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# Study Antagonistic Activity, the Level of Resistance to Hydrochloric Acid and Bile Probiotic Strain *Escherichia coli*

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**Intestinal infections of young farm animals have very importance among the infectious diseases. In the list of measures to combat with them the main methods are treatment sick animals with antibiotics, sulfa drugs, and nitrofurans. However, the use of antibacterial agents often leads to the death of the normal microflora, breaking microbiocenosis of gastrointestinal tract, the appearance of microorganisms having resistance to drugs to lower quality products. In this regard, the direction of biotechnology, which designs and creation of environmentally friendly microbial drugs for prophylactic efficiency is very important.**

**Key words:** Newborns, *Escherichia coli*, antagonistic properties, bile, hydrochloric acid.

Saving of newborn animals and growing it healthy, well-developed and adapted to the new conditions of detention of young animals is the basis for increasing of livestock products. Major losses of the newborn animals caused by gastrointestinal diseases. Published data of foreign sources, as well as our research shows that such diseases of young newborn animals are found in 70 and even in 100% of cases with significant mortality. The most difficult to retain newborn animals in the first 6-15 days. During this period, accounting for about 40% mortality, also recover early juvenile's developing worse in the future, and reducing its resistance to the weight of 15-20%[1, 2, 3].

The neonatal and colostric period (till birth to 10 days of age) has a special place in terms of prevention of gastrointestinal diseases, which is associated with a number of physiological characteristics of newborns.

Anatomical and physiological structure of placenta of cows, sheep and pigs impedes the flow of antibodies from mother to fetus. Therefore, they are boned without the immunoglobulins in the blood and are immunologically and genetically unprotected from foreign substances including infectious agents. In this state they remain until sufficient quantity of maternal colostrum is obtained. Colostrum contains in its composition 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 greatest number of immunoglobulins and cellular elements contained in the first portion of colostrum. The above factors contributing the emergence and spread of gastrointestinal illnesses, make the animals in postnatal period maximum vulnerability for the etiologic agents. Among them, first of all it should

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be noted pathogenic serovars of E. coli, Salmonella, Klebsiella, Proteus, streptococci (diplococci), Yersinia, Staphylococcus, rotavirus, coronavirus, enterovirus, parvovirus. Because of this complexity, the etiological structure of gastrointestinal diseases is difficult and proper organization and effective treatment - preventive measures, which should be based on accurate diagnosis and tailored to the specific epidemic situation[4, 5, 6, 7].

World experience shows that in the prevention and treatment of gastrointestinal diseases of young animals high value replacement therapy aimed at restoring intestinal biocenosis by the regulatory introduction of live bacteria - representatives of the normal intestinal microflora[8, 9]. To restore the damaged of microbiocenosis in the gastrointestinal tract of the newborn animals in veterinary practice used probiotic preparations manufactured on the basis of strains of lactobacilli, bifidobacteria. However, monitoring the probiotic market shows that the vast majority of development is not in demand practice.

In the works of many researchers indicate that for the manufacture of veterinary probiotics should be used microorganisms isolated from the intestines of animals. Therefore, the selection of strains of probiotics for this criterion is important[10, 11].

This circumstance gives reason to believe that the approach to the development of probiotics should be based on the study of many parameters including the first comprehensive assessment of the properties of microorganisms - probiotics. These characteristics are antagonistic activity, a certain level of resistance to hydrochloric acid and bile.

In this regard, the development of probiotic strains E. coli, isolated from healthy young farm animals and having bacteriocin properties is the main focus of our research.

## METHODS

Material for bacteriological examination were parenchyma organs (heart, liver, spleen, kidneys), mesenteric lymph nodes, brain cortical bone and the contents of the small intestine of the fallen and slaughtered lambs, calves and piglets with signs of acute disorders of the gastrointestinal tract, as well as samples feces from healthy newborn animals. Of parenchyma organs, mesenteric lymph nodes, brain cortical bone and the contents of the small intestine seeding done on MPA, MPB, Kitt-Tarocci, Endo agar and Ploskirev agar.

Isolation and identification of the cultures was carried out according to the following procedure: crops from pathological material and fecal were incubated at $37^0$C for 18 -24 hours. In the absence of growth after incubation on agar Endo and Ploskirev and its presence in MPLB under paraffin oil, broth cultures investigated with microscope to study the morphology of the bacteria, and then replanted them on blood agar Ceyssler. After growing for 24 hours at $37^0$C in microanaerostate the cultures studied for colony growth nature, smears were prepared and stained by Gram stain.

Identification selected cultures was performed by determinant of Bergey (1984). The significance of differences was set using the G-test method of Student (Stanton Glantz, 1999).

## RESULTS AND DISCUSSION

The objects of study were strains of Escherichia coli, isolated from healthy lambs, calves, piglets.

In our research interest to Escherichia coli strains with properties to produce colicines associated with the important role of this bacterium as a component of the intestinal microflora of human and animals. The initial task was to determine the etiologic landscape of intestinal infections of young farm animals.

Studies of pathological material and fecal of healthy and sick animals 220 cultures were allocated, 123 of which crops belonging to the genus Escherichia, 66 cultures - Salmonella, 18 cultures - Klebsiella, 5 cultures - Proteus and 8 cultures - Streptococcus (Diplococcus). Isolated cultures were studied by morphological, tinctorial, biochemical and antigenic properties.

It was established that in the event of gastrointestinal diseases are important bacteria of the family Enterobacteriaceae, which accounted for about 94% of the isolated cultures prevalent were Escherichia and Salmonella, respectively, 55.9% and 30.0%.

The aim of our study was the selection of strains - candidates for the manufacture of probiotic preparation. Of the inhabitants of the gastrointestinal tract of young farm animals of interest to us was E. coli. After serotyping of cultures they mounted accessory to 12 serological groups: 01, 02, 08, 078, 086, 0101, 0111, 0119, 0138, 0141, 0142, 0149.

As a result of our breeding were selected 4 Escherichia strain (E. coli 18, E. coli 25, E. coli 64G, E. coli 70), which were used in the experiments for the study of their biological properties. Taken to the study of culture are normal inhabitants of Escherichia gastro-intestinal tract of healthy lambs (25 - E. coli strains, E. coli 64G), calves (E. coli 18) and pigs (E. coli 70).

As a result of investigation strains of E. coli have the typical morphology, tinctorial, cultural, biochemical and antigenic properties of the genus Escherichia.

On MPA observed steady growth with a bluish tinge, formed round, smooth, translucent colonies. At Endo agar in Petri dishes strains E. coli 18, E. coli 25, E. coli 64G, E. coli 70 – red colonies.

A bacterium has good mobility. The optimum growth temperature of 37-38$^0$ C, pH 7,0-7,2. When smear microscopy can be seen randomly arranged sticks. Plated on MPB marked uniform turbidity of the medium to form on the bottom of the tube is easily broken sediment.

Determination of antigenic structure was carried out with common "0" - and monovalent agglutinating serum. It was founded that all strains agglutinated with serum above in four cross, indicating the usefulness of their antigenic properties and have: E. coli 25 - 078; E. coli 64G - 0111; E. coli 18 - 086; E. coli 70-0142.

The presence of an adhesive antigen examined in agglutination on glass with agglutinating serum – in first with common serum complex, and in the presence of a positive reaction - with monovalent serum. The studies found that the E. coli strain 25 gave a positive agglutination reaction with serum F 41, strain E. coli18 - 99 K, E. coli 70 - K 88, strain of E. coli 64G - in association of F 41 + 99.

Carbohydrate fermentation was determined with an indicator Andrede, various sugars and polyhydric alcohols. The reaction to form the acid gas was observed in media containing glucose, lactose, sucrose, maltose, mannitol, sorbitol and dulcitol. One of the most valuable features of a probiotic microflora is antagonistic activity against pathogenic saprophytes' microorganisms.

Antagonistic activity of strains of E. Coli 18, E. coli 25, E. coli 70 and E. coli 64G was studied on dense nutrient mediums. The degree of antagonist activity of the studied strains to each test microbe was judged by the width of the zone of growth inhibition last up to 10 mm, the average, more than 20 mm - High; no zone of growth retardation - zero antagonistic activity.

As a test cultures were taken of culture isolated from sick animals in farms of Almaty region having pathogenic properties and belonging to the genus Salmonella (S. dublin, S. abortusovis, S. typhymurium), Klebsiella (K. Pneumoniae - 3 strain), Streptococcus (Str. Pneumoniae - 3 strain) and Escherichia (E. coli- 3 strains).

**Table 1.**Antagonistic activity of E. coli strains with respect to various microorganisms

| Strains | The diameter of the growth inhibition zones of test-cultures (mm) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *S. dublin* | *S. abortusovis* | *S. typhymurium* | *K.pneumoniae* 11 | *K.pneumoniae* 23 | *K.pneumoniae* 19 | *Str.pneumoniae* 6 | *Str.pneumoniae* 9 | *Str.pneumoniae* 14 | *E. coli* 73 | *E. coli* 31 | *E. coli* 47 |
| *E. coli*18 | 21 | 20 | 19,5 | 19 | 17 | 18 | 20 | 16 | 15 | 22,5 | 19 | 20 |
| *E. coli*25 | 13 | 12 | 9 | - | 11 | 13 | 9 | 11 | 8 | 17 | 18 | 10,5 |
| *E. coli*70 | 12 | 14 | 11 | 11,5 | 7 | 14 | - | 9 | 8 | 12 | 9 | 18 |
| *E.coli*64G | 18,5 | 16 | 19 | 17 | 19 | 15 | 18 | 14 | 16 | 20 | 20,5 | 18 |

Our studies have established significant variation in the level of antagonistic activity of different strains of Escherichia in the spectrum of suppressed microorganisms. Most possess high antagonistic activity E. coli strains E. coli 64 G and 18, which inhibited the growth of all samples in the test culture experience. The aforementioned strains also showed high antagonistic activity against virulent E. coli cultures.

Investigation of E. coli strains, with properties to produce colicines, to bile and hydrochloric acid.

Each analyzed strain E. coli 18, E. coli 25, E. coli 64G, E. coli 70 was cultured at MPB (pH 7.0-7.4) containing 1, 5, 10, 20, 30, and 40% bile. In the experiments used the bile Medical (Cholemedicata), containing natural gallbladder bile of cattle.

Test cultures E. coli 18, E. coli 25, E. coli 64G, E. coli 70 were cultured to 24 hours and 48 hours. Resistance to bile was determined in terms of biomass accumulation, changes in the number of CFU and pH. The number of viable bacterial cells in 1 ml of the suspension (the number of colony forming units - CFU) were determined by the method of limit dilutions ($10^2$ to $10^9$) for plating on solid nutrient medium (MPA).

Readings are taken immediately after the introduction of the suspension cultures at various dilutions at 24 MPA culturing them in a thermostat at $37^0$ C for 18-20 hours, then tabulating the grown colonies.

A similar experiment was carried out with the test cultures -E. coli 18, E. coli 25, E. coli 64G, E. coli 70 after culturing them for 48 hours in the MPB.

The results showed that the seeding suspension cultures studied in accounting – $10^2$ on MPA (the definition of living cells) grown in the MPB with the contents of various concentrations of bile after 24 and 48 hours there was an increase strains E. coli 18 (85 colonies), E. coli 25 (72 colonies), E. coli 64G (91 colonies), E. coli 70 (70 colonies) with medium containing 1%, 5%, 10% and 20% bile.

Thus, the accumulation of biomass tested strains E. coli 18, E. coli 25, E. coli 64G, E. coli 70 depended on the concentration of bile in the medium. Most biomass accumulated for 1 day (24 hours) culture, titer of bacteria (CFU) was equal to at sowing in cultivation: $10^2$ (100 CFU) - strain E. coli 18 - 85 CFU of 100, strain of E. coli 25 - 72 CFU of 100, strain E. coli 64G – 91 CFU of 100, strain E. coli 70 - 70 CFU of 100.

Coli bacteria at sowing crops studied in 48 hours amounted to an average of 64-72 out of 100 CFU.

Growth strains E. coli18, E. coli 25, E. coli 64G, E. coli 70 on a medium containing 30 and 40% of bile was observed.

Thus, the analysis results of stability studies of selected strains of E. coli to different concentrations of bile showed that four of the most highly resistant strains to 1%, 5%, 10% and 20% bile possesses E. coli strain 64G.

Determination of the sensitivity of strains of Escherichia, with properties to produce colicines, to hydrochloric acid.

Determination of the sensitivity of strains E. coli 18, E. coli 25, E. coli 64G, E. coli 70 to hydrochloric acid was performed by photo calorimeter method to change the optical density of the broth cultures by the addition of different concentrations of hydrochloric acid, which was compared with the control, where there is a reproduction of the test cultures without the presence of hydrochloric acid. As reference strain of E. coli 04 culture was used (S - form).

At the same time, resistance to hydrochloric acid studied cultures of E. coli was determined by the level of biomass accumulation, changes in the number of CFU and pH. The number of viable bacterial cells in 1 $cm^3$ of the suspension (the number of colony forming units - CFU) were determined by limiting dilution ($10^2$ to $10^9$) for plating on solid nutrient medium (MPA).

Each strain was grown in cultures studied on MPB (pH 7.0-7.4) containing 0.1%, 0.2%, 0.5%, 0.8%, 1.0%, 1.2%, 1.5%, 1.8%, 2.0% and 2.5% hydrochloric acid. Strains E. coli 18, E. coli 25, E. coli 64G, E. coli 70 were cultured for to 24 hours and 48 hours. Results of growth cultures studied in a nutrient medium (MPB) content and free of hydrochloric acid for 24 and 48 hours in an incubator at $37^0$C.

The results of the study of resistant of strains Escherichia to hydrochloric acid by photo colorimetric method indicate that the growth of E. coli 18, E. coli 25, E. coli 64G, E. coli 70 observed in mediums containing hydrochloric acid at concentrations of 0.1%, 0.2 %, 0.5%, 0.8%, 1.0%, 1.2% (study of growing of strains after 24 hours)

and in medium containing hydrochloric acid at a concentration of 0.1%, 0.2%, 0 , 5%, 0.8% (study of growing of strains after 48 hours). In the control group there was growth in all the cultures in medium not containing hydrochloric acid.

Simultaneously was studied resistance to hydrochloric acid of studied cultures of Escherichia at accumulation level of biomass, changing the number of CFUs by limiting dilution ($10^2$ to $10^9$) for seeding cultures grown (at 24 and 48 hours) on solid nutrient media (MPA).

Results of the experiments were conducted after seeding suspension cultures of different dilutions of the test on the MPA, after 24 hours of culture them in an thermostat at $37^0$ C, then tabulating the grown colonies.

The results showed that the 24 hour seeding suspension of cultures in account $10^2$ on MPA (determination of living cells) grown in the MPB containing hydrochloric acid in concentrations of 0.1%, 0.2%, 0.5%, 0 , 8%, 1.0%, 1.2%, 1.5%, 1.8%, 2.0% and 2.5% increased observed E. coli strains 18 (70 to 81 colonies), E. coli 25 (from 61 to 67 colonies), E. coli 64G (from 89 colonies 78), E. coli 70 (from 58 to 67 colonies) with medium containing 0.1%, 0.2%, 0.5%, 0.8%, 1.0%, 1.2%, 1.5% hydrochloric acid. Similar results were obtained with 48 hour seeding suspension cultures studied at the same concentrations. Growth of E. coli strains CFU averaged between 55 and 64 colonies.

Seeding with medium containing 1.8%, 2.0% and 2.5% hydrochloric acid gave no growth cultures, regardless of the exposure of the culture.

## CONCLUSIONS

It was established that in the event of gastrointestinal diseases are important bacteria of the family Enterobacteriaceae, which accounted for about 94% of the isolated cultures prevalent were Escherichia and Salmonella, respectively, 55.9% and 30.0%.

As a result of our research were selected 4 Escherichia strain (E. coli 18, E. coli 25, E. coli 64G, E. coli 70), which were used in the experiments for the study of their biological properties. Research showed that strains of E. coli have typical morphological, tinctorial, cultural, biochemical, adhesive and antigenic properties of the genus Escherichia.

Study of the antagonistic activity of their level of resistance to hydrochloric acid and bile found that selected E. coli 64G strain has a antagonistic properties, which inhibited the growth of all samples in the test culture experience titer E. coli 64G growth culture under study at seeding 24 hours amounted to 91 CFU of 100, the highest growth was observed at E. coli strains 64G (from 78 to 89 colonies out of 100) at seeding in the MPB with the content of hydrochloric acid in concentrations of 0.1%, 0.2%, 0.5%, 0 , 8%, 1.0%, 1.2%, 1.5%, 1.8%, 2.0% and 2.5%.

Our studies have shown that the most high antagonistic activity had strain E. coli 64G which inhibited the growth of all the commitments in the experience of the test culture, was resistant to bile and hydrochloric acid and in the future we used it for the manufacture of probiotic preparation.

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