The etiology of the disease of the newborn young farm animals

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Abstract. The aim of our research was the study of species composition of conditionally-pathogenic and pathogenic microflora at the gastrointestinal diseases of the newborn young animals. The objects of our research were the healthy, sick and dead lambs, calves, piglets and foals. The samples of pathological material and feces of the sick and healthy young farm animals were transported to the antibacterial biotechnology laboratory of Kazakh National Agrarian University of Almaty region. After the isolation of microorganisms the cultures, obtained only from the isolated colonies, were subjected to the identification. In the studies of pathological material and samples of feces of sick and healthy animals 210 cultures were isolated in total, of which 123 cultures belonged to the genus of Escherichia, 66 cultures – Salmonella, 8 cultures – Klebsiella, 5 cultures – Proteus and 8 cultures – Streptococcus (Diplococcus). The isolated cultures were studied by the morphological, tinctorial and biochemical properties. Of 210 cultures, isolated from the dead and sick animals, 58.5% of cultures were referred to the genus of Escherichia, 31.3% – to the genus of Salmonella, 3.8% – of Klebsiella, 2.4% – of Proteus and 3.8% – of Streptococcus by its biochemical properties. Thus, the results of bacteriological research testify that the bacteria of Enterobacteriaceae family which accounted for about 90% of the isolated cultures, the predominant ones were Escherichia and Salmonella, accordingly, 58.5% and 31.5%; they play an important role in contraction of the gastrointestinal diseases.

Keywords: conditionally-pathogenic and pathogenic microflora, pathological materials, nutrient media

Introduction

Diarrhea of infectious origin is the main cause of mass death and morbidity of the young farm animals in the first days of life [1]. The damage from these diseases is composed not only of the costs of the dead animals but also of the costs of medical treatment and deterioration of feed conversion, that is, the recovered calves give much smaller increase at the same ration [2]. The accumulated factual material and numerous scientific publications of the last years testify that the characteristic feature of the modern infectious pathology of the young animals is strict increase in the intestinal infections the pathogenic organisms of which are conditionally-pathogenic bacteria. These microorganisms are widely circulating in households, they possess a broad spectrum of virulence (enterotoxigenic, adhesiveness, hemolytic activity, antibiotic resistance) [3-5].

The main biotopes of conditionally-pathogenic bacteria of genera Escherichia, Proteus, Citrobacter, Klebsiella, Peptococcus, Bacillus, Clostridium, Bacteroides, Yersinia, Erwinia, Salmonella, Streptococcus, Staphylococcus, Pseudomonas are the intestines of warm-blooded animals. The high adaptability of the conditionally-pathogenic bacteria to the environment allows them to remain for a long time. Against the high contamination of feed and various objects of the environment by the conditionally-pathogenic microorganisms the process of colonization of the intestinal wall by the normal microflora is disrupted – lactobacilli, bifidobacteria, propionic acid bacteria and enterococci, ahead colonizations occur in the intestines of the newborn animals by enterobacteria. Not by chance the diseases of the young animals, accompanied by diarrhea syndrome, remain the most complicated problems of veterinary medicine [6-8]. The practice shows that the current complex of technological, zoo-hygienic, veterinary and sanitary techniques in rearing of the young animals does not allow to maintain a high level of resistance to the bacterial infections caused by the conditionally-pathogenic microflora. The use of the antibiotics for prophylaxis and treatment of the gastrointestinal diseases lowers the quality of the products and is becoming less effective.

This gives grounds for reviewing the methods of treatment and prophylaxis of the gastrointestinal diseases and aims at creation of the ecologically safe products, aimed, first of all, at restoring the normal microflora of the animals’ intestines [9, 10]. The acute gastrointestinal diseases of the calves, lambs, piglets, foals and chickens are widely spread in Kazakhstan. According to the statistics, they occupy the first place among the currently registered in the Republic diseases of the newborn animals [11, 12]. The task of the research was to study the species composition of conditionally-pathogenic and...
pathogenic microflora at the gastrointestinal diseases of the newborn young animals.

**Methods**

The object of the research was the healthy, sick and dead lambs, calves, piglets and foals. The material for the bacteriological research was the parenchymatous organs (hearts, livers, spleens, kidneys), mesenteric lymph nodes, marrow of the cortical bones and contents of the small intestines of the dead and forcibly slaughtered lambs, calves, piglets and foals with the symptoms of acute disorders in the gastrointestinal tract and also samples of faeces from the healthy newborn animals. From the parenchymatous organs, mesenteric lymph nodes, marrow of the cortical bones and contents of the small intestines the seeding was carried out in meat-and-peptone agar (MPA), meat-peptone broth (MPB), Kitt-Tarozzi medium, Endo’s and Ploskirev’s media.

In the study of faeces the samples were taken from the rectum into the glass test tubes by the sterile individual rubber catheters, the samples were suspended in a physiological solution (pH-7.0), then the suspension was poured out into 2 tubes, after this from the 1st tube the sieving into the cups fractionally for Endo’s and Ploskirev’s media was performed, and from the 2nd test tube the seeding in Kitt-Tarozzi medium was carried out after heating at 700C for 30 min.

The isolation and identification of the cultures were carried out according to the following procedure: the seeds from the pathological material and feces were have been incubating for 18-24 hours at temperature of 370C. At the absence of growth after the incubation in Endo’s and Ploskirev’s media and the presence of it in meat-liver peptone broth (MLPB) under the paraffin oil, the broth cultures were microscoped to study the morphology of germs and then they were reseeded for Zeissler’s blood-agar. After the 24-hour cultivation in the micro anaerobic culture apparatus at 370C the nature of growth of colonies was studied, the smears were prepared and they were stained by Gram.

**Results**

We have studied the biological properties of the conditionally-pathogenic and pathogenic cultures isolated from the intestines of the healthy and sick young farm animals by the morphological, physiological-biochemical and antigenic properties.

The gastrointestinal diseases are diagnosed on the pathological-anatomical data, the characteristic clinical signs and on the basis of bacteriological, virological, parasitological and chemical research. It is not complicated to diagnose the gastrointestinal diseases. It is more difficult to identify the etiologic agent, especially as in 70-80% of cases the disease has the polyetiologic nature.

The aim of our research was to study the species composition of conditionally-pathogenic and pathogenic microflora at the gastro-intestinal diseases of the newborn young animals. The objects of our study were the healthy, sick and dead lambs, calves, piglets and foals. The samples of pathological material and feces of the sick and healthy young farm animals were transported to the antibacterial biotechnology laboratory of Kazakh National Agrarian University of Almaty region. After the isolation of microorganisms the cultures, obtained only from the isolated colonies, were subjected to the identification.

The morphological, tinctorial and cultural properties of the isolated cultures were studied by the methods generally accepted in Microbiology. The nature of growth in MPA, MPB, MLPB, Zeissler’s blood-agar, in elective Endo’s medium, Levin’s, Ploskirev’s media and Wilson-Blair medium was taken into consideration. The nature of growth, shape, size and colour of the colonies were considered in solid media, and in liquid media – the presence of sediment, film on the surface, formations of gas and parietal ring.

All the cultures had the signs typical of the corresponding genus by the morphological and tinctorial properties. The results are presented table 1.

**Table 1. The identification of the cultures isolated from the dead, sick and clinically healthy calves, lambs, piglets and foals**

As we can see from table 1, 210 cultures were subjected to the identification of which the ones were isolated from the dead, sick and clinically healthy newborn animals – lambs, calves, piglets and foals.

In the course of the research 123 cultures belonging to the genus of *Escherichia*, 66 cultures – of *Salmonella*, 18 cultures – of *Klebsiella*, 5 cultures – of *Proteus* and 8 cultures of *Streptococcus* (Diplococcus) were isolated. The isolated cultures were studied by the morphological, tinctorial and biochemical properties.

The representatives of the genus of *Escherichia* are polymorphic rod cells with the rounded ends, are arranged singly, less often in pairs, gram-negative, do not form spores and capsules. In MPA all cultures formed the transparent with bluish tint or greyish round colonies with the smooth surface and even edges. When growing in MPB the
uniform turbidity of the medium with the formation of the loose whitish sediment, easily broken at shaking, was observed, 95% of the studied cultures were movable. In elective Endo’s medium the cultures formed mainly the round, smooth with even edges and convex surface colonies, mainly of crimson red colour with the metallic glitter or without it; sometimes the colonies of pink colour with a red centre were met.

The formation of hydrogen sulphide of different intensity was observed in 12 of 123 Escherichia cultures. The presence of indole was observed in 25 of 123 strains. The positive and doubtful Voges-Proskauer reaction was observed in 10 of 123. When setting the reaction with methyl red, the positive reaction was observed in 110 of 123. All the cultures under study did not dilute gelatin, did not possess urease activity, curdled milk. The formations of acid and gas or only of acid were marked in the media with glucose, maltose, lactose, saccharose, mannitol, salicin (in all 210 strains). They did not ferment arabinose, xylose, rhamnose.

Salmonella is a small rod cell with rounded ends, it is arranged singly in the smears, disorderly, does not form spores and capsules, gram-negative, it is an aerobe. The cultures grew well in MPA, MPB, Endo’s medium. In MPA all the cultures formed the round colonies with even edges, of greyish-bluish colour, in Endo’s medium – the transparent colonies of pinkish colour, in MPB – the intensive turbidity of the medium, the sediment of grey-whitish colour was formed on the bottom of the tube. Almost all the cultures under study fermented glucose, maltose, mannitol and did not decompose lactose and saccharose. In respect of other carbohydrates (arabinose, xylose, rhamnose, dulcete, etc.) the cultures showed different enzymic activity. Salmonella, isolated from the dead and sick newborn animals, was characterized by the especially expressed variability. The studied cultures did not break urea up, gave the positive reaction with methyl red and negative Voges-Proskauer reaction.

Proteus is a thin mobile rod cell, gram-negative. The cultures grew well in MPA, MPB. In MPA they formed the colonies of greyish-bluish colour, creeping growth, in Endo’s medium formed colorless or slightly pink colonies covering all the surface of the nutrient medium with an entire layer. All the strains of Proteus fermented glucose, galactose, glycerin with formation of acid and gas. Urease activity, the ability to form hydrogen sulphide were well expressed. Reactions with methyl red were positive and Voges-Proskauer reaction – negative.

Streptococcus (Diplococcus) grew well in MPA, MPB with 1% glucose in the form of the small, rich colonies, reminiscent of a dew-drop, and in the broth – in the form of the uniform turbidity with the small sediment. In semiliquid agar the characteristic stalactite-shaped growth was marked. All the cultures stained positively by Gram. Streptococci fermented lactose, saccharose, glucose, maltose, salicin without gas formation. They did not ferment arabinose, dulcete and mannitol. In the medium with 10% of bile growth was not marked.

Klebsiella is a thick short gram-negative rod cell; it is arranged singly or in the form of short chains. They form a capsule, have no spores, immovable. All the cultures grew well in MPA, MPB. In MPA they formed the dome-shaped brilliant mucous colonies. In MPB the intensive turbidity of the medium was observed. The studied cultures fermented glucose, lactose, dulcete, formed urease.

In the study of species composition of pathogenic microflora, isolated from the sick, dead of the acute gastrointestinal diseases as well as from the healthy newborn lambs, calves, piglets and foals, infectious disease etiology of young animals was established due to the Association of microorganisms (table 2).

Table 2. Etiological structure of microorganisms isolated from the dead, sick and healthy lambs, calves, piglets and foals

<table>
<thead>
<tr>
<th>Type of animal</th>
<th>Salmonella</th>
<th>Escherichia</th>
<th>Klebsiella</th>
<th>Proteus</th>
<th>Streptococcus</th>
</tr>
</thead>
<tbody>
<tr>
<td>From the dead</td>
<td>14 (11.9%)</td>
<td>9 (7.9%)</td>
<td>26 (21.8%)</td>
<td>15 (12.7%)</td>
<td>1 (0.8%)</td>
</tr>
<tr>
<td>From the sick</td>
<td>11 (9.3%)</td>
<td>8 (6.8%)</td>
<td>9 (7.5%)</td>
<td>9 (7.7%)</td>
<td>1 (0.8%)</td>
</tr>
<tr>
<td>From the clinically healthy</td>
<td>36 (30.3%)</td>
<td>22 (18.4%)</td>
<td>21 (17.2%)</td>
<td>16 (13.7%)</td>
<td>1 (0.8%)</td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>39</td>
<td>56</td>
<td>40</td>
<td>3</td>
</tr>
</tbody>
</table>

On the basis of the conducted research, as we can see from table 2, of 210 cultures, isolated from the dead, sick and healthy, 123 (58.5%) cultures were referred to the genus of Escherichia by its biochemical properties, 66 (31.5%) – to the genus of Salmonella, 8 (3.8%) – of Klebsiella, 5 (2.4%) – of Proteus and 8 (3.8%) – of Streptococcus.

Discussion

Saving the newborn animals and rearing the healthy, well-developed young animals, adapted to the new conditions of keeping, are the bases of increase in output of the livestock products [13, 14]. The main losses of the young animals are due to the gastrointestinal diseases. The literary data from the foreign sources and also data of our research show that such diseases of the newborn young animals
occur in 70 or even in 100% of cases at significant mortality [15]. It is the most difficult to save the young animals in the first 6-15 days. There is about 40% of mortality in this period. Besides, the young animals, recovered at an early age, develop worse in the future, their resistance reduces and the weight increase – for 15-20%.

Prophylaxis of the intestinal infections acquires social significance because in parallel with the increase in the consumption of the livestock, pig and poultry products increases the risk of their contamination by enterobacteria and other microorganisms – foodborne toxic infection pathogens in human bodies [16, 17].

In this regard, the development of effective methods and means of prophylaxis of these infectious diseases of the newborn young animals is very actual.

Conclusion

In the studies of pathological material and samples of feces of the sick and healthy animals 210 cultures were isolated in total, of which 123 cultures belonged to the genus of Escherichia, 66 cultures – Salmonella, 8 cultures – Klebsiella, 5 cultures – Proteus and 8 cultures – Streptococcus (Diplococcus). The isolated cultures were studied by the morphological, tinctorial and biochemical properties.

Of 210 cultures, isolated from the dead and sick animals, 58.5% of cultures were referred to the genus of Escherichia, 31.3% – to the genus of Salmonella, 3.8% – of Klebsiella, 2.4% – of Proteus and 3.8% – of Streptococcus by its biochemical properties.

Thus, the results of bacteriological research testify that the bacteria of Enterobacteriaceae family which accounted for about 90% of the isolated cultures, the predominant ones were Escherichia and Salmonella, accordingly, 58.5% and 31.5%; they play an important role in contraction of the gastrointestinal diseases.

We will continue research on the development of means of substitution therapy aimed at restoring intestinal bioecosystem by regulatory intake of live bacteria – the representatives of the normal intestinal microflora.

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