

Some Peculiarities of Epizootic Process during Enterobacteriosis in Small Ruminants on the Territory of the Chechen Republic

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Abstract

Enterobacteriosis in agricultural animals are quite widespread. These are human and animal infectious diseases caused by pathogenic or opportunistic pathogenic representatives of various geni of the Enterobacteriaceae family, that often lead to the death of infected individuals. Enterobacteria capable of causing infectious diseases are widespread in nature, as they are isolated from soil, water, fruit, vegetables, grains, plants, representatives of various systematic groups of animals and humans. Among these microorganisms there is a wide variety of strains that differ by ecological properties, hosts range and pathogenicity for plants, animals and humans. A number of species cause gastrointestinal diseases, i.e. are enteropathogenic. Representatives of 12 geni out of the 30 from the Enterobacteriaceae family - Citrobacter, Escherichia, Enterobacter, Hafnia, Klebsiella, Morganella, Proteus, Providencia, Salmonella, Serratia, Shigella, Yersinia, are responsible for different pathologies in animals. There is lack of research data on the main pathogens diagnostics, peculiarities of the epizootic process at these diseases, the mechanisms of circulation and the species composition. The purpose of the present work is to evaluate the spread

of enterobacteriosis and to assess some peculiarities of the epizootic process at these diseases on the territory of the Chechen Republic.

Key-words: Epizootology, Farm Animals, Enterobacteriosis, Disease, Small Ruminants, Pathogen, Skin, Wool.

1. 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. Meat and milk consumption per person is 80% of the norm and fish consumption is 55% of the norm.

«Only innovation-driven growth of APC can provide Russia with food supply security. 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».[1,2, 3,4]

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 fourth 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 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. This is expected to develop animal breeding industry and increase its competitive advantages on Russian and world markets». [4,5, 6]

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

The diseases caused in animals and humans by different geni 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 geni of 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].

2. Materials and Methods

For evaluation and analysis of enterobacteriosis epizootic situation (colibacillosis, salmonellosis, serratiois, etc.) among small ruminants in the Chechen Republic, the authors used the data from annual reports from republic and regional vet laboratories. Epizootologic 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 methods.

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 methods, 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 activity of 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. Bukharina 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. Bukharina and V.Y. Sokolova in association with antibacterial effect of leucocytal human interferon drug [14].

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

3. 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. Their role and place in the formation of nosologic profile of the infectious pathology is quite significant [9,16].

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). Thoracic cavity contained hemorrhages under serous membranes, significant deviations from the norm were not observed.

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 colispecies*, 124 cultures

of *Enterobacter cloacae*, 74 cultures of *Morganella morganii*, 51 cultures of *Citrobacterfreundii* and 29 cultures of *Serratia marcescens*. These microorganisms had a wide range of pathogenic factors: antilysozymic, anticomplementary, antiinterferon, adhesive, hemolytic activity and multiple resistance to antibiotics [17, 18, 19].

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

Antiinterferon activity was minimum 28.4% in *Morganella* and maximum in *Escherichia* representatives. Minimal number of cultures with anticomplementary activity was among representatives of *Enterobacter* and maximum number of strains with this feature was in *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 *Morganella* strains 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 *Enterobacter* and the highest amount of representatives with this feature was found in *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, rifamycins and glycopeptides. 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 multiresistance to the used antibacterial drugs.[20]

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

High ratio of the strains with multiresistance to drugs 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 to gentamicin, kanamycin and laevomycetin. 82% of the studied *Morganella* cultures were multiresistant to antibiotics.

Among the studied *Citrobacter freundii* cultures, there were many strains with multiresistance to antibiotics (49.7%).

4. 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 multiresistance 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 methods, 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]

5. Conclusion

Antibiotics are widely used in different spheres of animal production as a feed additive for growth stimulation in many countries. This practice brought certain positive results. However, some data was obtained that indicated on the negative consequences of this practice. Animals, that receive antibiotics, grow better and faster and do not get sick in comparison with similar animals that do not receive antibiotics. Still, this practice resulted in selection of microorganisms with multiresistance to drugs. Such microorganisms can cause epidemic outbreaks in animals and humans.

Microorganisms with increased resistance to antibiotics can spread through soil, water, air, infected animal and poultry meat and fish. Such microorganisms can be a source of horizontal spread of this feature on other bacteria through plasmids, bacteriophages, etc.

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