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STUDYING THE ANTAGONISTIC PROPERTIES OF LACTOBACTERIN-TK^2 PROBIOTICS ON PATHOGENIC STRAINS CAUSING GASTROINTESTINAL PATHOLOGY IN CALVES AND LAMPS

Abstract. This article contains the researchesof antagonistic activity of pathogenic bacteria causing a gastro-intestinal disorder in calves and lambs in their first days of life. The antagonistic activity on the pathogenic intestinal microflora has been studied *in vitro*.

Key words: *Escherichia Coli, Salmonella AbortusOvis, Proteus Vulgaris,* Lactobacterin-TK^2, calves and lambs, gastrointestinal pathology, research.

Introduction. Recently, among infectious diseases of calves and lambs, gastrointestinal diseases of bacterial etiology occupy a special place.

Pathogenic strains of Escherichia, Salmonella and Proteus, which cause gastrointestinal disorder in calves and lambs in their first days of life, play a leading role in the nosological profile of these diseases [1].

Pathogenic strains of *Escherichia Coli, Salmonella AbortusOvis* and *Proteus Vulgaris* belong to Enterobacteriaceaefamily and, by morphology, are small gram-negative straight rods [2].

According to the official definition, escherichiosis or colibacteriosisin animals is an acutely occurring zoonotic disease, which usually infests young animals, and characterized by diarrhea, septicemia, toxemia and enteritis, general dehydration of the body, disorders of the central nervous system, symptoms of gradually increasing depression and weakness.

Escherichia are permanent inhabitants of the intestines of warm-blooded animals, generally, they live in the lower parts of the gastrointestinal tract. Some of them can cause lesions of the gastrointestinal tract that was experimentally proven by G. N. Gabrichevsky in 1894 and confirmed clinically by A. Adam. Calves and lambs become ill with escherichiosismainly in the first 2-7 days of life, but also the disease often affects one- and two-month-old calves and lambs [3]. The incidence of disease can reach up to 90%, and the mortality rate is about 30-50%. The incubation period of the disease lasts from several hours to 1-2 days. Escherichiosisin calves and lambs can occur in the septic, enteritic and enterotoxemic forms [4].

A characteristic symptom of the disease is the feces color, which in most cases has a white, yellowish or bright yellowish color with a greenish tinge and a sour odor. In calves and lambs affected by escherichiosis, subfebrile temperature reaches 40-40.5°C, the rapid toxicosis development is manifested by the rejection of colostrum, lethargy and lying long. [5]

Salmonellosis is an infectious disease in young farm animals, which is characterized, when in acute form, by fever, septicemia and diarrhea, and when in chronic form – by pneumonia.

The sources of pathogen of this infection are sick and ill animals. Adult animals can be salmonella carriers, releasing the pathogen with milk and feces, abortus fetus, amniotic fluid, and outflows from the birth canal. Sick young animals produce a pathogen with feces, urine, nasal effusion, saliva [6].

In case of delayed medicated treatment, the condition of sick animals worsens by the end of the first or second day of disease and is characterized by a complete lack of appetite and sucking reflex, adynamia, anuria, a decrease in body temperature to 36-37.7°C, cyanosis and dry mucous membranes, deep drooping of the eyeballs, involuntary outflow of watery feces from the anus. Calves and lambs predominantly die from dehydration.

Proteas are one of the most well-known genera of the Enterobacteriaceae family.

All types of proteas belong to the group of conditionally pathogenic microorganisms, which exhibit a negative effect on the body while reducing antimicrobial protection. For the development of infection, the virulent properties of bacteria are important. The most important pathogenic factors of the proteas are: fimbriae, bacterial proteas and urease, hemolysins, hemagglutinins, and the ability to "swarm".

Dysbiotic disorders of the gastrointestinal tract can cause acute intestinal diseases in newborn calves and lambs due to the prevalence of enterobacteria over the symbiotic flora, that is a feature in the development of intestinal microbiocenosisin calves and lambs in the first 7 days of life [7].

Enteropathogenic bacteria are able to show resistance to certain types of antibiotics. Recently, one of the important advances in the field of medicine is the establishment of the fact that the microflora inhabiting the organism of animals is not beneficial only, but necessary for its vital activity as well. It is known that the most numerous and complex in terms of its composition is the bacteria population in the intestine, especially in its lower parts [8].

Therefore, the search for effective forms of prophylaxis, using the beneficial microflora, which are able to stop the spread and development of dysbiotic conditions of the gastrointestinal tract of calves and lambs in time, is a hot topic.

Relevance of research. In our country, gastrointestinal diseases of bacterial etiology in calves and lambs remain relevant and continue to cause significant economic damage, since without introducing new means of prevention and therapy of these diseases it will be impossible to obtain high-quality animal products [2].

These pathologies also affect the normal gastrointestinal microflora of the animal organism. A number of scientists believe that one of the stimulating factors for occurrence of gastrointestinal pathologies contributes to the disorders in the gastrointestinal microbiocenosis. These disorders causing this series of disorders are united by a common name - dysbacteriosis [9].

A number of authors believe that the cause of dysbacteriosis is the excessive use of drugs, in particular antibiotics and other antimicrobial drugs. Their accumulation in the body, especially in young animals, leads to a weakening of natural resistance and, as a rule, leads to the formation of infectious processes [1, 3, 9, 10].

It is well known that probiotics are one of the most promising agents for treatment and prevention of dysbiotic conditions. Probiotic drugs or food products contain live microorganisms, most often lactobacteria (lactobacilli, bifidobacteria, enterococci). World experience shows that in the prevention and treatment of gastrointestinal diseases in young animals, replacement therapy is important, which is aimed at restoring the intestinal biocenosis through the regular introduction of live bacteria, representatives of the normal intestinal microflora. The drugs they contain are known as probiotics. Probiotics are used to stimulate the immunity, prevent and treat mixed gastrointestinal infections, digestive disorders resulting from a sudden change in the diet composition, disturbed feeding regimes, technological stress, etc. [1, 11].

Currently, many farms successfully practice of calf growing [12].The advantage of probiotic agents containing lactobacilli is that they are harmless to the organism and there is no addiction to them during prolonged use, there are no side effects completely [13].

Purpose and methods of research. Antagonistic activity of associations of probiotic bacteria was determined by co-cultivation in relation to the culture of Escherichia Coli, Salmonella Abortus Ovis and Proteus Vulgaris.

On dense nutrient media, these pathogens form round colonies of 2-4 mm in diameter, with a smooth, convex surface and a smooth edge. Escherichia on MPA (Meat-Peptone Agar) - the colonies are translucent, grayish. On Levin's medium – they are dark blue, purple, black with or without metallic luster; on Mc-Conkey medium - pink, red (some strains of Escherichia may not ferment lactose and form colorless colonies on the listed media). Proteas and colonies of pathological microorganisms of other serogroups on BGA (Brilliant Green Agar) form pale pink or red-crimson colonies, transparent, surrounded by a brilliant

a) Salmonella Abortus Ovis and *Proteus Vulgaris*

b) Stains of Escherichia, *Salmonella Abortus Ovis* and *Proteus Vulgaris*

c) Stain of *Escherichia Coli*

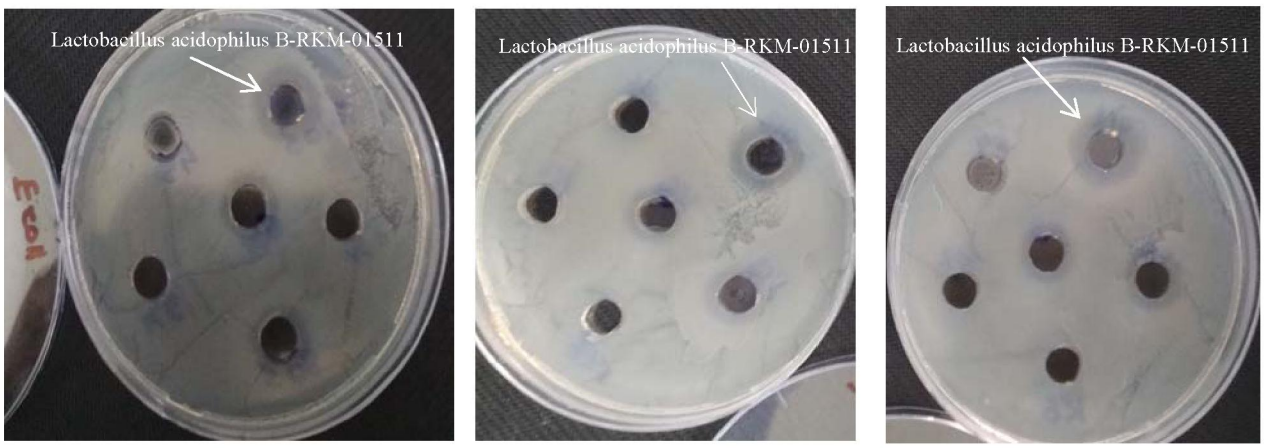
Figure 1 – Colonies of *Escherichia Coli, Salmonella Abortus Ovis and Proteus Vulgaris* on a dense nutrient medium

red halo. Salmonella on BSA (Bismuth Sulphite Agar) usually form black colonies with metallic luster, surrounded by blackening as a result of hydrogen sulfide production and recovery of sulphite to ferrous sulphide, which is black.

For the researches, the deposited passported lactobacillus strain, *Escherichia Coli, Salmonella Abortus Ovis and Proteus Vulgaris,* contained in Lactobacterin-TK[2], were used. Cultivation of the Lactobacillus acidophilus B-RKM-01511 strain of probiotic Lactobacterin-TK[2] was carried out under aerobic conditions at a temperature of 37°C for 24-48 hours in a milk hydrolyzate medium. Enterobacteria were cultured under aerobic conditions at 37°C for 24 hours in Müller Hinton agar medium.

A diffusion well method was used. Separately, 1 ml of cultures of Escherichia Coli, Salmonella Abortus Ovis and Proteus Vulgaris grown within 24 hours were added to sterile Petri dishes, having a titer of 105 microbial cells per ml according to the turbidity standard for opportunistic strains of these bacteria, and then 20 ml of molten and cooled to 40-45°C MPA. When the coating of dishes has harden in the form ofmetal stamp, wells with a diameter of 10 mm were cut out, and 100 µl of probiotic bacteria associations, which are part of Lactobacterin-TK[2], were introduced thereinto. After incubation at room temperature, the dishes were placed in a thermostat (37°C) for 24-48 hours. Then, the diameter of growth inhibition areas of tested microorganisms around the well, including its diameter, was determined.

The results of research of the probiotic activity of Lactobacterin-TK[2] are shown in table.



a) Escherichia Coli,

b) Salmonella Abortus Ovis

c) Proteus Vulgaris,

Figure 2 – Determination by the diffusion method of the well diameters of growth inhibition areas of tested microorganisms

Zone of growth inhibition of the antagonistic activity of the culture of probiotic bacteria Lactobacillus acidophilus B-RKM-01511 against *Escherichia Coli, Salmonella Abortus Ovis and Proteus Vulgaris*

Culture	Antagonistic activity of the probiotic drug (growth ingibition zone in mm)		
	Lactobacillus acidophilus B-RKM-01511		
	Sensibility	Dose-dependent zone	Resistance
Escherichia Coli	≥22	20-21	≤19
Salmonella Abortus Ovis	≥26	23,5-25	≤22,5
ProteusVulgaris	≥21	20-20	≤19

From the data in table it can be seen that the probiotic strain of Lactobacterin-TK^2- Lactobacillus acidophilus B-RKM-01511 has antagonistic activity to strains Escherichia Coli, Salmonella Abortus Ovis and Proteus Vulgaris.

It was found that the strains, which are part of probiotic Lactobacterin-TK^2 are able to show antagonism with regard to *Escherichia Coli, Salmonella Abortus Ovis and Proteus Vulgaris*that determines the high efficacy of the drug.

Results. Made researches have shown that Lactobacterin-TK^2 probiotic strain – Lactobacillus acidophilus B-RKM-01511 has a high antagonistic activity and is able to succeed in the gastrointestinal tract of calves and lambs. The results can serve as a basis for the inclusion of Lactobacterin-TK^2 in the scheme for complex use in the prevention of dysbiotic conditions of the gastrointestinal tract in sick animals.

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БҰЗАУЛАР МЕН ҚОЗЫЛАРДЫҢ АСҚАЗАН-ІШЕК ЖОЛЫ ПАТОЛОГИЯСЫН ТУДЫРАТЫН ПАТОГЕНДІ ШТАММДАРҒА ҚАРСЫ "ЛАКТОБАКТЕРИН-ТК^2" ПРОБИОТИГІНІҢ АНТАГОНИСТІК ҚАСИЕТТЕРІН ЗЕРТТЕУ

Аннотация. Мақалада жаңа туылған бұзаулар мен қозылардың асқазан-ішек жолы қызметінің бұзылуына әкелетін патогенді бактериялардың антагонистік белсенділігі зерттелген.In vitro жағдайында ішектің патогенді микрофлорасына қарсы антагонистік белсенділік зерттелді.

Түйін сөздер: *Escherichia Coli, Salmonella Abortus Ovis, Proteus Vulgaris,* Lactobacterin-TK2, бұзау және қозылар, асқазан-ішек патологиясы, зерттеу.

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ИЗУЧЕНИЕ АНТАГОНИСТИЧЕСКИХ СВОЙСТВ ПРОБИОТИКА «ЛАКТОБАКТЕРИН-ТК^2» НА ПАТОГЕННЫЕ ШТАММЫ ВЫЗЫВАЮЩИЕ ЖЕЛУДОЧНО-КИШЕЧНУЮ ПАТОЛОГИЮ У ТЕЛЯТ И ЯГНЯТ

Аннотация. В статье изучена антагонистическая активность патогенных бактерий, вызывающих расстройство деятельности желудочно-кишечного тракта у телят и ягнят в первые дни жизни. В условиях *in vitro* изучена антагонистическая активность на патогенную микрофлору кишечника.

Ключевые слова: *Escherichia Coli, Salmonella Abortus Ovis, Proteus Vulgaris,* препарат «Лактобактерин-ТК^2», телята и ягнята, желудочно-кишечная патология, исследования.

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REFERENCES

[1] Suvorov A., Gromova L., Borschev Y., Ermolenko E., Karaseva A., Gruzdkov A. Influence of probiotic enterococci on the gastrointestinal tract of rats before andafter the treatment of antibiotic associated dysbiosis // The Joint Meeting of The XVIIth International Symposium on Gnotobiology and The XXXIVth Congress of theSociety for Microbial Ecology and Disease Navios Yokohama. Japan: Yokohama, 2011. P. 108.

[2] Bondarenko V.M., Rubakova Je.I., Lavrova V.A. Immunostimulirujushhee dejstvie laktobakterij, ispol'zuemyh v kachestve osnovy preparatov probiotikov // Mikrobiol. zhurn. 1998. N 4. P. 107-111.

[3] Gaggìa F., Mattarelli P., Biavati B.Probiotics and prebiotics in animal feeding for safe food production // International Journal of Food Microbiology. 2010. Vol. 141. P. 15-28.

[4] Gavrilova N.N., Ratnikova I.A., Bajakyshova K., Utegenova N.M., Belikova O.A. Sposobnost' molochnokislyh bakterij probiotika polilaktovit sintezirovat' biologicheski aktivnye veshhestva // Izvestija NAN RK. Serija biologicheskaja i medicinskaja. 2017. N 6. P. 112-119. ISSN 2518-1629 (Online), ISSN 2224-5308 (Print). https://doi.org/10.32014/2018.25181629

[5] Tulemisova Zhet al. Prophilaxy of gasstro-intestinal diseases of young animals // Jour. of Anim. and Vet. Ad. 2013. Vol. 12. P. 1645-1650.

[6] Zhang X.S., Chase-Topping M.E., McKendrick I.J., Savill N.J., Woolhouse M.E.J. Spread of E. coli O157 infection among Scottish cattle farms:stochastic models and model selection // Epidemics. 2010. Vol. 2, N 1. P. 11-20.

[7] Vlková E., Rada V., Trojanová I., Killer J., Smehilová M., Molatová Z. Occurrence of bifidobacteria in faeces of calves fed milk or a combined diet // Arch AnimNutr. 2008. Vol. 62, N 5. P. 359-365.

[8] García-Hernández Y. et al. Isolation, characterization and evaluation of probiotic lactic acid bacteria for potential use in animal production // Res. Vet. Sci. 2016. Vol. 108. P. 125–132.

[9]Von Baum H., Marre R. Antimicrobial resistance of Escherichia coli and therapeutic implications // Int. J. Med. Microbiol. 2005. Vol. 295. P. 503-511.

[10] Ripamonti B. et al. Screening of species-specific lactic acid bacteria for veal calves multi-strain probiotic adjuncts // Anaerobe. 2011. Vol. 17, N 3. P. 97-105.

[11] Bayatkouhsar J., Tahmasebi A.M., Naserian A.A., Mokarram R.R., Valizadeh R. Effects of supplementation of lactic acid bacteria on growth performance, blood metabolites and fecal coliform and lactobacilli of young dairy calves // Anim. Feed Sci. Technol. 2013. Vol. 186, N 1–2. P. 1-11.

[12] Semenov V.G. at al. Activation of adaptogenesis and bioresource potential of calves under the conditions of traditional and adaptive technologies // Bulletin of NAS of RK. 2019. Vol. 1, N 377. P. 175-189. 2518-1467 (Online), ISSN 1991-3494 (Print). https://doi.org/10.32014/2019.2518-1467.20

[13] Masucci F., De Rosa G., Grasso F., Napolitano F., Esposito G., Di Francia A. Performance and immune response of buffalo calves supplemented with probiotic // Livest. Sci. 2011. Vol. 137, N 1-3. P. 24-30.