes, and lymphocytes increased, while the hematocrit value decreased. These indicators suggest the presence of anemia in the sheep's organism.
2. According to the hematological test results, on the 9th day of the experiment, the hemoglobin level in Group I sheep was 9,4±0.27 x10g/L, erythrocytes 9,81±0,41 $x10^{12}$g/L, leukocytes 11,9±0,46 $x10^{9}$/L, and lymphocytes 71,5±1,97 %$x10^{9}$/L, showing an increase. The hematocrit value was found to decrease to 29,5±1,24 $x10^{-2}$ L/L on the 9th day of the experiment.
3. After the sheep in the experiment were infected with the E. coli strain, and after the full clinical signs of the disease appeared, the ETIS-2 complex preparation was administered subcutaneously once daily at a dose of 3 ml per sheep for 7 days. By the third day of treatment, the sick sheep's body temperature returned to normal (+38,3°C), appetite improved, and diarrhea stopped. In the following 6-7 days and throughout the rest of the experiment, no signs of the disease were observed in the treated sheep.
4. When treating colibacteriosis with the ETIS-2 complex preparation, there is no need to use other types of antibiotics or treatment methods.

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