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THE NEW TREATMENT REGIMEN OF CALVES' DIARRHEA CAUSED BY MIXED INVASION OF *EIMERIA* AND *GIARDIA*

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Abstract

The article described a farm experiment provided in July-August of 2019 on 32 diarrheal calves of two and three-month-old, spontaneously infected with *Eimeria* and *Giardia*. The animals were selected by coproscopic methods and divided into four groups with eight individuals in each. The first control group calves were untreated. The second group animals treated orally by Metronidazole-25% (100 mg/kg), the third group – by probiotic Vetom-1 (50 mg/kg) for seven days. The fourth group calves were fed by a new treatment regimen with Metronidazole-25% at the

first three days, and additionally with Vetom-1 at the next four days in above dosage. The efficacy determined at the 10th day after treatment according to shedding cysts' level and weight gain per day. Diarrhea symptoms assessed on a 4-score scale before and after the treatment. As a result, during the experiment the control animals' parasite shedding level and diarrhea signs did not changed; the weight gain was minimal compared to other groups (0.66±0.26 kg). In the second group intensity efficiency (IE) against *Eimeria* was 90.9±9.3% and extensive efficiency (EE) – 75%, while against *Giardia* – 91.3±4.8% and 37.5%, respectively. The weight gain during the experiment was approximately same with the control group, and diarrhea remained at 2-3 scores. In the third group the infection intensity remained at the same level, body weight was higher (0.78±0.29) compared with two previous groups, and diarrhea decreased to 1-2 scores. In the fourth group, IE against *Eimeria* and *Giardia* was 99.9±0.2% and 98.9±1.5%, respectively, and EE against both parasites was 87.5%; weight gain was higher than in other groups and reached 0.89±0.06 kg, and there were no diarrhea signs. Thus, the new treatment regimen, including the combined use of Metronidazole and Vetom-1, showed high efficiency against calves' diarrhea caused by mixed invasion of *Eimeria* and *Giardia*.

Key words: Calves; *Eimeria* spp.; *Giardia* spp.; Metronidazole; Vetom-1; intense efficiency; extensive efficiency.

Introduction.

Diarrhea of calves is a common and significant disease in beef and dairy cattle farming. Economic losses from the disease are due to poor weight gains, high level mortality of calves, diagnosing and treatment costs [1].

Protozoal nature diarrhea of calves is caused by Eimeriidae, Giardiidae and Cryptosporidiidae families' pathogens and is considered the main reasons for the livestock's productivity declining. For example, an infected with eimeriosis calve under one year age have a weight for 27 kg less than a healthy one. So, over \$1.5 billion losses per year after eimerioses are documented in the United States cattle farms [2].

Infected by *Giardia* spp. animals demonstrated the smoothed clinical signs but the mass diarrhea is noted in calves, moreover, *G. duodenalis* is a dangerous zoonotic pathogen [3].

In different Kazakhstan regions prevalence with these parasites reached

81.8% among cattle. In the central region, prevalence with intestinal protozoan of young cattle (from the first day to 12 months) was 35.6%, including *Giardia* spp. – 12%, and *Eimeria* spp. – 15.7%. In intestine of animals these enteropathogens occurred in the form of monoinvasion or formed multicomponent parasitocenoses and caused associative diseases with signs of diarrhea [4]. Therefore, search the effective and affordable technologies for prevention and treatment of this disease is considered a novel problem [5].

Modern veterinary medicine market is offered several compounds for treatment of animal giardiasis, namely Chimcoccidium, Amprolium and other Nitrofurans, Sulfanilamide preparations as well as agents belonging to groups of Tinidazole, Furazolidone, Mepacrine, and sometimes Benzimidazole. These medicines are mostly used to treat domesticated pets.

Eimeria are often treated with such coccidiostats as Stop-coccid, Solicox, Baycox, etc. [6]. However, the action spectrum of these drugs is focused on certain groups of pathogens, while intestinal diseases are caused by various mixed infections [7]. Therefore, developing the methods for treatment of gastrointestinal associative diseases that help suppress various groups of pathogenic bacteria, fungi, parasites and restore intestinal function is considered a rational direction [8].

Thus, in the last decade, probiotics have been used to control intestinal infections of bacterial and parasitic etiology, including a such zoonosis as cryptosporidiosis. They are

Material and methods.

An experiment to assess the therapeutic efficiency of drugs was provided in July-August of 2019 at a commercial farm where samples from calves of local and black-and-white breeds aged 2-3 months were studied using coproscopic methods. Cysts of *Giardia* spp. were detected by staining a native faecal smear with Lugol's solution, and oocysts of *Eimeria* spp. – by Fülleborn and McMaster methods [11, 12]. As a result, 32 calves, simultaneously infected with *Eimeria* and *Giardia*, with signs of diarrhea were isolated, and four experimental groups with 8 animals at each group were formed according to the analogue principle.

The first group calves were remaining untreated.

The second group animals treated orally by Metronidazole-25% (100 mg/kg), the third group calves – by probiotic Vetom-1 (50 mg/kg) for seven days.

The fourth group calves were treated by the new treatment regime with using

recommended as an alternative to specific drugs against protozoa [9]. It was reported that monthly feeding of newborn *Cryptosporidium*-infected calves with *Saccharomyces cerevisiae* fermentation products resulted in less fragmented and atrophied villi of intestines in comparison to untreated controls, suggesting a preventive effect of these products against the infection [10].

The purpose of this study was to evaluate the efficiency of the new treatment regime by using a probiotic in combination with a specific antibiotic against the calves' diarrhea caused by a mixed infection with coccidia and giardia.

Metronidazole-25% at the first three days, and additionally – Vetom-1 at the next four days in above dosage. Diarrhea symptoms assessed on a 4-score scale before and the next day after the treatment.

During the experiment drugs were mixed with feed and given to animals in the morning and evening.

The efficacy determined at the 10th day after treatment according to shedding cysts' level. For determination the intensity of invasion (II) by *Eimeria* McMaster method was used for counting the oocysts 'number per g of faeces (COG). The II by *Giardia* was fixed by the number of cysts in 10 random fields of view of the eyepiece at a microscope magnification of ×400. In accordance with these variables, intensity efficiency (IE, %) and extensive efficiency (EE, %) were determined according to generally accepted methods [12].

Table 1 – The treatment regimen

	Experimental groups			
	the first (control)	the second *	the third *	the fourth**
Number of calves	8	8	8	8
Metronidazole-25%, mg/kg	-	100	-	100
Vetom-1, mg/kg	-	-	50	50
Frequency of processing per day	-	2	2	2

Notes: * - Medicines were given for 7 days

** - Metronidazole was administered on the first three days, Vetom-1 was additionally fed from the fourth to 7th days.

To control the clinical condition before and at the next day after the end of treatment the diarrheal status of each calve was assessed by 4-score scale, according to the consistency of faeces: 1 – hard-formed, 2 – soft-formed, 3 - semisolid, 4 - liquid [1]. Weight gain of animals was measured before and 10 days after the end of treatment.

The obtained quantitative results were processed statistically in a Microsoft Excel table.

Results

During the experiment, in the first group the intensity of oocysts and cysts shedding by calves before and after treatment remained at a similarly high level (table 2). In the second group of animals the dynamics of excreted number of *Eimeria* oocysts and *Giardia* cysts decreased by 86.6 and 53.4%, respectively, and the number of oocysts and cysts in the third group remained at the level that it was before treatment. In the fourth group of calves, where treatment was carried out using metronidazole and vetom, the level of excretion of *Eimeria* oocysts after treatment decreased by 98.5%, and *Giardia* cysts - by 88.5% (Table 2). In the second experimental group, the IE of the drug against *Eimeria* spp. was

90.9±9.3% and EE was 75%, while against *Giardia* spp. – 91.3±4.8% and 37.5%, respectively. In the third group, the intensity indicators remained unchanged. In the fourth group, IE against *Eimeria* and *Giardia* reached 99.9±0.2% and 98.9±1.5%, respectively, and the elimination of both parasites by EE was 87.5% (Table 2).

With regard to clinical indicators, the rate of calves' weight gains per day in the control group was at the lowest level, and signs of diarrhea remained unchanged (table 3).

The animals' weight gain level in the second group was approximately equal to the first group (0.70 ± 0.05 kg), and the diarrhea's indicator remained in most of calves (2, 3 scores).

The third group's calves, treated with the probiotic Vetom-1, the weight increased to 0.78±0.29 kg compared to the two previous groups, among animals the diarrhea varied in 1 and 2 scores.

In the fourth group, it was found that the calves' daily weight gain increased, on average, to 0.89 ± 0.06 kg, and the signs of diarrhea stopped (Table 3).

Table 2 – Efficiency the regimes at the 7th day of treatment

The group №	Number of calves	Treatment efficiency against							
		<i>Eimeria</i> spp.				<i>Giardia</i> spp.			
		II, COG, M±m		IE, %, M±m	EE, %	II, number of cysts in fields of view, M±m		IE, %, M±m	EE, %
		before treatment	after treatment			before treatment	after treatment		
1	8	637.5±190.8	718.7±118.0	-	-	42.1±8.7	51.0±9.2	-	-
2	8	1025.0±163.9	137.5±140.4	90.9±9.3*	75	38.6±7.9	18.0±4.3	91.3±4.8*	37.5
3	8	887.5±198.8	750.0±176.1	0	0	50.6±5.8	41.5±11.4	0	0
4	8	793.7±220.5	6.3±9.5	99.9±0.2*	87,5	51.8±9.9	0.75±1.2	98.9±1.5*	87.5

Table 3 – Influence the treatment regimes on clinical indicators

The group №	Number of calves	Indicators				
		weight gain, kg			diarrhea, scores	
		mass of a calve, M±m		weight gain for a day, kg, M±m	before treatment	at the next day after treatment
		before treatment	at the 10th day after treatment			
1	8	97.6±6.7	105.7±7.1	0.66±0.26	3, 4	3, 4
2	8	90.1±8.4	102.0±1.4	0.70±0.05	3, 4	2, 3
3	8	98.9±9.8	112.9±6.8	0.78±0.29	3, 4	1, 2
4	8	93.8±8.9	116.6±4.5	0.89±0.06	3, 4	1

Discussion

In cattle farms contagious intestinal diseases of newborn calves are caused by several infectious and invasive pathogens. They develop pathological processes manifested by diarrhea, which in most cases is due to the action of enterotoxic microorganisms (the strains of *Escherichia coli*, *Eimeria*, *Giardia*, *Cryptosporidium*). The protozoan are triggered development of the intestinal destructive processes, and then lead to disorders of bacterial and viral flora [2].

The chemical treatment is considered as the main measure for control of parasitic diarrhea. Antiprotozoal drugs make up a significant proportion of the modern veterinary market [5]. For elimination of protozoans, causing the calves diarrhea, the metronidazole from the nitroimidazole group is widely

used. The drug refers to the pharmacological group of antibiotics. It inhibits nucleic acid synthesis of microbial cells by forming [nitroso radicals](#), which disrupt the DNA and caused the death of anaerobic bacteria and protozoans. Metronidazole is primarily used to treat [trichomoniasis](#), [giardiasis](#), and coccidiosis of cattle. For example, it is proved that efficacy of 12.5-25% metronidazole against animals' giardiasis is at a high level [13]. When it was used against the calves' eimeriids twice a day for five days the drug rendered a coccidostatic effect. The animals' general condition has improved the next day, but the diarrhea signs were observed within 4-5 days [14].

In our experiment there was also shown that Metronidazole-25% in recommended doses has a high eliminative activity against a mixed invasion of calves: its IE against *Eimeria* spp. was $90.9 \pm 9.3\%$ and EE – 75%, and against *Giardia* spp. – $91.3 \pm 4.8\%$ and 37.5%, respectively. However, the diarrhea indicator among calves of this experimental group during the observation period changed slightly and was 2-3 scores. The daily weight gain of this group's calves also corresponded to such a control group, where the treatment of animals did not conduct.

According to literary sources, by treatment with drugs against protozoans the useful intestinal microflora is also destroyed which contributes to dysbacteriosis, and long-term diarrhea occurs in young animals.

As a rule, probiotics are used for prevention the diarrhea during treating with antibacterials [14]. The coccidostatic effect of some lactic acid bacteria was also described. For example, when a fermented milk was added in the feed of calves, as a protective factor, a shedding of *Cryptosporidium parvum* oocysts reduced. This is due to the fact that the fermented milk contains probiotics (like *Lactobacillus* sp.), and the modifying activity of these bacteria prevents colonization and reproduction of pathogens. When laboratory animals were received the feed with *L.acidophilus* and *L.reuteri*, the *C.parvum* oocysts' excretion decreased [14].

However, in our experiment when the group of calves with mixed infection by *Eimeria* and *Giardia* was treated by Vetom-1, the probiotic did not show an

antiparasitic action. Nevertheless, the diarrheal symptoms of animals in this group stabilized, and the faeces consistency came to normal. The animal weight gain indicator of the group was higher compared to the control group.

In the fourth experimental group the new developed scheme with combined application of the antibiotic and probiotic for calves' treatment had shown the highest level of efficiency against *Eimeria* spp. and *Giardia* spp. pathogens. The calves' diarrhea stopped and the weight gain increased compared to animals of other groups.

Conclusions

The new treatment scheme with combination of Metronidazole and Vetom-1 that was offered for control the calves' diarrhea caused by the mixed infection has shown a high efficiency against *Eimeria* spp. and spp. *Giardia* (IE, respectively, 99.9±0.2% and 98.9±1.5%). Among calves treated by this regime the diarrheal symptoms completely disappeared, and the daily weight gain increased, on average, above 25.8% in compared to the untreated animals.

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ІРІ ҚАРА МАЛ ТӨЛІНІҢ ЭЙМЕРИЯЛАР МЕН ГИАРДИЯЛАР ТУДЫРАТЫН ДИАРЕЯНЫ ЕМДЕУ ҮЛГІСІ

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Түйін

Мақалада эймерия мен гиардиялармен табиғи залалданған, диарея белгісі бар 32 бас 2-3 айлық бұзауларға 2019 ж. шілде-тамызында шаруашылық жағдайында қойылған тәжірибе сипатталады. Бұзаулар копроскопиялық әдістерімен таңдалынды және 8 бастан төрт топқа бөлінді. Эксперимент төменгі ретпен қойылды: бірінші бақылау тобындағы төлдер дәріленбеді. Екінші топтағы төлдерге Метронидазол-25%, үшінші топтағы жануарларға Ветом-1 пробиотигі жеті күн бойы жеммен қосып дәріленді. Төртінші топтағы бұзауларға жаңа емдеу үлгісі қолданылды: оларға алғашқы үш күні Метронидазол-25%, келесі төрт күні Ветом-1 пробиотигі қоса берілді. Емдеу тиімділігі (интенсэфективтілігі – ИЭ,%; экстенсэфективтілігі – ЭЭ,%) дәрілеу тоқтатылғаннан кейін 10-шы күні паразит цисталарының шығу қарқыны, сонымен қатар, бір күндік салмақ қосуы арқылы анықталды. Дәрілеуге дейін және емдеу аяқталған соң келесі күні әр бұзаудың диарея белгілері 4-балдық жүйе бойынша бағаланды. Нәтижесінде бақылау тобындағы төлдерде паразиттерді бөлу қарқыны өзгермеді; күндік дене салмағын қосуы ең төмен деңгейде болды, ал іш өту белгілері өзгермеді. Екінші топта *Eimeria* spp. қарсы дәрілеудің ИЭ $90.9 \pm 9.3\%$ және ЭЭ 75%, ал *Giardia* spp. қарсы, сәйкесінше, – $91.3 \pm 4.8\%$ және 37.5% болды. Осы топта салмақ қосу деңгейі бақылау тобымен шамалас болды, ал диарея сақталды. Ветом-1 пробиотигі берілген топта ИЭ және ЭЭ бірыңғай деңгейде қалды, дене салмағы алдыңғы екі топқа қарағанда жоғарылады, іш өту 1-2 балға дейін бәсеңдеді. Төртінші топта дәрілеудің эймерия және гиардияларға қарсы ИЭ, сәйкесінше, $99.9 \pm 0.2\%$ және $98.9 \pm 1.5\%$ болды, ал ЭЭ – екі паразитте де 87,5% құрады; төлдердің күндік салмағы, орта есеппен, 0.89 ± 0.06 кг-ға дейін жоғарылады, ал диарея белгісі тоқтады. Сонымен, *Eimeria* spp. және *Giardia* spp. микст-инвазиясы тудыратын ірі қара мал төлінің диареясы кезінде ұсынылған метронидазол мен ветом-1 пробиотигін қоса қолданатын жаңа емдеу схемасы жоғары тиімділік көрсетті.

Кілт сөздер: бұзаулар; *Eimeria* spp.; *Giardia* spp.; Метронидазол; Ветом-1; интенс эфективтілік; экстенс эфективтілік

СХЕМА ЛЕЧЕНИЯ ДИАРЕИ, ВЫЗВАННОЙ ЭЙМЕРИЯМИ И ГИАРДИЯМИ МОЛОДНЯКА КРУПНОГО РОГАТОГО СКОТА

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Аннотация

В статье описывается производственный эксперимент, который был проведен в июле-августе 2019 года на 32 телятах 2-3-месячного возраста с признаками диареи, спонтанно зараженных эймериями и гиардиями. Больных животных выделили копроскопическими методами и разделили на четыре группы. Телят первой контрольной группы не обрабатывали. Животным второй группы в течение семи дней задавали с кормом Метронидазол-25% в дозе 100 мг/кг, третьей группы – пробиотик Ветом-1 из расчета 50 мг/кг. Телятам четвертой группы применяли новую схему лечения: им в первые три дня задавали Метронидазол-25%, в последующие

четыре дня дополнительно пробиотик Ветом-1 в указанной дозировке. Эффективность лечения определяли на 10-й день после лечения по интенсивности (ИЭ,%) и экстенсивности (ЭЭ,%) согласно уровня выделения цист паразитов, а также привесу за сутки. Симптомы диареи до и на следующий день после окончания лечения оценивали по 4-балльной шкале. В результате установили, что до и после применения препаратов уровень выделения паразитов у молодняка контрольной группы был на одном уровне; привес тела за сутки – минимальным по сравнению с другими группами (0.66 ± 0.26 кг), а признаки диареи не менялись. Во второй группе ИЭ против *Eimeria* spp. составила $90.9 \pm 9.3\%$ и ЭЭ – 75% , а *Giardia* spp. – $91.3 \pm 4.8\%$ и 37.5% , соответственно. В этой группе привес в период эксперимента был примерно одинаковым с контрольной группой, а диарея сохранялась на уровне 2-3 балла. В группе, где применяли Ветом-1, интенсивность инвазии оставалась на одинаковом уровне, масса тела по сравнению с двумя предыдущими группами была выше (0.78 ± 0.29), а показатель диареи снижался до 1-2 балла. В четвертой группе наблюдали ИЭ против эймерий и гиардий $99.9 \pm 0.2\%$ и $98.9 \pm 1.5\%$, соответственно, а ЭЭ против обоих паразитов составила $87,5\%$; привес животных был выше, чем в других группах, и достигал, в среднем, 0.89 ± 0.06 кг, а признаки диареи отсутствовали. Таким образом, при диарее молодняка крупного рогатого скота, вызываемой микст-инвазией *Eimeria* spp. и *Giardia* spp., предлагается новая эффективная схема лечения, которая включает сочетанное применение препаратов Метронидазол и Ветом-1.

Ключевые слова: молодняк крупного рогатого скота; *Eimeria* spp; *Giardia* spp; Метронидазол; Ветом-1; интенсивность; экстенсивность