Effect of probiotic bacteria of the genus Bacillus on gnorobic pathogens of surgical infections

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Abstract. As a result of the study of the antimicrobial activity of selected probiotic preparations with isolated culture of Str.pyogenes, it has been found that the probiotic preparation Inoprovet2, prepared from Bac.subtilis, Bac. licheniformis, had the earliest and highest activity (starting from 6 hours the reduction of streptococcal culture was noted by 39 ±0.9, after 24 hours - 78 ±2.6, after 96 hours - 100%, i.e. destroys the pus microbe) compared to the others. Inoprovet1 differed from Inoprovet2 only by the quality of the excipient, but its activity was slightly lower (6 hours - 22 ±1.1, 24 hours - 53 ±3.9 and 96 hours - 100%), the third most active was Vitasporin12B probiotic (6 hours - 21 ±1.8, 24 hours - 51 ±2.3 and 96 hours - 98 hours - 98 ±4.2%), followed by Vetom 1. 2 (16±2.9 after 6 hours, 51±0.5 after 24 hours and after 96 hours 96±1.8%) and probiotic Sporovetin which showed antibacterial activity on killing streptococcal culture after 6 hours 16 ±2.9, after 24 hours 45 ±3.3 and after 96 hours 89±2.6%. The obtained results, the possibility of using Inoprovet2 probiotic in clinical practice not only for the treatment of gastroenteric infections but also for wound infections

1 Introduction

At present, increasing the number of farm animals and providing the population with high-quality, safe, and environmentally friendly products is impossible without the active intervention of veterinary specialists in the prevention of infectious, non-infectious and invasive animal diseases. Attempts to enlarge farms by small farms, transition to industrial production of agricultural products, in some cases, unprofessional approach to the cultivation and maintenance of livestock and poultry, nutritional disorders, postvaccinal stress, and regrouping, active use of antibiotics as prophylactic agents for young animals, as well as their incorrect use for treatment inevitably disrupt the microbiological balance in the gastrointestinal tract and lead to a widespread distribution of antibiotic-resistant livestock and poultry. Surgical diseases, both in medicine and in veterinary medicine, in 30 - 35% of the cases can be accompanied by infections in the form of acute and chronic purulent diseases. Lack of comprehensive treatment of wound processes leads to disruption of the

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body's homeostasis and the functions of internal organs, which reduces the effectiveness of drugs and contributes to complications of diseases [3].

In this regard, the search and development of environmentally safe therapeutic and prophylactic means for the treatment of surgical diseases is currently an urgent problem.[4].

To achieve these aims authors offer various approaches, for instance, improvement methods for topical therapy and using effective antimicrobials. Antibiotics and antiseptics are known to inhibit local and general, specific and non-specific immune, which is a complication in the treatment and worsens the course of the disease. For this high frequency and variability of infectious complications (sepsis, meningitis, mediastinitis, etc.) and the frequency of microorganisms acquiring resistance by using the used drugs, lead to a decline in the level of general and local immunologic reactivity of the animal organism under certain conditions, require further study, development and improvement of ways and means of treatment of wounds [5].

Lately, the focus of attention of scientific researchers in this matter has shifted to probiotic preparations, the antibacterial activity of which is conditioned by the ability to produce amino acids (asparagic, glutamic, folic acids, glycine, serine, proline, histidine, tyrosine, tryptophan, valine, phenylalanine, isoleucine, lysine, alanine); low molecular weight proteins (peptides); organic acids (lactic acid, formic acid, acetic acid, propionic acid, folinic acid); naturally occurring antibiotics, including thermostable antibiotics (acidophilin, lactocidin, lactolin, nisin); vitamins of groups C, D, B; enzymes, in particular lysozyme, alcohols, hydrogen peroxide, and, in addition. actively compete for nutrients and receptors for adsorption on host cells with subsequent colonization, which gives probiotic microorganisms the opportunity to temporarily, and this most often falls during the period of treatment, to be present in the body of a sick animal and suppresses the development of putrefactive and pathogenic organisms, address the weaknesses of normal microflora, promotes the destruction of toxic metabolic products with carcinogenic effect of nitrites, reduces cholesterol and oxalate levels, promotes the breakdown of lactose [6-8].

Numerous wound pathogens multi-antibiotic resistance. Ideal means, possessing antibacterial and regenerating effect could be the probiotics genus Bacillus, capability to produce antibiotics and bacteriocins. This type of bacteria increases healing wounds with its antioxidant activity and working out proteolytic ferments due to their thrombolytic action and prevention of scarring. For some authors, the quality of funds to treat non-infection wounds, purulent-necrotic processes, burns, and dermatitis was used Bioseptin, Bactisporin, Sporobacterin, and Bactisporinlast.

Our work aimed to design, characterize, and study the antimicrobial properties of probiotic preparations such as Innoprovet1, Innoprovet 2, Sporovetin, Vetom 1.2 and Vitasporin prepared based on Bac. subtilis, Bac. licheniformis, Bac.amyloliquefaciens on gnorobic pathogens of surgical wounds. Tasks accomplished included:

- Isolation of gnorobic pathogens from various sources, study of their properties and identification.
- Study of in vitro antimicrobial activity of selected probiotic preparations on isolated gnorobic microorganisms.
- Study of the effectiveness of probiotic preparations on wound healing under "in vivo" conditions.

2 Materials and methods

The isolation of gnorobic microorganisms was performed from abscesses of 4 cats. The wool the abscess area was clipped, treated with alcohol, opened and the material was pipetted into a sterile tube, the preparation was prepared and stained by Gram's method. The material in the tube was diluted with sterile physiological solution 1:20 and sown on

simple nutrient media as well as media with 5% defibrinated blood, serum and sugar broth. The isolated culture of gnorobic microorganisms was identified by culture and biochemical properties.

To determine the microbe species, the cultures were grown at +10 º and 45 ºC, on medium with 6.5% NaCl, on medium with pH 9.6, on medium with 40% bile, in milk with 0.1% methylene blue and after heating for 30 min at 60 ºC. The cultures were examined for the formation of gas and hydrogen sulfide, indole, catalase with 3% hydrogen peroxide and fermentation of galactose, maltose and mannitol. The isolated cultures were examined for the formation of gas and hydrogen sulfide, indole, catalase with 3% hydrogen peroxide and fermentation of galactose, maltose, mannitol when grown on carbohydrate media.

2.1 Study of antimicrobial activity of selected probiotic preparations

Antimicrobial activity of selected probiotic preparations was studied on isolated gnorobic microbes, which were sown on Petri dishes with meat-peptone agar with blood in the volume of $1.5x10^8$ CFU/mL, kept for 10 min to adapt microbes to the medium, excess liquid was taken away with a pipette. Pure daily culture grown on solid nutrient medium was used for inoculation, identical, clearly isolated colonies were selected. A set of cells from a single colony cell was transferred to a test tube with sterile physiological saline solution and seeded turbidity to McFarland's standard. One drop (0.2 ml) of probiotic preparation in the volume of $1.5x10^9$ CFU/ml was dripped on the surface of agar with culture, left for 2-3 min, then the Petri dish was inverted and placed in the thermostat for 1-2 days at 37 º C. A total of six Petri dishes were used for the experiment. The result was evaluated visually by the presence of a “clean zone” in the place of probiotic action.

The study of bactericidal activity of probiotic preparations was carried out in test tubes, for which an experiment in broth with isolated culture ($1.5x10^8$ CFU/mL) and probiotics ($1.5x10^9$ CFU/mL) was made. The result was recorded after 6 and 24 hours by sowing 1 ml of liquid from each tube into a Petri dish with nutrient medium and then counting the number of CFU from the mixture of pus microbes with each probiotic in comparison with the number of pus microbes without probiotics by the percentage of dead cells relative to the growth of live cells (CFU/ml) in the control tube with the culture of isolated microorganisms. The bacterial reduction ratio was estimated using the following equation: R (%): A-B/A ≈ 100%, where R is the percent reduction ratio, A is the number of bacterial colonies from the untreated bacterial suspension (control tube to which no probiotics were added) and B is the number of bacterial colonies of gnorobic bacteria grown with the presence of one of the probiotics. Usually, the bactericidal effect is considered 90% lethality in 6 hours and 99% in 24 hours. Based on the results of this experiment, we determined the most effective probiotic in relation to the microorganisms we isolated and further studies were conducted with this probiotic preparation.

3 Results and Discussion

Results of isolation of gnorobic pathogens from various sources, study of their properties and identification.

Results of microscopy of material containing pus. The preparation prepared from the selected material from abscesses of four cats of different breeds and ages was Gram stained. According to the outcome microscopy, a picture of various microbes (rod-shaped gram (-) and coccoid (gram (+)) was observed in one of the cats, but since gram-positive coccoid microbes predominate in the acute stage of the development of the purulent process and in 50% of cases at the initial stages of the formation of a chronic wound, gram-negative species of the genus E.coli and Pseudomonas they begin to develop at later stages,

subsequently enter into the deep layers of the skin and cause significant tissue damage, as a result of which this material was not used in the future. The other three cats were observed to have mono, cum, and streptococcal, gram-positive-stained microorganisms. Additional coloring by various methods made it possible to judge that these microbes do not form spores and capsules.

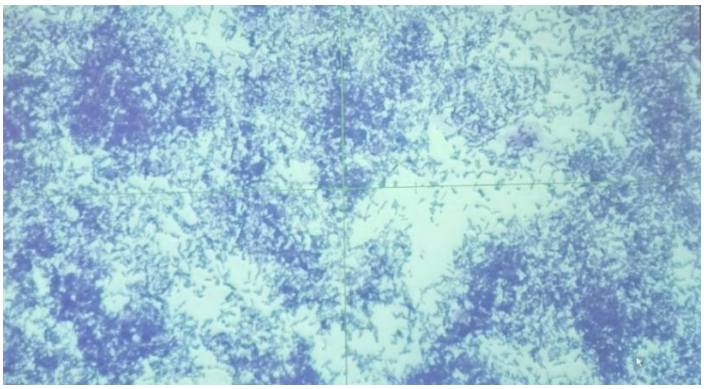

Fig. 1. Gram staining of microbes isolated from feline abscesses.(OPTA-TECH /POLISH AID 40*/0.65).

Then pus taken with a sterile pipette from the opened abscess was transferred to a sterile tube, diluted with sterile physiological solution 1:20 and sown on simple nutrient media as well as media with 5% defibrinated blood, sugar broth. The results of cultivation of the isolated cultures are presented as follows: Two cultures showed crumbly wall growth on sugar broth, one culture showed benthic-pristine growth in the form of crumbly sediment, all 3 cultures had clear broth. The growth of delicate transparent small colonies with flat rough surface and irregular openwork edges was observed on agar. To determine the microbe species, the culture was grown at +10o and 45 ° C, on medium with 6.5% NaCl, on medium with pH 9.6, on medium with 40% bile, in milk with 0.1% methylene blue, and after heating for 30 min at 60 ° C. The culture was then grown on medium with 6.5% NaCl, on medium with pH 9.6, on medium with 40% bile, in milk with 0.1% methylene blue and after heating for 30 min at 60 ° C. Negative results were obtained for cultures isolated from all three cats. The isolated cultures were studied according to their biochemical properties. The studied three cultures in serum media did not form gas, hydrogen sulfide, indole, were catalase-negative, well fermented: galactose, maltose, mannitol. Accordance with the results culture morphological and biochemical properties, cultures isolated from purulent material from three cats were attributed to the genus Streptococcus pyogenes.

The results of the study of antimicrobial activity of selected probiotic preparations were carried out on six Petri dishes, each of the five dishes was sown a culture of Streptococcus pyogenes at a volume of $1.5x10^8$ CFU/mL, incubated for 10 min and 1 drop (0.2 ml) of probiotic preparation at a volume of $1.5x10^9$ CFU/mL was dropped on the surface of agar with the culture and placed in the thermostat for 1-2 days at a temperature of 37 ° C. The results of the study of the antimicrobial activity of selected probiotic preparations were analyzed. In addition, a disk impregnated with probiotics was placed on the agar surface with the seeded culture. In the sixth cup, the seeded agar surface was not affected by the probiotic. The result was assessed visually by the presence of a "clear zone" of inhibition of the streptococcal culture by the probiotic. Probiotic Inoprovet1 was dropped into the first petri dish, Inoprovet2 - into the second, Sporovetin - into the third, Vetom 1.2 - into the fourth and Vitasporin - into the fifth. When examining the seeded cups on the second day after sowing, rapid growth of streptococcal culture was observed in all cups, except for the

second cup with Inoprovet2. Around the disk with Inoprovet2 probiotic a weaker growth of streptococci was noted, which was manifested in a smaller number of microorganisms colonies.

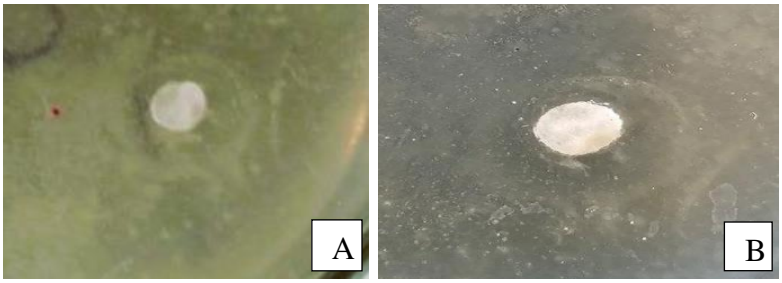

Fig. 2. Antimicrobial activity of the probiotic preparation Inoprovet2 on Streptococcus pyogenes isolated from abscess: A - after 6 hours; B - after 96 hours.

Table 1. Results of the study of bactericidal activity of probiotic preparations on Streptococcus pyogenes (in %).

Reduction of Str. pyogenes with probiotics	Decrease in Str.pyogenes count after 6 hours	Decrease in Str.pyogenes counts after 24 hours	Decrease in Str.pyogenes counts after 96 hours
Inoprovet1	22 ± 1.1	53 ± 3.9	100
Inoprovet2	39 ± 0.9	78 ± 2.6	100
Sporovetin	16 ±2.9	45 ± 3.3	89 ±2.6
Vetom 1.2	16 ± 2.9	51 ± 0.5	96 ±1.8
Vitasporin 12B	21 ±1.8	51 ± 2.3	98 ± 4.2

It follows from the data in the table that the probiotic preparation Inoprovet2 prepared from Bac.subtilis, Bac. licheniformis had the earliest and the highest activity (starting from 6 hours the reduction of streptococcal culture was noted by 39 ±0.9, after 24 hours - 78 ±2.6, after 96 hours - 100%, i.e. completely destroys the pus microbe) compared to the others. Inoprovet1 differed from Inoprovet2 only by the quality of the excipient, but its activity was slightly lower (6 hrs 22 ±1.1, 24 hrs 53 ±3.9 and 96 hrs 100%), the probiotic Vitasporin12B prepared from Bac.subtilis, (6 hrs 21 ±1.8, 24 hrs 51±2.3 and 96 hrs 98 ±4.2%), followed by Vetom 1. 2 prepared from probiotic microorganism of genus Bac.amyloliquefaciens (16 ±2.9 after 6 hours, 51±0.5 after 24 hours and after 96 hours 96 ±1.8%) and probiotic Sporovetin prepared from Bac.subtilis which showed antibacterial activity on killing streptococcal culture after 6 hours 16 ±2.9, after 24 hours 45±3.3 and after 96 hours 89 ±2.6%.

The data obtained indicate that all probiotic preparations prepared from Bac.subtilis are active against gnorobic streptococcal microorganisms. The probiotic with both Bac.subtilis and Bac. licheniformis had the highest activity, allowing it to be used as a treatment for purulent wounds. However, also Bac.amyloliquefaciens Vetom 1.2 was active against suppurative Streptokokkus pyogenes after Inoprovet2, Inoprovet1, Vitasporin 12B with Bac.subtilis, which suggests the active fraction of its effect on the microbes of purulent wounds.

It is believed that the use of Bacillus subtilis spore culture contaminates wounds, complicating the healing process. However, the works of many authors proved the opposite. that proteases produced by Bacillus subtilis are able to kill pathogens, while proteolytic enzymes of this probiotic microbe necrotize injured tissues, promoting wound healing [1].

Vegetative forms of Bacillus subtilis that have entered the wound form spores that contain dipicolinic acid, which has antimicrobial activity. These spores subsequently germinate and this process is repeated many times until the wounds are completely healed. The infection is cured. [2].

The antimicrobial activity of this probiotic biopreparation containing spore cultures of Bacillus subtilis is explained by the action of dipicolinic acid and the mechanism of increasing local immunity.

4 Conclusion

Cultures were isolated from abscesses of four cats, from which three cultures homogeneous by microscopic analysis were selected for examination. Based on the results of culture and biochemical studies, the isolated culture was identified as belonging to the species Str.pyogenes. Account of the results of the study of antimicrobial activity of selected probiotic preparations with isolated culture of Str.pyogenes, it was found that the probiotic preparation Inoprovet2 prepared from Bac.subtilis, Bac. licheniformis had the earliest and the highest activity (starting from 6 hours the reduction of streptococcal culture was noted by 39±0.9, after 24 hours - 78±2.6, after 96 hours - 100%, i.e. completely destroys the pus microbe) in comparison with the others. Inoprovet1 differs from Inoprovet2 only by the quality of the excipient, but its activity was slightly lower (6 hours - 22±1.1, 24 hours - 53±3.9 and 96 hours - 100%), the probiotic Vitasporin12B, prepared from Bac.subtilis, was ranked third in activity (6 hours - 21±1.8, 24 hours - 51±2.3 and 96 hours - 98 hours - 98±4.2%), followed by Vetom 1. 2 prepared from probiotic microorganism of genus Bac.amyloliquefaciens (16±2.9 after 6 hours, 51±0.5 after 24 hours and after 96 hours 96±1.8%) and probiotic Sporovetin prepared from Bac.subtilis which showed antibacterial activity on killing streptococcal culture after 6 hours 16±2.9, after 24 hours 45±3.3 and after 96 hours 89±2.6%. The data obtained indicate that all probiotic preparations prepared from Bac.subtilis are active against gnorobic streptococcal microorganisms. The probiotic, which contains both Bac.subtilis and Bac. licheniformis, had the highest activity, which allows it to be used as a treatment for purulent wounds. The obtained results, the possibility of using the probiotic preparation Inoprovet2 probiotic in clinical practice not only for the prevention and treatment of dysbacteriosis, gastroenteric infections, but also in wound infections.

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