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THE PROTOSTOP DRUG EFFECTIVENESS IN DIARRHEA OF MIXED PARASITIC-BACTERIAL ETIOLOGY IN CALVES

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Abstract

In cattle breeding, diseases of the gastrointestinal tract (GIT) of calves with diarrheal syndrome, which are widespread almost everywhere and involve most of the livestock, remain a serious problem. Gastrointestinal diseases of calves of mixed, parasitic-bacterial and parasitic-viral etiology are often noted, a combination of *Cryptosporidium* spp. with *Escherichia coli*, enterobacteria of the genus *Salmonella*, *Klebsiella* have been detected. Thereof, it is optimal to use broad-spectrum drugs that have antimicrobial and antiprotozoal effects. This article presents for the first time the effect of the drug paromomycin sulfate on pathogens of gastrointestinal diseases of both parasitic and bacterial origin. The minimum inhibitory concentration (MIC) of paromomycin sulfate has been established for pathogens of gastrointestinal tract diseases of calves of bacterial etiology, i.e., *Salmonella* Dublin, *Escherichia coli* with hemolytic properties, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Citrobacer freundii*. The aim of study was to examine the therapeutic effectiveness of the drug Protostop (the active substance is paromycin sulfate) for diseases of the gastrointestinal tract with diarrheal syndrome in calves. The research was carried out at a cattle farm (Leningrad Province, Lomonosovsky District) in July-August 2021. The experiments involved calves (*Bos taurus*) of the black-white breed, up to 5 months old, weighing from 30 to 50 kg. Fecal samples weighing 20-40 g were taken from the rectum of calves with clinical signs of gastrointestinal dysfunction. To detect oocysts of *Cryptosporidium* spp. fecal smears were prepared on a glass slide, and stained using a Diakhim-Kit for Ziehl-Neelsen staining (NPF Abris+, Russia). Microscopy was performed using a Primo Star microscope (Carl Zeizz, Germany). To establish the quantitaty and species of enterobacteria, 10-fold dilutions of feces in saline solution were sown in 0.05 ml quantities on the Endo nutrient medium (LLC Research Center for Pharmacotherapy, Russia). To assess the therapeutic effectiveness of the drug Protostop (powder, 1 g of the drug contains 100 mg of paromomycin sulfate; AVZ Ltd, Russia), animals in whose feces consist oocysts of *Cryptosporidium* spp., strains of hemolytic *E. coli* and opportunistis microorganisms were selected. We formed 5 groups of animals of 10 animals in each (4 experimental, 1 control). All calves in experimental groups were given the drug Protostop individually, orally, once a day. Before use, a single dose of the drug was dissolved in water by adding the liquid to the powder. Animals from the 1st group received the drug at a dose of 250 mg/kg of body weight for 3 days, calves from the 2nd group were given the drug at the same dose for 5 days. Animals of the 3rd and 4th groups received Protostop at a dose of 350 mg/kg body weight, respectively, for 3 and 5 days. Calves from the control group were treated with an analog drug Parofor 70 (powder, 1.0 g of the drug contains 100.0 mg of paromomycin sulfate; Biovet AD, Bulgaria). The drug was administered at a dose of 350 mg/kg body weight orally once a day for 5 days. During a clinical examination on the 3rd, 5th, 10th and 14th days from the start of treatment, the health condition of the animals, their consumption of water and feed, changes in the GIT function, the condition of the mucous membranes and coat were examined. Coprological studies for the presence of oocysts of *Cryptosporidium* spp. were carried out on the 8th and 12th days of treatment, bacteriological on the 10th day after the end of treatment. To assess the health condition, blood samples for examination was taken in all groups. The MIC of paromomycin sulfate,

the active substance of the drug Protostop, was determined by the method of serial dilutions according to GOST R ISO 20776-1-2010. Strains for which the MIC of paromomycin did not exceed 4 μg/ml were classified as susceptible, and those above 4 μg/ml were classified as resistant. A parasitological study revealed more than 25 oocysts of *Cryptosporidium* spp. in the feces of calves in the field of view, which indicated a high intensity of invasion. A bacteriological study revealed *Escherichia coli* strains with hemolytic activity and other strains of opportunistic microflora. After treatment, all calves in the experimental groups showed significant improvements in their health condition: the animals became more active, diarrhea stopped, and the feces became a mushy consistency, characteristic of cattle feces. The drug Protostop had a pronounced therapeutic effect at a dose of 350 mg/kg of animal weight, administered orally with water, once a day, for a course of 5 days. 8 days after the start of treatment according to the indicated regimen, there were no *Cryptosporidium* spp. oocysts in the feces; 10 days after the end of treatment, strains of hemolytic *Escherichia coli* and other opportunistic enterobacteria were absent in a 1:10 dilution of feces. The therapeutic effectiveness of the drug Protostop at the indicated dosage and duration of use is higher than the effectiveness of the drug Parofor 70 at a dose of 350 mg/kg. The MIC of paromomycin for *Salmonella* Dublin and *Citrobacter freundii* strains was 2 μg/ml, which allows them to be classified as microbiologically susceptible to this drug. The remaining strains were microbiologically resistant, for hemolytic *Escherichia coli* strains the MIC ranged from 128 to more than 256 rg/ml, for *Klebsiella pneumoniae*, *Proteus vulgaris* MIC was more than 256 μg/ml.

Keywords: paromomycin, cryptosporidiosis, minimal inhibitory concentration, *Escherichia coli*, calves, gastrointestinal diseases, gastrointestinal tract

In industrial livestock farming, diseases of the gastrointestinal tract (GIT) with diarrheal syndrome in young cattle are widespread and cause significant economic damage due to mortality, low weight gain and treatment costs [1-4]. The main etiological agents of diarrhea in calves in the first 3 weeks of life are *Cryptosporidium* spp., rotaviruses, coronaviruses and *Escherichia coli*. Diseases of the gastrointestinal tract of calves of mixed parasitic-bacterial and parasitic-viral etiology are quite often [5-8]. Variants of combinations of etiological agents have regional peculiarities [9-11]. The combination of *Cryptosporidium* spp. with *E. coli* is one of the most common, accounting for 12 to 27.8% of calf diarrhea incidence [12-14]. Associations of *Cryptosporidium* spp. with viruses, giardia, *Klebsiella* spp. have also been noted [11, 15, 16]. Coinfection with *Cryptosporidium* spp. and *Salmonella* spp. was detected in 0.5-40% of cases [17, 18]. The relationships between the components of the parasitic-bacterial system are not yet well understood, but there is evidence that coinfection may aggravate the course of diarrhea [19, 20].

The spread of diarrhea of mixed etiology in young cattle also poses a threat to human health. According to some publications, young ruminants serve as a reservoir of *E. coli* strains pathogenic to humans [11, 15]. It has been proven that cattle can be a source of the causative agent of cryptosporidiosis for humans, which is especially important for children under 5 years of age and adults with a weakened immunity, especially those infected with HIV [19, 21].

Treatment of animals with mixed infections is expensive and difficult [22, 23]. For the treatment of young cattle, it is optimal to use broad-spectrum drugs that act simultaneously on protozoa and microorganisms [24-26]. One of the side effects of using chemotherapy drugs to treat animals with infectious and invasive diseases is the development of drug resistance. Many chemotherapy drugs used in veterinary and human medicine cause resistance, so the choice of effective drugs becomes very problematic [27]. The effectiveness of antibiotics that have not been used for many years is currently being reconsidered because microorganisms have lost the genetic determinants of resistance to these drugs [28, 29].

Aminoglycosides are among such antibiotics, including paromomycin sulfate (syn. aminosidine) which have an antibacterial effect against strains of *E. coli*, *Salmonella* spp. and other microorganisms that populate the gastrointestinal tract of humans and animals [8, 24, 30]. Aminoglycosides have a bactericidal effect due to disruption of protein synthesis by blocking the 30S ribosomal subunit [31]. The

degree of antibacterial activity of aminoglycosides depends on their peak concentration in blood. When used together with penicillins or cephalosporins, synergism occurs against some gram-negative and gram-positive aerobic microorganisms [31]. The mechanism of the antiprotozoal action of paromomycin has not been sufficiently studied, although it serves as one of the optimal drugs for the treatment of animals with diarrheal syndrome. Just like other drugs from the aminoglycoside group, paromomycin is poorly absorbed from the gastrointestinal tract and is mostly excreted in feces. Preparations containing paromomycin are used in the European Union in veterinary medicine and in medicine for diseases caused by protozoa (*Cryptosporidium* spp., *Eimeria* spp.) and multidrug-resistant microorganisms [24, 32, 33].

This work is the first demonstration of the effect of a drug containing paromomycin sulfate on causative agents of gastrointestinal tract diseases of both parasitic and bacterial origin. The minimum inhibitory concentration of paromomycin sulfate has been established for causative agents of gastrointestinal diseases of calves of bacterial etiology *Salmonella* Dublin, hemolytic *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, and *Citrobacer freundii*.

Our goal was to assess the therapeutic effectiveness of the drug Protostop (active ingredient paromomycin sulfate) in calves suffered from gastrointestinal diseases with diarrheal syndrome.

Materials and methods. The studies were carried out on Black-and-White 30-50 kg cattle (*Bos taurus*) up to 5 months old (livestock breeding complex, Leningrad Province, Lomonosov District, July-August 2021).

With a sterile glove, 20-40 g fecal samples were taken from the rectum of calves with clinical signs of gastrointestinal dysfunction (watery feces with mucus, depression, dehydration and cachexia). The samples were placed in sterile isothermal containers at +2-6 °C, labeled and delivered to the laboratory. Parasitological and bacteriological studies were performed on the date of sampling.

Oocysts of *Cryptosporidium* spp. were detected in smears of feces on a glass slide [34] stained with reagents Diachim-Set for Ziehl-Neelsen staining (NPF Abris+, Russia) as per the manufacturer's instructions. Preparations were examined at a magnification of 10×40 and 10×100 (a Primo Star microscope, Carl Zeizz, Germany). The intensity of invasion (II) was considered high for more than 25 oocysts per field of view (+++), moderate with up to 25 oocysts per field of view (++), low at 1-3 oocysts per field of view (+); "±" corresponds to one oocyst in the field of view when viewing several fields, and "–" meams the absence of oocysts of *Cryptosporidium* spp. [35].

To assess the quantitative and species composition of enterobacteria, 0.05 ml of 10-fold dilutions of feces in physiological solution were plated on Endo nutrient medium (LLC Research Center for Pharmacotherapy, Russia). The cultures were incubated at 37±2 °C for 18-24 h. The colonies were counted and identified to species using the biochemical tests DIS-DIF-ENTERO-24 and the Biochemical Plate Differentiating Enterobacteriaceae (NPO Diagnostic Systems LLC , Russia) according to the manufacturer's instructions. The hemolytic activity of *E. coli* was assessed by inoculating 10-fold dilutions of calf feces in physiological solution on commercial Colombian sheep blood agar (Biomedia LLC, Russia) [36].

To evaluate the therapeutic effectiveness of the drug Protostop (powder, 1 g of the drug contains 100 mg of paromomycin sulfate, lactose monohydrate as an excipient; NVC Agrovetzashchita LLC, Russia), animals were selected in whose feces oocysts of *Cryptosporidium* spp., strains of hemolytic E. coli and opportunistic microorganisms were detected. Calves were individually given the preparations orally once a day. Before use, a single dose of the drug was dissolved by adding

water to the powder. In groups 1 and 2, animals received Protostop at a dpsage of 250 mg per 1 kg bodyweight for 3 and 5 days, respectively, in groups 3 and 4, at 350 mg per 1 kg bodyweight for 3 and 5 days, respectivel. Calves from the control group were treated with an analogue drug Parofor 70 (powder, 1.0 g of the drug contains 100.0 mg of paromomycin sulfate, excipients colloidal anhydrous silicon dioxide and glucose monohydrate, Biovet AD, Bulgaria). The drug was administered at a dose of 350 mg/kg bodyweight for 5 days. In each group, $n = 10$.

During a clinical examination on days 3, 5, 10 and 14 from the start of treatment, the condition of the animals, water and feed consumption, changes in gastrointestinal function, the condition of the mucous membranes and coat were examined. Coprological analysis for the presence of *Cryptosporidium* spp. oocysts was carried out on days 8 and 12 of treatment, bacteriological analysis on day 10 after the end of treatment. To assess animal's condition, blood was sampled from the subcaudal vein into K3 tubes with EDTA before treatment and on day 10 after using the drug Protostop. The number of blood cells was determined as generally accepted. Erythrocytes and leukocytes were counted in a Goryaev chamber, erythrocyte sedimentation rate (ESR) was measured by the Panchenkov method [34].

The minimum inhibitory concentration (MIC) of the active ingredient of the drug Protostop (paromomycin sulfate) was determined by serial dilutions according to GOST R ISO 20776-1-2010 [37]. Two *Salmonella* Dublin strains previously isolated from the corpses of calves with salmonellosis (the collection of strains of St. Petersburg State University of Veterinary Medicine) and hemolytic *E. coli*, *Citrobacter freundii*, *Enterobacter cloaceae*, *Klebsiella pneumoniae*, *Proteus vulgaris* we isolated during bacteriological examination of feces served as tes cultures. Strains for which the MIC of paromomycin did not exceed 4 μg/ml were classified as susceptible, those with MIC above 4 μg/ml were considered resistant [33].

For statistical processing, the $\chi 2$ was calculated with Yates' continuity correction (the Biometrica online calculator, http://www.biometrica.tomsk.ru). The mean values (M) and standard deviations (±SD) were calculated using Microsoft Excel 2016 program. The differences were considered statistically significant at $p \leq 0.05$.

Results. Calves resived Protostop tolerated it well. Based on regular clinical examinations, no negative effects were identified. On day 3 of the treatmen, diarrhea stopped in all calves, regardless of the dose, the animals became more active, and feed and water consumption increased. The fecal masses acquired a mushy consistency, characteristic of cattle feces, and there were no mucus impurities. Calves from the control group retained mucus in their feces.

Based on the results of a clinical blood analysis before treatment, the hemoglobin content of calves in all groups was below the reference values. In addition, we established thrombocytopenia (Table 1). Leukograms indicated an inflammatory reaction in the body, since neutrophilia and an increase in ESR occurred.

1. Clinical blood analysis of black-and-white calves (*Bos taurus*) with gastrointestinal disease of mixed parasitic-bacterial etiology before and after the use of the Protostop drug (M±SD, Leningrad Province, Lomonosovsky District, 2021)

Parameter	Group ($n = 10$ each)					Reference values
	1	2	3	4	control	
Leukocytes, $\times 10^9$/l						4,5-12,0
before treatment	9.2±0.14	9.6±0.43	7.3±0.40	11.1±0.60	8.2±0.50	
day 10 of treatment	7.2±0.70	8.4±0.80	6.7±0.30	8.3±0.50	9.7±0.80	
Erythrocytes, $\times 10^{12}$/л						5.0-7.5
before treatment	6.9±0.27	7.0±0.52	7.0±0.81	6.6±0.05	6.6±0.65	
day 10 of treatment	6.2±0.70	6.9±0.40	6.4±0.60	6.2±0.09	6.2±0.30	

Continued Table 1

Hemoglobin, g/l						99-129
before treatment	81.8±2.72	92.8±3.45	76.6±4.30	80.6±2.11	106.8±2.80	
day 10 of treatment	83.8±1.73	93.8±1.87	76.8±0.92	82.2±2.52	103.6±2.79	
Platelets, $\times 10^9$/л						260-700
before treatment	217.2±1.63	18.3±8.20	203.0±2.64	204.0±4.69	236.6±4.44	
day 10 of treatment	223.6±5.26	185.6±3.02	212.2±4.90	208.4±5.30	249.2±3.90	
Eosinophils, %						5-8
before treatment	5.0±0.05	6.0±0.09	7.0±0.10	8.0±0.40	6.2±0.40	
day 10 of treatment	5.4±0.10	5.5±0.30	5.8±0.20	6.1±0.40	5.8±0.09	
Band neutrophils, %						2-5
before treatment	4.2±0.97	2.8±0.38	2.2±0.10	1.8±0.05	2.3±0.07	
day 10 of treatment	4.2±0.10	4.6±0.50	2.2±0.20	2.2±0.20	2.5±0.08	
Segmented neutrophils, %						20-35
before treatment	36.0±3.10	40.8±2.87	38.4±1.20	32.0±1.30	38.6±1.30	
day 10 of treatment	30.0±1.24	33.8±2.50	28.2±1.30	29.6±2.30	35.4±2.80	
Lymphocytes, %						40-65
before treatment	51.0±1.40	43.6±1.30	48.6±1.02	50.4±1.93	50.9±1.80	
day 10 of treatment	55.1±2.10	48.4±2.30	58.0±3.50	54.1±3.80	51.2±1.96	
Monocytes, %						2-7
before treatment	2.6±0.14	6.2±0.70	3.8±0.64	6.0±0.16	3.4±0.04	
day 10 of treatment	4.1±0.09	6.2±1.30	4.4±0.80	6.4±0.70	4.2±1.20	
ESR, mm/h						0.5-1.5
before treatment	1.6±0.39	1.8±0.60	1.5±0.06	1.8±0.06	1.7±0.06	
day 10 of treatment	1.2±0.09	1.4±0.13	1.3±0.36	1.4±0.33	1.5±0.08	

N o t e. For a description of the groups, see the Materials and methods section. When statistically analyzing the results, no significant differences were found between the values of indicators before and after treatment ($p > 0.05$).

In 10 days after the start of the therapy, a slight increase in hemoglobin tending to the reference value and an increase in the number of platelets to the reference values ocurred. The leukogram showed a decrease in the number of neutrophils and ESR to reference values.

In groups 1 and 3, on day 8 after the treatment started, the number of oocysts of *Cryptosporidium* spp. in feces decreased significantly, up to 25 oocysts per field of view in group 1 and 1-3 oocysts in group, compared to that before using Protostop. After drinking 250 mg/kg Protostop for 5 days in group 2, on day 12 oocysts of *Cryptosporidium* spp. were single. In group 4, on days 8 and 12 protozoa were not found (Table 2).

2. Intensity of invasion by *Cryptosporidium* spp. oocysts of black-and-white calves (*Bos taurus*) with gastrointestinal disease of mixed parasitic-bacterial etiology before and after the use of the Protostop drug (Leningrad Province, Lomonosovsky District, 2021)

Group	*Cryptosporidium* spp. oocysts		
	before treatment	day 8 of treatment	day 12 of treatment
1(n = 10)	+++	++	–
2 (n = 10)	+++	–	±
3 (n = 10)	+++	+	–
4 (n = 10)	+++	–	–
Control	+++	–	±

N o t e. For a description of the groups, see the Materials and methods section, +++ — high intensity of invasion (II), ++ — medium II, + — low II, ± — single oocysts in the microscope field of view, «–» — no *Cryptosporidium* spp. oocysts.

In control fecal samples of calves which received a drug with the same active ingredient, single oocysts of *Cryptosporidium* spp were identified on day 12 of the treatment

In bacteriological studies of fecal samples [38], hemolytic *E. coli* strains were identified in all animals. Their presence indicates a parasitic-bacterial etiology of gastrointestinal disease. Strains of opportunistic microflora *Citrobacter freundii*, *Enterobacter cloaceae*, *Klebsiella pneumoniae*, and *Proteus vulgaris* were also discovered that serve as potential causative agents of the diseases studied (Table 3) [38].

3. Qualitative and quantitative composition of the intestinal microbiota of black-and-white calves (*Bos taurus*) with gastrointestinal disease of mixed parasitic-bacterial etiology before and after the use of the Protostop drug ($M \pm SD$, Leningrad Province, Lomonosovsky District, 2021) [38]

Group	Hemolytic *Escherichia coli*, %			Opportunistic microflora, CFU/g			Obligate *E. coli*, CFU/g		
	before treatment	10 days after treatment	p	before treatment, $\times 10^7$	10 days after treatment, $\times 10^7$	p	before treatment, $\times 10^7$	10 days after treatment, $\times 10^7$	p
1 ($n = 10$)	24.1±2.2	16.8±1.8	> 0.05	7.2±0.9	6.2±0.7	> 0.05	4.3±0.7	3.9±0.8	> 0.05
2 ($n = 10$)	22.2±1.9	14.7±1.1	> 0.05	8.3±1.2	7.1±0.8	> 0.05	3.9±0.8	3.3±0.7	> 0.05
3 ($n = 10$)	23.4±2.0	9.8±0.9	> 0.05	6.5±1.2	4.9±0.8	> 0.05	4.5±0.8	3.9±0.9	> 0.05
4 ($n = 10$)	24.1±1.4	0	< 0.05	8.1±1.1	0	< 0.05	4.0±0.9	3.9±0.7	> 0.05
Control	23.2±1.5	16.5±1.4	> 0.05	7.3±0.6	6.2±0.9	> 0.05	4.2±0.8	4.1±0.8	> 0.05

During a bacteriological study of feces on day 10 after the end of treatment, we revealed the absence of hemolytic *E. coli* strains and opportunistic microorganisms in a 1:10 dilution of feces from calves of group 4, while in the feces of calves of other groups, these microorganisms were present [38].

4. Minimum inhibitory concentration (MIC) **of paromomycin sulfate for causative agents of gastrointestinal tract diseases of black-and-white calves (*Bos taurus*)** (Leningrad Province, Lomonosovsky District, 2021)

Species (serological variant)	MIC, μg/ml	Susceptibility
Salmonella Dublin	2	S
Escherichia coli hemolytic (strain 1)	128	R
E. coli hemolytic (strain 2)	> 256	R
E. coli hemolytic (strain 3)	> 256	R
E. coli hemolytic (strain 4)	> 256	R
Klebsiella pneumoniae	> 256	R
Proteus vulgaris	> 256	R
Citrobacter freundii	2	S

N o t e. R — resistant strain, S — susceptible strain.

When studying the inhibitory effect of paromomycin sulfate, the active ingredient of Protostop, only strains *Salmonella* Dublin and *C. freundii* were susceptible to this drug (MIC 2 μg/ml), strains of hemolytic *E. coli*, *K. pneumoniae*, *P. vulgaris* were resistant (MIC 128 μg/ml or more) (Table 4).

Diseases of the gastrointestinal tract of young cattle, which remain a serious problem for veterinary specialists almost worldwide, are multi-etiological [1]. Therefore, for the treatment of animals, it is desirable to use drugs that are effective against several etiological factors, in particular aminoglycosides that act on microorganisms and single-celled protozoa [33].

When assessing the effect of Protostop from the group of aminoglycosides on calves, we did not identify statistically significant deviations in clinical and morphological blood parameters after treatment compared to the values obtained before it began. The content of hemoglobin, platelets and leukogram values before and after treatment remained within the reference values. That is, Protostop did not have a negative effect on calves. We previously reported that the use of Protostop for 5 days at a dose of 350 mg/kg leads to a significant decrease in the proportion of hemolytic *E. coli* and opportunistic microflora. Indicators for obligate *E. coli* strains remained without statistically significant changes [38].

We also determined the MIC of Protostop paromomycin sulfate for the isolated in the study hemolytic *E. coli* (potential causative agents of colibacillosis in calves) [39] and strains *C. freundii*, *E. cloaceae*, *K. pneumoniae*, *P. vulgaris* (a conditionally pathogenic microflora, which can cause diseases of the gastrointestinal tract of young cattle) [40]. In addition, we used *Salmonella* Dublin strains which serve as the causative agent of salmonellosis in calves. W. Chen et al. [41]

determined the MICs of various chemical isomers of paromomycin for *E. coli* and showed that MIC varied from 2-4 to >128 μg/ml depending on the drug isomer. Our data are comparable with these findings and indicate different sensitivity of microorganisms from the gastrointestinal tract of calves to the active ingredient of Protostop.

According to the CLSI standards (Clinical and Laboratory Standards Institute, https://clsi.org/), only strains with an MIC of no more than 4 μg/ml can be classified as susceptible to paromomycin [33]. However, the MIC value determines the so-called microbiological sensitivity. Often it does not coincide with the clinical sensitivity which indicates the effectiveness of treatment [42]. There is no information on clinical sensitivity to paromomycin in the treatment of farm animals in the scientific literature. For the first time, our data indicate the clinical effectiveness of Protostop, containing 100 mg of paromomycin sulfate per 1 g, for diseases of the gastrointestinal tract of calves not only of bacterial, but also of parasitic etiology.

Based on the data that the mechanism of action of aminoglycoside drugs on microorganisms is to disrupt protein synthesis by ribosomes [31, 41], we assume that paromomycin sulfate interferes with protein synthesis not only in microorganisms, but also in forms of *Cryptosporidium* spp. with a thin cell wall, leading to their elimination from the animal body. In calves, auto invasion by cryptosporidium oocysts occurs, that is why their elimination reduces the intensity of invasion and shortens the recovery time of animals. We found that Protostop at a daily dose of 350 mg/kg for 5 days significantly reduces the number of oocysts of *Cryptosporidium* spp. in the feces, which indicates its therapeutic effectiveness. Similar results were obtained by A. Aydogdu et al. [43] who used paromomycin at a lower dose (100 μg/kg bodyweight), but for a longer period, for 7 days vs. 5 days in our study. General condition of the animals improved on day 2 of treatment, the number of oocysts in feces decreased on day 3. O.A. Hameed [44] noted that paromomycin was effective in the treatment of cryptosporidiosis in lambs and camels.

Thus, Protostop, 1.0 g of which contains 100.0 mg of paromomycin sulfate, at a dose of 350 mg/kg of animal weight, when administered orally with water once a day for 5 days, has a pronounced therapeutic effect in gastrointestinal tract diseases of calves of parasitic-bacterial etiology. Eight days after the start of treatment, there were no *Cryptosporidium* spp. oocysts in the feces; after 10 days, hemolytic strains of *Escherichia coli* and other opportunistic enterobacteria were absent in 1:10 dilutions of feces. The therapeutic effectiveness of Protostop at the specified dosage and duration of use was higher than that of the drug Parofor 70 at a dose of 350 mg/kg. Minimum inhibitory concentration of paromomycin for *Salmonella* Dublin and *Citrobacter freundii* was 2 μg/ml which classifies them as microbiologically sensitive to this drug. Other strains were microbiologically resistant, for hemolytic *Escherichia coli* the MIC ranged from 128 to more than 256 μg/ml, and for *Klebsiella pneumoniae* and *Proteus vulgaris*, the MIC values were more than 256 μg/ml.

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