The effect of probiotic Vetom 2 on the microbial intestinal landscape in calves after antibiotic therapy

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Abstract. Intestinal microflora is directly and actively involved in ensuring the constancy of the internal environment of the microorganism. Disruption of the normal composition of the microflora of the gastrointestinal tract is accompanied by the development of dysbacteriosis. The most important role in restoring the normal intestinal microbiocenosis belongs to bacterial probiotic preparations. The aim of our study was to study the effect of Vetom 2 on the microbial intestinal landscape in calves after antibiotic therapy. Studies were conducted in AO Uchkhoz Prigorodnoye on calves of black pied breed. Five groups of 5 calves each were formed: healthy calves, calves with dyspepsia before antibiotic therapy, calves with dyspepsia during antibiotic therapy, calves after completing antibiotic therapy who did not receive Vetom 2, calves receiving Vetom 2 after completing antibiotic therapy. Feces of calves of all groups were bacteriologically investigated on the content of Escherichia, Streptococcus, Staphylococcus, Salmonella, Pseudomonas aeruginosa. We found that in animals of all experimental groups, the microbial landscape of the intestine is not the same. In diseased calves, before antibiotic therapy, the number of opportunistic microorganisms is much higher relative to the group of healthy animals. During treatment, these figures decrease. The number of conditionally pathogenic microorganisms in calves treated with Vetom 2 during rehabilitation after the end of antibiotic therapy was lower—unlike in the group of calves that did not receive Vetom 2 during rehabilitation—as a result of suppression of growth and development of conditionally pathogenic microorganisms in the intestines of calves after antibiotic therapy by bacterial strains included in Vetom 2.

1. Introduction

Normal functioning of animals requires ensuring optimal microbiocenosis in their bodies. Microorganisms, constantly residing in the intestines of an animal, bring enormous benefits by participating in the processes of its vital activity. Intestinal microflora is directly and actively involved in ensuring the constancy of the internal environment of the microorganism. Some types of bacteria are involved in the synthesis of vitamins and essential amino acids. The intestinal microflora contributes to
the normal intestinal peristalsis, as well as to the breakdown and absorption of metabolic products of lipids, proteins and carbohydrates [1].

Normal intestinal function in animals, despite the continuous entry of pathogenic bacteria into the body, can be maintained only under the condition of equilibrium of the natural microbiocenosis of the gastrointestinal tract. Disruption of the normal composition of the intestinal microflora is accompanied by the development of dysbacteriosis [2]. Dysbacteriosis reduces the protective function and the natural resistance of the body, which can lead to the emergence of various diseases, impaired digestion and metabolism in animals, and consequently, to reduce productivity and product quality.

The most important role in restoring the normal intestinal microbiocenosis belongs to bacterial probiotic preparations [3, 4].

The use of probiotics is one of the most effective and physiological ways to prevent and correct disorders of the microbiocenosis of the gastrointestinal tract, and also as a result of a number of secondary disorders not only of the digestive and endocrine systems, but also of the immune system [5].

Currently, many probiotic preparations are used in veterinary practice. One of them are probiotics based on bacteria of the genus Bacillus. Non-pathogenic bacilli can significantly increase the nonspecific resistance of the organism [6, 7].

Such preparations include probiotic Vetom 2. It consists of two bacterial strains Bacillus amyloliquefaciens VKPM B-10642 (DSM 24614) and VKPM B-10643 (DSM 24615). These strains are able to selectively inhibit the growth and development of pathogenic, conditionally pathogenic and putrefactive microflora due to the ability to produce specific biological active substances.

The aim of our study was to study the effect of Vetom 2 on the microbial intestinal landscape in calves after antibiotic therapy.

2. Materials and Methods
The studies were conducted in the Prigorodnoye educational farm in the autumn-winter period on calves of the black pied breed. For the experiment, 5 groups of calves with 5 animals in each were formed. The first group contained healthy calves; the second group contained calves with dyspepsia before antibiotic therapy; the third group contained calves with dyspepsia during antibiotic therapy. To assess the effectiveness of the process of rehabilitation of calves after antibiotic therapy and determine the effect of Vetom 2 on the intestinal microbial flora after antibiotic therapy, two additional groups of animals were formed. The fourth group contained calves who had dyspepsia, which were treated with antibiotics; the fifth group contained calves who had dyspepsia after antibiotic therapy, after which Vetom 2 was prescribed at a dose of 50 mg/kg of body weight once a day for 10 days.

Groups were formed as calves were born. In the calves of all groups, samples of feces from the rectum were taken for bacteriological examination of the content of Escherichia, Salmonella, Staphylococcus, Streptococcus, Pseudomonas bacillus.

The first group consisted of healthy calves that did not have dyspepsia. The feces of calves of this group was samples once at the age of 14-15 days. The feces of calves of the second group was sampled once before the start of antibiotic therapy. The feces of calves of the third group was sampled once during the antibiotic therapy. At this farm, the antibiotic Rificyclinum was used for treatment in a dose of 200-300 mg/kg orally 2 times a day and the antibiotic Enroxil subcutaneously with 1 ml/20 kg of calf mass once a day. In the fourth group of calves, to assess the process of restoring the microbial landscape of the intestine after antibiotic therapy, feces samples were taken on days 3, 6, 9 after the completion of the antibiotic therapy. In the fifth group of calves, feces samples were also collected on day 3, 6, 9 after the completion of the antibiotic therapy. Bacteriological studies of calf feces were carried out in the Altai Regional Veterinary Center for the Prevention and Diagnosis of Animal Diseases.

3. Results and Discussion
Studies have shown that Salmonella and Pseudomonas aeruginosa were not detected in fecal samples from calves of all experimental groups. On the contrary, non-pathogenic strains of Escherichia coli were
found in the calves of all experimental groups. In the group of healthy calves, they amounted to 1.1±0.5·10^8 Colony-forming units (CFUs) in 1 g of feces (p < 0.05).

In the 2nd group of calves, the CFU of Escherichia was 4.0±0.1·10^8 in 1 g of feces (p < 0.001). The increase in the number of CFU of Escherichia in this group, in our opinion, is associated with a change in the intestinal microbial flora, due to a violation of the norms of feeding calves, which increased the content of putrid and conditionally pathogenic microflora, and, therefore, caused dysbacteriosis. One calf of this group was found to have a pathogenic strain of Escherichia coli, which suggests the bacterial etiology of dyspepsia in this household.

In calves of the 3rd group, the CFU of Escherichia coli was 1.7±0.7·10^8 in 1 g of feces (p < 0.05). Lowering the CFU of Escherichia in this group is associated with the action of the antibiotic on the intestinal microflora.

In the 4th group of calves who had recovered after dyspepsia and underwent a course of antibiotic therapy, the following indicators were observed: the number of CFU of Escherichia 0.2±0.2·10^8 (p < 0.05); 0.07±0.004·10^8 (p < 0.01); 2.1±0.3·10^8 (p < 0.05) in 1 g of feces on 3rd, 6th and 9th day after cessation of antibiotic therapy, respectively. The decrease in the CFU of Escherichia on days 3 and 6 after the end of antibiotic therapy is probably due to the residual effect of the antibiotic in the intestines of calves even after the completion of antibiotic therapy. Indeed, according to the description, Rificyclinum remains in the body in therapeutic concentrations for at least 12 hours after a single use and is eliminated mainly with feces and urine, while Enroxil remains in the therapeutic concentration in the body for another two days after application. In this farm, Rificyclinum treatment was carried out for at least 3-5 days, depending on the severity of the disease. These features of the pharmacokinetics of antibiotics used, in our opinion, explain that for some time the growth and development of Escherichia in the intestine decreases after the end of antibiotic therapy. At the same time, by day 9, the CFU of Escherichia rises up to 2.1±0.3·10^8 (p < 0.05) in 1 g of feces. It should be noted that a pathogenic strain of Escherichia coli was also found in one calf in this group, although no clinical manifestations of calf dyspepsia were found.

In the 5th group of calves, which immediately were prescribed to take Vetom 2 after the end of antibiotic therapy, the following results were obtained: the CFU of Escherichia in 1 g of feces on 3th, 6th and 9th days after antibiotic therapy equaled, respectively, 1.1±0.5·10^8 (p < 0.05); 0.4±0.2·10^8 (p < 0.05); 1.6±0.1·10^8 (p < 0.01). The number of CFU of Escherichia on days 3 and 6 after the end of antibiotic therapy was significantly higher, while on day 9 it was significantly lower compared to the 4th group of calves. At the same time, in calves of the 5th group treated with the probiotic, on days 3 and 6 there was a decrease in the CFUs and an increase in it on day 9. However, on day 9 of the study in the 5th group of calves, the CFU was still 24% lower than that in the 4th in the group of calves who after antibiotic therapy did not receive Vetom 2. In our opinion, this is due to the antagonistic activity of bacteria strains included in Vetom 2 in relation to conditionally pathogenic strains of colibacillosis in the intestine.

Studies on the determination of streptococci in the feces of calves showed that when inoculating samples from feces from healthy calves on nutrient media, there was no growth of streptococci. The group of sick calves from which samples were taken before antibiotic therapy (group 2), on the contrary, had continuous growth of characteristic streptococcal colonies. In calves from which samples were taken during treatment (group 3), the number of streptococcal colonies was noticeably reduced; in some calves the growth of these bacteria was absent, which is explained by the effect of antibacterial drugs during treatment of calves. In the groups of calves from which samples were taken on 3rd, 6th and 9th days after the end of antibiotic therapy, even with a visual assessment, it was seen that on nutrient media when inoculating from samples of feces of calves treated with Vetom 2, the growth of streptococcal colonies was less pronounced relative to calves not treated with Vetom 2 after completion of antibiotic therapy. This circumstance, in our opinion, is associated with the antagonistic effect of the bacterial strains that are included in Vetom 2, in relation to opportunistic pathogens of the intestine.

During the study of samples of feces of calves for the content of staphylococci, it was found that when inoculating from samples of healthy calves on glucose-blood agar, the growth of staphylococci would be
was absent. At the same time, in the group of sick calves, in which samples were taken before antibiotic therapy (group 2), a characteristic growth of non-plasma-coagulating staphylococcus (S. Xylosus) on nutrient media was observed. In calves during treatment with antibiotics (group 3), this type of bacteria disappears, and when inoculated from samples of calves of this group on nutrient media, the growth of staphylococci was not observed, which is explained by the action of antibiotics used in the course of treatment. In the group of calves who were given probiotic (group 5) after antibiotic therapy on days 3 and 6 after the end of antibiotic therapy, the growth of staphylococci on nutrient media was absent, and on day 9, 20% of calves showed characteristic staphylococcal colonies. In the group of recovered calves that did not receive Vetom 2 (group 4), on day 3 after the end of antibiotic treatment there was no growth of staphylococci, on day 6, 20% of the calves on nutrient media showed colonies of non-plasma-coagulating staphylococci, and on day 9 of the study the number of such calves increased up to 40%.

4. Conclusion
1. In animals of all experimental groups, the intestinal microbial flora is not the same. In sick calves, prior to antibiotic therapy, the number of conditionally pathogenic microorganisms was higher relative to the group of healthy animals. During treatment, under the action of antibiotics, the number of these microorganisms decreased, or was completely absent.
2. In calves that received probiotic (Vetom 2) during rehabilitation after the end of antibiotic therapy, the number of conditionally pathogenic microorganisms was lower by 24% (1.6±0.1·10^8 CFU) relative to the group of calves who did not receive this probiotic (2.1±0.3·10^8 CFU).
3. The bacterial strains included in Vetom 2 inhibit the development of conditionally pathogenic microorganisms in the intestines of calves after antibiotic therapy, providing favorable conditions for the restoration of the natural intestinal microflora after the use of antibiotics.

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