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Prophilaxy of Gasstro-Intestinal Diseases of Young Animals

Zh. Tulemisova, K. Biyashev, B. Biyashev, G. Kasenova, Z. Kozhakhmetova and D. Sarybaeva
Department of Biological Safety, Kazakh National Agrarian University, Almaty, Kazakhstan

Abstract: Fundamental studies of modern medical and veterinary science, advances in the knowledge of the multifaceted aspects of the relationship of macro and microorganisms allowed developing and introducing new biologics class probiotics which are based on live microbial cultures that have complex properties beneficial to the microorganism. The problem of sanitation, safety and productivity of the animals as well as get full on the biological quality of livestock products with probiotics is very promising, it requires profound both basic and applied research in biotherapy problem with the use of live cultures of micro-organisms that make up the human bodys and animals normal ecological community. The use of bio-safe products-probiotics become a priority in the livestock industry of Kazakhstan.

Key words: Lactic acid bacteria, yeast, Escherichia, antagonism, probiotics

INTRODUCTION

Saving newborn animals and growing a healthy, well-developed and adapted to the new conditions of detention of young animals is the basis of increasing the output of livestock products. The main losses are caused by gastro-intestinal diseases. Foreign literature data sources and the data from the studies indicate that such a disease in young infants animals found in 70 and even 100% of cases with significant mortality. The most difficult to keep young animals in the first 6-15 days of life. During this period, accounting for about 40% of the case of mortality. In addition, recover from an early age young animals develop worse in the future, reducing its resistance and increase its weight of 15-20% (Polotskii and Aveeva, 1981; Malik and Panin, 2001; Tarakanov, 2000).

The neonatal period and colostric unsoldering (from birth to 10 days old) has a special place in terms of prevention of gastrointestinal disease which is associated with a number of physiological characteristics of newborns (Gilbert *et al*., 1993; Vatasescu-Balcan *et al*., 2006; Lin *et al*., 2001; Nielsen *et al*., 2003; Grzybowski *et al*., 1998; Dring *et al*., 2006; Agerholm, 2007; Biyashev *et al*., 2010a-c).

Anatomical and physiological structure of the placenta of cows, ewes, pigs prevents the entry of antibodies from the mother to the fetus in uterus development. Therefore, they are has borned without the immunoglobulins in the blood and are exposed immunologically to genetically foreign substances including infectious agents. In this state, they remain until yet have sufficient maternal colostrum. Colostrum contains in its composition is 10-20 times more gamma globulin than in plasma; it contains a large number of macrophages, T and B-lymphocytes and other biologically active substances. The largest number of immunoglobulins and cellular elements contained in the colostrum of the first milking. The above factors contributing to the emergence and spread of gastrointestinal illnesses, make the animals of the early postnatal period as vulnerable to the etiological agents. Among them, it is first necessary to note pathogenic strains of Escherichia, Salmonella, Klebsiella, Proteus, Streptococci (diplococci), Yersinia, Staphylococcus, Rotavirus, Corona virus, Enterovirus and Parvovirus. These microorganisms are widely circulated in the farms have a wide range of virulence (enterotoxigenic, adhesiveness, hemolytic activity, antibiotic resistance).

Because of this complexity, the etiological structure of the gastro-intestinal disease is difficult to organize a regular and effective system of treatment and preventive measures that should be based on an accurate diagnosis and constructed taking into account the specific situation of the epizootic.

Acute diarrheal diseases of calves, lambs, pigs and chickens are widespread in Kazakhstan. According to statistics, they rank first among the currently registered in the republic of diseases of newborn animals and remain the most difficult problem of veterinary medicine.

Prevention of intestinal infections acquired social significance since in parallel with the increase in consumption of cattle, pig and poultry products increases the risk of contamination of the Enterobacteriaceae and other microorganisms food toxic infections pathogens in humans. This is evidenced by the massive human cases

Corresponding Author: Zh. Tulemisova, Department of Biological Safety, Kazakh National Agrarian University, Almaty, Kazakhstan

in 2011 across Europe escherichiosis and is therefore one of the key objectives of public health and veterinary medicine.

Practice shows that existing at present, a complex of zoohygenic, veterinary practices in the rearing of animals can not maintain a high level of resistance to bacterial infections caused by pathogenic microflora. The use of antibiotics for the prevention and treatment of gastrointestinal diseases of animals often leads to the emergence of the pathogenic micro-organisms to antibiotics polyresistance, lowers product quality and become less effective.

With the accession of Kazakhstan to the WTO will need to introduce a system of quality management, the basic principles of which are enshrined in the ISO9001: 2000 which required the use of real declaration of pharmaceutical products.

The veterinary services of many countries have a sufficient number of different species of probiotic preparations (biosporin, bacterin-SL, endosporin, sporolakt, bifikol, SBA, etc.) for the prevention of gastrointestinal diseases of young animals and birds. However, monitoring the probiotic market shows that the overwhelming majority uncalled practices by Tarakanov (2000), Malik and Panin (2001, etc., Bifidobacteria and their use in the clinic, the medical industry and agriculture: Blokhin, I., V. Dorofeychuk, L. Disbacteriosis in 1979; Antipov, V., V. Subbotin, performance and prospects of application of probiotics. Veterinary Medicine, 1980, No. 12, Works of MSRIEM by the name G.N. Gabrichevski, 1986; US Patent No. 3876807, cl. S12N1/20; Patent No. 2018313, cl. C 12N1/20, 1994).

This is explained by the fact that most of the probiotics include strains isolated from human intestinal or taken from a collection of strains of food biotechnology. This applies to strains *Bif. lactis*, *Bif. bifidum*, *Bif. adolescentis*, *Bif. longum*, *L. acidophilus*, *L. plantarum*, *L. fermentum*, etc. Note those lactobacilli and bifidobacteria as well as other microorganisms not all the same. *Bifidobacterium* genus for example, unites 24 species of microorganisms. Genus of *Lactobacillus* more. Some species are only certain animal species other in many animal species and others in humans and animals, the fourth only in humans. Accordingly, the ability to colonize the intestine in humans, animals or birds is different. In selecting for strains of probiotics necessary to consider these and many other biological properties (Biyashev *et al*., 2011a-c).

Moreover, modern probiotics often have several disadvantages: duration of use, poor survival in the intestines due to the low colonizing ability (adhesiveness and low growth rate) used medicines act only on single species of pathogenic microorganisms, various methods for screening probiotic strains, poor the quality of the medicines (small or large number of living microbial cells in a dose or extraneous microbial contamination); unsatisfactory safety of the medicines in the field. Therefore, the physiological activity and the effectiveness of different medicines depend on the composition of the strains, production technologies.

This requires revision of the existing methodological approaches to prevention and treatment for gastro-intestinal diseases and the need to develop a new generation of environmentally friendly products aimed at correcting the intestinal biocenosis of animals and increase colonization resistance of the intestinal mucosa to the contamination of the conditional-pathogenic microorganisms.

Probiotics help to restore the digestive system, the biological status, immune response in food-producing animals, increases the effectiveness of vaccination. In their application, decreases the incidence, the number of veterinary treatments and related material costs. Using these medicines stimulate the immune response of animals, normobiocenosis resumed while livestock products are environmentally friendly. In connection with this animal products is competitive in quality and price.

The aim of this research: The study of the biological properties of complex high-performance environmentally safe preparation of the normal flora, containing the association of lactic acid bacteria, yeast and genetically characterized safety strain of Escherichia, complementary to each other on the spectrum of the specific activity and aimed to correct the host's homeostasis.

MATERIALS AND METHODS

Objects and methods: The objects of the study was a new eco-friendly complex probiotic preparation Torukol includes two cultures of lactic acid bacteria (*Lactobacillus acidophilus* B-143, *Lactococcus lactis* B-263), yeast (*Torulopsis kefir* Y-252) and genetically characterized safety strain of Escherichia (*Escherichia coli* 64). The research was performed using methods that are used in international scientific practice and constantly improved by the patent information elaborations.

Researchers used advanced certified and standardized biochemical, microbiological, molecular biological research: culturing microorganisms on different composition nutrient media, identifying the antimicrobial activity by the agar diffusion method, determination of resistance of microorganisms to antibiotics the method of standard discs; electrophoresis, PCR, gas chromatography, electron microscopic studies of antibiotic selection and separation systems and flash column chromatography followed by bioautography against the test microorganisms.

The study of the genomic characteristics of strains of lactic acid bacteria, yeast and Escherichia was conducted at the Laboratory of Molecular Biology and Genetics Research Institute by the name NF Gamalei (Moscow), acting as a taxonomic Centre, Institute of Microbiology and Virology, Research Institute for Biological Safety Problems MO and S of RK.

Scientific studies conducted in research and diagnostic laboratories of Kazakhstan-Japan Innovation Center in the laboratories of the Department of Biological Safety of the Kazakh National Agrarian University which are equipped with modern facilities for molecular genetic studies: an atomic absorption spectrometer (Shimadzu AA-7000F), a gas chromatograph (Shimadzu GC-2010 Plus), liquid chromatograph Lc20AD, EPR-spectrometry, transmission electron microscope JEM-1011, scanning electron microscope JSM-65101LA, system identification of microorganisms and pathogenic fungi Sherlock (USA), real-time PCR for detection and quantification of specific DNA sequences in the sample (QPCR System Mh3005R 230), automatic immunoanalizator FT-2 (produced by AMS, Italy); ultramicrotome Leika-IMUC7; hardware system for photomicrography leika dim 4000B, etc.

The laboratory of the department has a museum of strains of microorganisms. Experiments on farm animals will be held in different farms of the Republic of Kazakhstan. Carrying out research based on the existing standards in the Republic of Kazakhstan, regulations, rules, guidelines and directives and decisions of the european communities (http://www.Eu.Int.), office international des epizooties (http://www.oie. int), the world trade organization, international standards (http://www.gost.int) have been studied over the internet.

Metrological control devices and laboratory equipment was provided in accordance with the requirements of state standard of the Republic of Kazakhstan in accordance with established regulations right. For the mathematical treatment of the results of standard methods of finding the mean values and their average errors.

Characteristics used cultures: Strain *Lactobacillus acidophilus* B-143 isolated from milk and using for production of fermented milk products, particularly high acidophilus milk. The strain was deposited in the collection of the museum of RSE research and production center of processing and food industry. Strain *Lactobacillus acidophilus* B-143 was grown on liquid medium from the hydrolyzed milk for 24 h at 37°C. For this purpose, pasteurized milk at 34-35°C added a 1% yeast, stirred and kept at this temperature until formation of a clot. Fermentation occurs after 9 h, the acidity of the bunch at the end of fermentation is 60-65°C. The strain is protected by a patent of the committee on intellectual property rights of the Republic of Kazakhstan, No. 15969.

The strain *Lactococcus lactis* B-263 was grown on skim milk at 37°C to formation of clot (24-48 h). The strain was deposited in the museum of cultures of microorganisms for the food industry of state enterprise KazSRIFI ME and S of RK. The strain is protected by a patent of the committee on intellectual property rights of the Republic of Kazakhstan, No. 41176.

Strain *Torulopsis kefir* Y-252 grown on skim milk at 37°C to formation of clot (24-48 h). The strain was deposited in the museum of cultures of microorganisms for the food industry of State Enterprise KazSRIFI ME and S of RK. The strain is protected by a patent of the committee on intellectual property rights of the Republic of Kazakhstan, No. 41176.

Strain *Escherichia coli* 64 are deposited in the collection of microorganisms SRAI of the National Center for Biotechnology of the Republic of Kazakhstan. Strain *E. coli* 64 is typical of the genus of *Escherichia*. *E. coli* strain 64 has a genetic marker that allows distinguishing it from the natural prototypes. A genetic study of strains of *E. coli* 64, carried out by using the transducting bacteriophage showed that the phenotype defines by the presence of two independent mutations, each of which can reduce the virulence is convincing a proof of the stability and security of the strain *E. coli* 64. The strain is protected by a patent innovation committee on intellectual property rights of the Republic of Kazakhstan, No. 23876. For the mathematical treatment of the results of standard methods of finding the mean values and their average errors.

RESULTS AND DISCUSSION

Production of complex bacterial medicine Torukol includes the following stages: manufacturing, the definition of preventive and therapeutic efficiency. A process for preparing probiotic Torukol includes separate culturing of strains *Lactobacillus acidophilus* B 143, *Lactococcus lactis* B-263, *Torulopsis kefir* Y-252 and *Escherichia coli* 64 in a medium, concentration, packaging into vials and lyophilizing.

Lactobacillus acidophilus B-143, *Lactococcus lactis* B-263, *Torulopsis kefir* Y-252 separately grown on skim milk at 37-38°C before clot formation (in 40-50 h). The resulting cultured mass was adjusted to pH 6.0-6.2, the number of viable cells was determined and then it was added with stirring components serving as protective medium with subsequent lyophilizing: molasses in an amount of 15 g L^{-1} and corn flour in amount of 50 g L^{-1}.

Strain *Esherichia coli* 64 grown on Hottinger broth for 16-18 h at 37-38°C. After verification of the resulting

microbial mass for purity and typicality growth was adjusted to a concentration of 10^{10} CFU in 1 mL of the optical turbidity standard SRI by the name of Tarasevich. Sterile medium for the lyophilizing was then added. Medium for the lyophilizing comprises of 1.5-2% gelatin, 10% Sucrose, pH 7.8-8.0.

The obtained suspension cultures of *Lactobacillus acidophilus* B 143, *Lactococcus lactis* B-263, *Torulopsis kefir* Y-252, *Esherichia coli* 64 were mixed in the ratios 1:1:1:1, dispensed into vials 20.0 cm^3 and then was lyophilized to dry product-probiotic. The vials were sealed with rubber stoppers and aluminum caps and packed in boxes. Keep only at 4-6°C. Shelf life of 12 months. At all stages of manufacture the medicine was carried out a biological control for the specificity of cultures.

The control of the medicine Torukol and the preparation was tested for purity, genericity growth, antagonistic activity in comparison with test cultures. To determine the purity and typicality of growth used 5 vials of the medicine. The contents of each vial diluted in 4 mL of sterile salt solution and plated in amount of 0, 2 mL from each vial to special nutrient medium. The preparation does not contain extraneous microflora. Culture media stand for 10 days at a temperature of 37-38°C.

The antagonistic activity of the medicine was determined in comparison with test cultures: *E. coli*, *Salm. typhimurium*, *Salm. dublin*, *Salm. choleriaesuis*, *Proteus vulgaris*, *Staphylococcus aureus*, *Streptococcus pneumonia*, *Klebsiella pneumonia* and *Bacillus subtilis*.

The antagonistic activity of the medicine Torukol studied in comparison with the widely used medicine in practice from *B. longum* 379M. The antagonist activity was determined by Microbiological Method-Agar Diffusion Method (method of holes). In Petri dishes in wells were slurry preparation Torukol and preparation of B. 379M strain in rate of 1.0 cm^3. Plates were incubated at 37°C for 18-24 h and measured the diameter of the lack of growth of the test cultures (Table 1).

Table 1: The results of determination of the antagonistic activity of the Torukol and *B. longum* 379M

	Hold-up zone of test cultures (mm)	
Test cultures	Torukol	B 379M
E. coli	20.6	15.2
Salm. typhimurium	22.9	10.4
Salm. dublin	29.4	16.3
Salm. choleriaesuis	27.5	14.5
Proteus vulgaris	24.8	13.7
Staphylococcus aureus	18.6	10.6
Streptococcus pneumonia	22.6	13.7
Klebsiella pneumonia	23.9	14.4
Bacillus subtilis	24.6	16.5

Of material can be seen that the Torukol inhibits the growth of all investigated test cultures, the inhibition zone of most of them more than in the B379M. This indicates a higher antimicrobial activity of the Torukol. Harmlessness of preparation was checked on 10 white mice weighing 14-16 g. Application was administered orally at a dose of 10^7 CFU in volume of 0.5 mL. For the test used two vials of the medicine. Within 10 days of observation all experimental white mice were live. The medicine is considered harmless.

The medicine activity is checked at 10 white mice weighing 14-16 g which was administered orally at a dose 10^7 CFU in volume of 0.5 mL. After 24 h, all experimental (10) and control (10) mice were orally infected by virulent culture of *E. coli* in lethal doses. All test animals survived and at all control mice are fall. The observation period to 10 days.

The method of prevention and treatment of gastrointestinal diseases in young farm animals includes a single oral using of the Torukol to newborn calves, lambs, piglets before the first feeding not later than 30 min after birth in appropriate doses.

Determination of the effectiveness of prophylactic efficiency of Torukol was performed on newborn calves, lambs, piglets by a single oral watering in appropriate doses (Table 2). After 24 h the experimental and control animals were infected orally by a virulent culture in lethal dose. The results are shown in Table 2. As can be seen from Table 2, the optimal effective preventive dose of the medicine is a 4×10^{10} CFU for calves, lambs for -10^{10} CFU, for piglets 10^{10} CFU.

Determination of the therapeutic efficiency of Torukol was performed on newborn calves, lambs and piglets. At the beginning of the test animals were orally infected by culture of virulent Salmonella, Proteus, Klebsiela and Bacilli in infectious doses (Table 3). On day 3 after the introduction of virulent cultures test animals were orally applicated by a single dose of Torukol. The control animals did not receive the medicine. The results are shown in Table 3. As can be seen from Table 3 Torukol has a high therapeutic efficiency.

The microorganisms that are part of the Torukol are symbionts of the gastrointestinal tract are harmless to humans and animals. The therapeutic effect they exert antimicrobial activity and thanks to the normalization of the intestinal microflora.

As a result of the research is designed and prepared a highly environmentally friendly probiotic from representatives of normal microflora based on the use of symbiotic relationships of lactic acid bacteria, yeast and genetically characterized strain of Escherichia aimed at

Table 2: Determination of the effectiveness of prophylactic efficiency of Torukol

			Results of contagion by virulent *E. coli*			
Species of animal	Quantity	Dose of preparation (CFU)	Contagious dose (CFU)	Fall	Survived	Survival (%)
Calves	10	10^{10}	2×10^{10}	-	10	100
Calves	10	3×10^{10}	2×10^{10}	-	10	100
Calves	10	4×10^{10}	2×10^{10}	-	10	100
Calves	10	5×10^{10}	2×10^{10}	-	10	100
Calves (Control)	5	-	2×10^{10}	5	-	-
Lambs	10	3×10^{9}	10^{10}	-	10	100
Lambs	10	5×10^{9}	10^{10}	-	10	100
Lambs	10	10^{10}	10^{10}	-	10	100
Lambs	10	2×10^{10}	10^{10}	-	10	100
Lambs (Control)	5	-	10^{10}	5	-	-
Piglets	10	3×10^{9}	10^{10}	-	10	100
Piglets	10	5×10^{9}	10^{10}	-	10	100
Piglets	10	10^{10}	10^{10}	-	10	100
Piglets	10	2×10^{10}	10^{10}	-	10	100
Piglets (Control)	5	-	10^{10}	5	-	-

Table 3: Determination of the therapeutic efficiency of Torukol

		Results		Results of application of medicine				
Species of animal	Quantity	Species	Dose of preparation (CFU)	Dose of preparation, (CFU)	Fall	Survived	Survival (%)	Note
Calves	5	*S. typhimurium*	10^{9}	4.10^{10}	-	5	100	-
Calves	5	*S. typhimurium*	10^{9}	-	5	-	-	At 6-7 days
Calves	5	*P. vulgaris*	10^{9}	4.10^{10}	-	5	100	
Calves	5	*P. vulgaris*	10^{9}	-	5	-	-	At 6-9 days
Calves	5	*K. pneumonia*	10^{9}	4.10^{10}	-	5	100	
Calves	5	*K. pneumonia*	10^{9}	-	5	-	-	At 8-9 days
Calves	5	*B. subtilis*	10^{9}	4.10^{10}	-	5	100	
Calves	5	*B. subtilis*	10^{9}	-	5	-	-	At 5-6 days
Lambs	5	*S. typhimurium*	10^{8}	10^{10}	-	5	100	
Lambs	5	*S. typhimurium*	10^{8}	-	5	-	-	At 5-8 days
Lambs	5	*P. vulgaris*	10^{8}	10^{10}	-	5	100	
Lambs	5	*P. vulgaris*	10^{8}	-	5	-	-	At 6-8 days
Lambs	5	*K. pneumonia*	10^{8}	10^{10}	-	5	100	
Lambs	5	*K. pneumonia*	10^{8}	-	5	-	-	At 6-8 days
Lambs	5	*B. subtilis*	10^{8}	10^{10}	-	5	100	
Lambs	5	*B. subtilis*	10^{8}	-	5	-	-	At 6-8 days
Piglets	5	*S. typhimurium*	10^{8}	10^{10}	-	5	100	
Piglets	5	*S. typhimurium*	10^{8}	-	5	-	-	At 4-7 days
Piglets	5	*P. vulgaris*	10^{8}	10^{10}	-	5	100	
Piglets	5	*P. vulgaris*	10^{8}	-	5	-	-	At 7-8 days
Piglets	5	*K. pneumonia*	10^{8}	10^{10}	-	5	100	
Piglets	5	*K. pneumonia*	10^{8}	-	5	-	-	At 6-8 days
Piglets	5	*B. subtilis*	10^{8}	10^{10}	-	5	100	
Piglets	5	*B. subtilis*	10^{8}	-	5	-	-	At 6-8 days

correcting the host's homeostasis. When selecting plants for the preparation of probiotic preparation took into account the following basic requirements:

- Is a normal inhabitant of the gastrointestinal tract of healthy animals to be non-pathogenic and non-toxic as the use of other bacteria can lead to unintended effect
- Have the ability to adhere to the epithelium and engraftment in the gastrointestinal tract where the enzymatic activity associated with the digestion of feed is high and corrosive
- To be metabolically active in the ecosystem of the gastrointestinal tract, the transfer passage through the stomach have a positive effect on the intestinal microbiocenosis, increase the overall resistance of the organism
- To be stable and able to remain viable for a long time if stored in a production environment

CONCLUSION

Thus, the probiotic preparation Torukol comprising the cultures of lactic acid bacteria, yeast and Escherichia has adhesive ability, high adaptability, eliminate long

term, high antagonistic activity against pathogenic and conditionally pathogenic microorganisms, the presence of the genetic tag which allows to distinguish strains-producers of natural prototypes and proof of their safety may be used for the prevention and treatment of gastrointestinal diseases of the newborn young animals and birds.

The Torukol is used for prophylaxis in areas where the gastrointestinal diseases caused by pathogenic enteric microbes. The medicine is indicated to newborn animals until the first feeding no later than 30 min after birth. The medicine is used at once, orally in doses: 4×10^{10} CFU for calves, lambs for 10^{10} CFU and for piglets 10^{10} CFU. If necessary, re-use of the medicine is recommended in 2-3 days.

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