Some Features of the Bacteria of the Family Enterobacteriaceae Allocated from Small Cattle at Different Pathological Processes

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Abstract

Enterobacteria with a certain set of pathogenicity factors and capable of leading to the development of diseases of animals and humans are ubiquitous in nature, which can be recognized as the result of anthropogenic activity. Recently, they have been isolated with increasing frequency from soil, water, from fruits, vegetables, grains, plants, from representatives of various systematic groups of animals and humans. We have obtained bacteria from sheep with gastrointestinal pathologies. From sick animals, we have identified a large number of bacterial cultures belonging to species of different genera of the *Enterobacteriaceae* family. Among them, 190 cultures, species belonging to *Escherichia coli*, 124 cultures of *Enterobacter cloacae*, 74 cultures of *Morganella morganii*, 51 culture of *Citrobacter freundii*, 29 cultures of *Serratia marcescens*. For these microorganisms a large set of various pathogenicity factors was characteristic: antilysocyme, anti-complementary, anti-interferon activity, adhesive ability, hemolytic activity, multiple resistance to antibacterial drugs.

The aim of our work is to study the spread of enterobacteriosis, some features of the epizootic process in these diseases in the territory of the Chechen Republic

Keywords: *Epizoology, farm animals, enterobacteriosis, disease, small ruminants, pathogen, skin, wool.*

Introduction

Independence of the state is defined not only by the power of their military forces, but also by the capability to supply the population with food. According to the standards of UN FAO of the WHO, a person has to consume around 959.7 kg of plant and animal food products annually. In Russian Federation there are on average 701.6 kg of food per person annually[1]. Meat and milk consumption per person is 80% of the norm and fish consumption is 55% of the norm[2].

Only innovation-driven growth of APC can provide Russia with food supply security[3]. The issue of animal production intensification in Russian new economic conditions is one of the most acute, because it is directly connected with the quality of population nutrition and their life in general. Scientific approach to dealing with this acute issue is absolutely necessary, since food supply becomes a lever of economic and political pressure in international relations.[4]

At present, animal production industry is in crisis, it is considered unprofitable and has to cut the main qualitative parameters. This issue is especially acute in the present conditions of the imposed import substitution policy.

The popularity of import substitution policy in agricultural sector and on the food market is increasing. The problem of production intensification, based on innovation-driven technologies, in particular, on energy saving technologies, is very acute.

During the past years Russia has increased production rate, in particular, agricultural production rate, and, as a result, became one of the exporters. In 2014 export profit of agricultural production exceeded 20 billion dollars, which is higher by one forth than the profit from military equipment export and by one third than from gas resources export.

The concept of long-term social and economic

development of Russian Federation defined the main objective that population should be supplied with agricultural products and food by domestic industry. Thus, by 2020 Russia should reach the level of the recommended daily food norm of animal food products consumption. Meat production is expected to increase by 1.7 times and milk production is expected to increase by 27%. It is planned to provide the industry with breeding animals and increase their productivity[5] to the levels compatible with those of West European countries.

State support of establishment of the additional and modernization of the existing animal breeding genetic centers should provide the import substitution policy implementation by 80% and create conditions for parental and line breeding animals and their reproductive and productive characteristic improvement[4]. This is expected to develop animal breeding industry and increase its competitive advantages on Russian and world markets».[6]

«Successful animal production development is defined by veterinary policies that protect animal farms from infectious and parasitic diseases. Scientific research data[7] and practical experience in veterinary show that the majority of infectious diseases, as a rule, is diagnosed not as mono infection, but as associated parasite community[56]. It should be noted that parasite associations in farm animals are diverse in both taxonomic aspect (viruses, bacteria, protozoan, helminths)[8] and the pathologies caused».[9]

The diseases caused in animals and humans by different genera of the *Enterobacteriaceae* family, i.e. enterobacteriosis, are quite widespread. These diseases often lead to lethality in the infected individuals.

Enterobacteriosis pathogens are often isolated from soil, water, plant and animal products, domestic and wild animals, as well as humans. Among these microorganisms there are strains that differ significantly from each other by biological properties, ecological peculiarities, range of hosts, pathogenic factors and virulence. A number of species cause gastrointestinal problems. 12 out of 30 geniof the *Enterobacteriaceae* family–*Citrobacter*, *Escherichia*, *Enterobacter*, *Hafnia*, *Klebsiella*, *Morganella*, *Proteus*, *Providencia*, *Salmonella*, *Serratia*, *Shigella*, *Yersinia*,cause different pathologies. In the Chechen Republic these diseases were registered annually in farm animals from 2002 to 2015. The annual season dependent dynamics of morbidity was identified in young small ruminants[9].

Materials and Method

For evaluation and analysis of enterobacteriosis epizootic situation (colibacillosis, salmonellosis, serratiosis, etc.) among small ruminants in the Chechen Republic, the authors used the data from annual reports from republic and regional vet laboratories. Epizoologic screening was performed from 2002 to 2015 according to the common methodical guidelines [10].

Isolation of opportunistic and pathogenic enterobacteria, their cultivation and evaluation of pathogenicity factors were conducted according to the common method.

Cultivation of microorganisms was done on 1.5% meat and peptone nutrient agar and nutrient broth. Bacteria culture was cultivated in thermostat at 37°C within 18-24 hours. The bacteria were isolated and identified according to the "Methodical guidelines to bacteriological diagnostics of associated intestinal infections caused by pathogenic enterobacteria in animal growing stock[11].

Morphologic, tinctorial and cultural properties evaluation was performed according to the method, outlined in the reference books on microbiology[12].

Disc diffuse method was used for identification of sensitivity range of the studied bacterial culture, isolated from sheep, to antibiotics[13].

Lysozymic activityof the studied cultures was assessed by the microorganism lysozyme capacity to split β-(1-4)-glycoside bonds of mucopolysaccharide complex in cell walls of the reference strain *Micrococcus luteus var. Lysodeikticus*[13].

Assessment of antilysozymic activity was done by the method of O.V. Bukharin et al.[13].

Adhesive activity of the studied bacteria was evaluated by the reaction of hemagglutination with 3% goat erythrocyte suspension in the presence of D-mannose and without it[13].

Hemolytic activity of the studied cultures was assessed on nutrient agar in the presence of 3-5% rabbit washed erythrocytes. Thiol dependent hemolysines were identified by cultivation on trypticase soy agar in the presence of 3-5% rabbit washed erythrocytes (washed three times in Hank's solution) according to the recommendations of Albesa I. et al[13].

Anti-interferon activity in the isolated cultures was studied by O.V. Bukharin and V.Y. Sokolov in association with antibacterial effect of leucocytal human interferon drug [14].

Anticomplementary activity was evaluated by the method of O.V. Brudastovet al[15].

Results

The results of the present study showed that 80% of enterobacteriosis cases in small ruminants were diagnosed as associated diseases and epizootically appeared on a wide territory in the Republic[9]. Their role and place in the formation of nosologic profile of the infectious pathology is quite significant[16].

During the years of screening, small ruminants, morbidity rate varied from 0.01% to 7.3% at serratiosis, from 0.05% to 11.5% at colibacillosis, from 0.01% to 3.7% at enterobacteriosis. Lamb mortality rate during these years varied from 0.02% to 2.2%, from 0.2% to 6.2%, from 0.01% to 1.5%, respectively. The present study allowed the authors to identify the peculiarities of serratiosis, colibacillosis and enterobacteriosis disease development in small ruminants on the territory of the Chechen Republic.

The study of this pathology annual dynamics allowed the authors to identify significant seasonal dependence in lamb morbidity rate. Thus, in February-March enterobacteriosis morbidity rate varied from 0.055 to 0.1%, decreased during the following months and increased again in June to 0.05%. Consequently, seasonal epizootological changes in these diseases are characterized by infection rate increase in the end of summer and in autumn and infection rate increase at the end of winter and in spring.

These bacterial pathologies in small ruminants have the following symptoms: appetite decrease, fatigue, low mobility, permanent diarrhea, decrease of life mass gain in comparison with healthy animals, hyperthermia, heart rate increase and tachycardia, unfavorable outcome rate during the first days of disease reached 60%.

Flashness of animal corpses was below the average, skin and wool around anal orifice, on the tail, hips and back abdominal part were covered with liquid feces, mucous membranes had signs of anemia. Pathological study of fallen animals showed peculiar changes in the intestine and parenchymatous organs, which were primarily observed in abdominal cavity: forth stomach mucous was irritated, tremellose infiltrated with hemorrhages. Mesenterium lymphatic vessels were enlarged, often hyperemic, fleshy on section cuts. Serosal abdominal layers often contained hemorrhages.

Splinter was slightly enlarged without significant alterations in most cases. Liver and kidneys had signs of anemia, often with hemorrhages under their coats, gall-bladder was filled with dark-green bile. Bladder mucous layer did not have significant changes in rare cases it was hyperemic.

Intestine content was rare, mixed with mucus and was red because of blood presence (catarrhal hemorrhagic colitis).Mucous membranes of large and small intestine were swollen, covered with mucus, hyperemic and often with hemorrhages. Mucous intestine layer had signs of necrotic colitis and proctitis localized in different parts of intestine in different animals. In most cases blindgut was filled with hydrous content and was inflated because of gases (meteorism)[17]. Thoracic cavity contained hemorrhages under serous membranes, significant deviations from the norm were not observed[18].

Enterobacteriosis was diagnosed based on bacteriologic tests performed in bacteriologic laboratory.

A lot bacterial cultures from the *Enterobacteriaceae* family were isolated from infected animals. Among them there were 190 cultures of *Escherichia coli* species, 124 cultures of *Enterobacter cloacae*, 74 cultures of *Morganella morganii*, 51 cultures of *Citrobacter freundii* and 29 cultures of *Serratia marcescens*. These microorganisms had a wide range of pathogenic factors: antilysozymic, anticompiementary, antiinterferon, adhesive, hemolytic activity and multiple resistance to antibiotics[19].

Thus, the studied microorganisms antilysozymic activity varied from 43.5% in representatives of genus Enterobacter to 86.2% in ygenus Serratia bacteria.

Antiinterferon activity was minimum 28.4% in genus Morganella and maximum in genus Escherichia representatives. Minimal number of cultures with anticomplementary activity was among representatives of genus Enterobacter and maximum number of strains with this feature was in genus Enterobacter.[17]

The study of goat erythrocytes agglutinative capability in the presence of D-mannose showed that

this feature was present in all the species of the studied enterobacteria. However, in different species this feature was present at different rate. Hemagglutinating capability was quite expressed in 94.7% of Escherichia strains. The identified hemagglutinins in the studied Escherichia strains had different sensitivity to mannose. Mannose resistant hemagglutinins to goat erythrocytes were identified in 73 Escherichia strains (39.2%). 24 out of 29 Serratia strains had hemagglutinating capability in the absence of D-mannose among Serratia, which is 82.75%. D-mannose resistant hemagglutinating activity was observed in 24 of the studied Serratia strains. The lowest number of strains with hemagglutinating activity (42 out of 124 (33.84%)) was identified among Enterobacter cloacae cultures. Among the studied Morganellastrains hemagglutinating activity was observed in 58.9% of cultures. The studied Morganella cultures produced mannose resistant hemagglutinins more often (63.64%) than mannose sensitive ones (36.4%). Goat erythrocyte agglutinating activity was identified in 180 out of 190 (94.7%) Escherichia strains. Hemagglutinins, identified in Escherichia strains, had different sensitivity to mannose. Thus, human erythrocyte agglutination was inhibited by mannose in 84 cultures (44.7%), and mannose resistant hemagglutinins were identified in 68 strains of the studied bacteria. Goat erythrocyte mannose resistant hemagglutinins were observed in 73 strains (39.2%).[18]

Among the studied cultures of microorganisms, the lowest amount of hemolytically active strains (16.1%) were found in genus Enterobacter and the highest amount of representatives with this feature was found in genus Citrobacter (58.8%).

All the studied cultures had sensitivity to 9 groups of antibiotics: group I – β-lactam antibiotics of penicillin group, group II – β-lactam antibiotics of cephalosporin group, group III – tetracyclines, group IV – aminoglycosides, group V –macrolides, group VI – rifamycins, group VII– glycopeptides, group VIII– nitrobenzenes (laevomycetin group), group IX – polypeptides (polymyxins).

The studied Escherichia strains showed higher resistance to β-lactams, tetracyclines, macrolides, rifamycinsandglycopeptides. The highest sensitivity in these cultures was to polymyxin and laevomycetin. Among Escherichia strains half of the studied cultures (47.7%) were the strains with multiply resistance to the used antibacterial drugs.[20]

Evaluation of Enterobacter cloacae cultures sensitivity to antibiotics showed that there was a number of strains with multiply resistance to drugs (45.16%). The studied Enterobacter cloacaestrains were mostly sensitive tomonomycin (81%), carbenicillin (29.0%), laevomycetin (17.8%) andneomycin (8.1%), to other antibiotics they were highly resistant.[21]

High ratio of the strains with multiple antibiotic resistance was identified among Serratia marcescens strains (68.96%).[22]

The studied Morganella cultures had absolute resistance to methicillin, streptomycin, ampicillin, benzylpenicillin, rifampicin, ristomycin, neomycin and erythromycin. Morganellas were sensitive togentamicin, kanamycin and laevomycetin. 82% of the studied Morganella cultures were multiple antibiotic resistance.

Among the studied *Citrobacter freundii*cultures, there were many strains with multiple antibiotic resistance(49.7%).

Discussion

The results of the present study allowed the authors to conclude that the disease outbreak and development in lambs was associated with both environmental factors (sharp temperature fluctuations in winter-spring seasons, traditionally high air humidity, high level of solar activity, etc. in the Republic) and uncontrolled indication of antibiotics to animals as food additives for growth stimulation, that led to appearance of strains with multiple resistance to antibiotics and high range of pathogenic features. Environmental factors are accompanied by the conditions of sheep barn housing in the Republic. This period coincides with high lamb birth rate, which leads to sheep concentration increase on the limited barn area. This, in its turn, leads to worsening of sanitary conditions in sheep farms. Feed supply and feed quality reduction (feed with insufficient amount of nutrients, vitamins, microelements, etc.) in the end of winter-spring season leads to animal immune system weakening.

Often, water and food, contaminated with pathogenic or opportunistic pathogenic bacteria, can become a source of infection because of sanitation and hygiene standards of animal keeping violation.

The obtained data indicates on widespread and unfavorable conditions of animal farm keeping in the

Republic, which makes sick and recovered animals the main source of pathologic infection.

During the analysis of epidemiologic situation in Chechen Republic, it was important not only to isolate bacteria cultures and identify their species, but also to confirm the fact that those particular cultures were the etiological agents that caused the diseases, because they had a number of pathogenic features defining their virulence. Development of labelling method, as well as identification of the isolated bacteria sensitivity to antibiotics, has great practical significance for establishing antibiotic therapy regimen for the sick animals on the territory of the Chechen republic.[13]

Conclusion

1. The results of the studies showed that enterobacteriosis of small cattle in 80% of cases occurs as associative diseases and have wide territorial boundaries of epizootic manifestations in the republic, and their role and place in the formation of the nosological profile of the infectious pathology of sheep is very significant.
2. From sick animals, we isolated a large number of bacterial cultures belonging to species of different genera of the *Enterobacteriaceae* family. Among them 190 cultures belonging to the species *Escherichia coli*, 124 cultures of *Enterobacter cloacae*, 74 cultures of *Morganella morganii*, 51 cultures of *Citrobacter freundii*, 29 cultures of *Serratia marcescens*. These microorganisms were characterized by a large set of various pathogenicity factors: antilysocyme, anticomplementary, anti-interferon activity, adhesive ability, hemolytic activity, multiple resistance to antibacterial drugs.

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