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Persistence of the *Escherichia coli* 64G-Probiotic Strain in the Intestine of Calves

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Abstract

Fundamental research in modern biological and medical sciences and advances in the knowledge of multidimensional aspects of the relations between macroorganisms and microorganisms allowed the development and implementation of probiotics, a new class of drugs which is based on live microbial cultures and has complex properties beneficial to the microorganism, in public health and veterinary practices. In this article, the results of the experiment for studying the strain *E. coli* 64G persistence in 27 newborn calves with postmortem bacteriological investigation of the animals' intestine on the first, second, and third days are described. It was found that the strain has a high persistence rate in the intestine of calves due to the ability of bacteria to attach to the intestinal wall and reproduce in the inner intestine layer or chime.

Keywords

Probiotics; Colonization resistance; Intestine chimus; Identification; Persistence

Introduction

Prevention of gastrointestinal diseases gained social significance because the increase in consumption of animal products increases the risk of contamination by pathogenic and opportunistic microorganisms—agents of food poisoning in humans. In this regard, treatment and prevention of intestinal diseases requires the development of environmentally friendly products for correcting the animals' intestinal biocenosis and increasing the intestinal mucosa's colonization resistance to contamination by pathogenic microflora [1-3].

In modern livestock production, the probiotics, bacterial preparations of living microbial cultures, are widely used for the prevention of disease, the treatment of animals, and for increasing their productivity. Efficacy of probiotics is associated with favorable metabolic changes caused in the digestive tract, better absorption of nutrients, increasing the animals' resistance, as well as antagonistic effects on the pathogenic microflora. They do not cause side effects, have nocontraindications for use, and in conjunction with the veterinary sanitation interventions, could have a positive impact on the microbiocenosis in livestock buildings [4,5].

The probiotic microorganisms must be capable of actively multiplying in the gastrointestinal tract. They could produce biologically active metabolites to be resistant to gastric juice and bile. Probiotics should not have a contraindication for use and cause adverse reactions in an organism after introduction [6-8].

Microbial populations submit to the general environmental laws. The symbiosis between bacteria of different species in the intestine has many forms: neutralism, competition, parasitism, mutualism, and others. However, in spite of this scale variability, microflora is fast becoming a very stable population, which helps the animal to maintain a resistance to gastrointestinal infections. This phenomenon is called "bacterial antagonism," "bacterial interference," or "colonization resistance" [9,10].

Study of the above phenomena and their role in the digestive tract, necessary for the knowledge of microbial ecology of the intestine, and the results of research could be a basis for the creation of highly effective probiotics.

The purpose of the research was to study the persistence time of the probiotic *Escherichia coli* 64 G—strain in the intestine of calves.

Materials and Methods

The strain study was performed on 27 newborn calves, divided into three groups of nine animals each. Calves were orally administered the investigated culture of *Escherichia* immediately after birth before the first feeding.

The strain of *Escherichia coli* 64G, cultured on MPA, was used in the experiment. Cultures with significant growth were selected, and they were adjusted to a value of 109 CFU/ml according to the optical standard concentration of turbidity. Calves of the first group were desoldered once orally with 20 ml (2010 CFU) of the microbial culture; animals of the second group were desoldered once orally with 30 ml (3010 CFU) of the culture. Calves of the third group were used as a control group animals and were desoldered with saline solution.

In each group, after desoldering of the tested *Escherichia* culture, three calves were killed, and certain portions of their small intestine and colon were sampled. Under sterile conditions, intestinal segments were dissected, the surface was cleaned of food, and mucosal scrapings were done with capturing of the part of the intestinal chime. From these scrapings serial 10-fold dilutions to the value 10^{-6} at 0.9% sodium chloride solution were prepared. From each dilution, a drop of suspension was taken and applied to various solid nutrient mediums to determine the amount of bacteria of different taxonomic groups.

Inoculums streaked under the surface of the medium, after which the Petri dish with the inoculums was placed in a thermostat at 37-38°C for 24 h. For the cultivation and counting of the number of bacteria, the following media were used: Endo agar for *E. coli*, Ploskirev medium

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Group	*Escherichia*	Enterobacteria	Enterococci	Lactobacteria	Bifidobacteria
1 Experimental (after 24 h)	48 ± 0.04	14 ± 0.03	4 ± 0.02	3 ± 0.09	2 ± 0.02
2 Experimental (after 24 h)	55 ± 0.09	12 ± 0.02	6 ± 0.11	2 ± 0.11	1 ± 0.17
Control (background investigation)	18 ± 0.11	38 ± 0.11	22 ± 0.12	6 ± 0.14	4 ± 0.10
$p < 0.05$					

Table 1: The number of different groups of bacteria in the intestine chime of calves

for enterobacteria, Enterococcus agar for enterococci, and Blaurock medium for lactobacteria and bifidobacteria.

At the end of cultivation, counting the number of colonies of bacteria (CFU) of different groups in the intestinal chime of calves was carried out.

Results and Discussion

Bacteriological examination revealed that cultures of *E. coli*, enterobacteria, enterococci, lactobacteria, and bifidobacteria were allocated from the intestine of calves (Table 1).

In the results of bacteriological researches on the intestines of calves from control group, their chimes were allocated 14-18 cultures of *E. coli* and 32-45 cultures of enterobacteria.

Primary identification of isolated cultures on Ploskirev medium showed that 85-90% of them were *Salmonella* spp. (colonies are colorless, translucent, flat), 5-8%—*Klebsiella* spp. (pink with a yellow center, convex colonies), 1-2%—*Proteus* spp. (transparent, convex with yellowish-pink pearl shade colonies). Study of the antigenic properties of the enterobacteria isolated cultures confirmed their typical specificity. It should be noted that, in differentiation, the main part of *Salmonella* cultures was *Salmonella typhimurium* and *Salmonella dublin*.

Within 24 h after desoldering of the strain *E. coli* 64G, the amount of *Escherichia* increased considerably compared with the control group animals, regardless of the desoldering method of the test culture. Their numbers increased 5 and 7 times on the second and third days, accordingly.

Identification of *E. coli* cultures isolated from calves' intestines after desoldering of the probiotic strain showed that biochemical and cultural, antigenic and adhesive properties of all cultures corresponded to the strain *E. coli* 64G.

Within 24 h after administration of the probiotic strain the number of enterobacteriaceae in the intestine of calves significantly decreased 2-3 times compared to control group animals.

Enterococci in the intestinal chime were mainly represented by *Streptococcus faecalis*. The number of enterococci, lactobacteria, and bifidobacteria in the calves' intestinal chyme after 24 h of supplementation of *E. coli* strain decreased slightly compared with the control group, then on the second and third days after exclusion from diet the test strain increased 1.5-2 times than in control group animals.

Research results indicated that the *E. coli* strain in the intestinal content of the inoculated experimental animals and Escherichia (5-16 colonies on an average), enterococci (4-12), and lactobacteria (8-20 colonies) in the chyme of the inoculated control group animals were analyzed throughout the experiment.

Conclusion

The strain *E. coli* 64G had a high persistence rate in the calves' intestines, as evidenced by the terms of its release from the intestine and intestinal contents. The duration of persistence of the bacteria in the organism was conditioned by the ability of the strain to adhere to the intestinal wall and to reproduce in the wall layer or the intestinal contents. Currently, the strain of *E. coli* 64G bacteria is used to make the effective veterinary probiotic preparation "Antakon" against gastrointestinal diseases of newborn young animals. The created preparation "Antakon" is used in the farms of the Republic of Kazakhstan as a therapeutic and prophylactic drug against intestinal infections of farm animals and birds.

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