UDC 619:616.981.49:619:612.017:636.2
© 2016

P. Lavriv, Candidate of Veterinary Sciences, Doctor of Philosophy
R. Kravtsiv, Academician of the NAAS, Doctor of Biological Sciences
Lviv National University of Veterinary Medicine and Biotechnology named after SZ Gzhytsky
I. Avdosyeva, Candidate of Veterinary Sciences,
State Scientific-Research Control Institute for Veterinary Medicinal Products and Feed Additives

ROLE OF NANOPREPARATION HERMACAP IN PROPHYLACTICS OF SALMONELLOSIS AND HEIGHTENING STATUS AT CALVES

The purpose. To study role of new nanopreparation hermacap in prophylactics of salmonellosis of calves and heightening immune-and-physiological status at them. **Methods**. 2 groups of calves were formed using method of groups-analogs. Animals in control group got placebo — intramuscular 0,9% solution of sodium chloride in dose of 5 cm3 for an animal with an interval of 14 days doubly. Animals of experimental group got intramuscularly hermacap in dose of 5 cm3 for a head with the same interval. **Results.** Efficiency of hermacap is proved in prophylactics of contagions and salmonellosis at calves. **Conclusions.** Intramuscular injection to calves of nanopreparation hermacap positively influences their immune-and-physiological status, and also prophylactics of gastrointestinal diseases and salmonellosis, and heightening of productivity by increase of alive mass.

Key words: calves, germicidal, lysozyme and phagocytal activity of leucocytes, subpopulations, T- and B-lymphocytes, immune-and-physiological status, nanopreparation hermacap, salmonellosis.

Today, due to the development of industrial dairy cattle, calf disease in gastro-intestinal diseases, including salmonella, is a very topical and economically important problem. This problem is also attracted by considerable attention in many studies, both domestic and foreign scientists. It is necessary to point out that microbes tend to mutate changes more than macroorganisms, and, therefore, ahead of them in terms of evolution.

Thus, microorganisms may appear in the unprotected and vulnerable state of a new pathology. However, in this period it is possible to increase the pathogenic qualities in the microcosm due to the synergistic interaction of infectious agents in the created association in one or another parasitosis. This

fact emphasizes once again the need for a comprehensive and routine study of parasiticots, artificially created due to the high concentration of livestock. Thus, the establishment of a new approach in the study of etiology in contagious pathology led to a rethinking of the relationships that existed between the etiological complex and the macroorganism, that is, calves and cows-mothers. The function of the protective complex is to combine all the protective functions of the macroorganism, namely: immunity, nonspecific protection, medical care, the use of therapeutic and prophylactic means. Only thanks to adequate measures it is possible to overcome the associated microbiological population in modern industrial complexes, which are a high-tech branch [1, 2, 5, 6]. However, at present, in real production, it is impossible to quickly establish the entire spectrum of the pathogen-damaging complex due to a number of objective reasons, namely: lack of funding, lack of diagnostic test systems, shortage of certified laboratories, etc. However, there remains the daily task of industrial livestock, providing the food market with quality products. Due to this, in the countries with developed livestock raising the importance of preventive prophylactic schemes, which are aimed at microflora normoflorization among the livestock and in industrial premises, has considerably increased. It should also be noted that the presence in the arsenal of veterinary medicine of a large number of antibacterial drugs, their large variety of recommended schemes for the prevention and treatment of symptomatic diseases of the gastrointestinal tract in calves indicates a lack of effectiveness in the conditions of real production. However, due to the action of parasitocenoses, the correct prevention strategy remains relevant. In this case, the use of prevention should be aimed at preventing the increased pathogenicity of parasitoenoses and the reduction of the possibility of replenishment by newcomers. But today, due to restrictions on the use of antibiotics in livestock, which are used for therapeutic purposes and in the prevention of salmonella in accordance with the recommendations and requirements of the world agro-market, the increasing importance among appropriate means of increasing importance of the introduction of nanoparticles and feed additives, which are manufactured by modern nanotechnology from cheap and available source of trace elements

based on nanocarboxylates of germanium, zinc and silver. Nanopreparations, including nanoparticle Germancap, as noted by Avdosyev I. with co-authors [1] have the property of highly effective adsorbents and have a positive effect on the body tissues.

However, the ambiguity in the evaluation of the results of use for the prevention of gastrointestinal diseases, including salmonella in dairy livestock of nanoparticles, as evidenced by literary sources, will be of great importance in the development of veterinary and humane medicine and biology [7, 8, 9, 10, 11 , 12]. The purpose of our work To study the role of a new nano-agent of Germancaup in the prevention of calmonella calves and increase the immuno-physiological status of them. Germancap, a solution for injections, manufactured by LLC "Nanomaterials and Nanotechnologies" (Ukraine), which has active substances of both germanium and zinc (in the form of citrates) of 5.0 mg in 100 ml of the preparation, and the excipients polyethylene glycol 400 and water for injection are in favor. Material and methods. Clinical, biochemical and immuno-physiological studies of the Germancap drug were conducted in the conditions of the agricultural production cooperative named after Mykhailo Hrushevsky (St.No. 20536228) in the Rogatinsky district of the Ivano-Frankivsk oblast on calves 2 and 3 weeks after birth, black-and-white breed. The farm for five years is happy with the disease of calves for salmonella. After a clinical examination of calves, two groups of animals were formed in 10 heads each, at the age of 10-12 days from birth. Controlled calves were administered twice a placebo, intramuscularly 0.9% sodium chloride solution at a dose of 5.0 cm3 per animal at intervals of 14 days, and in the experimental group intramuscularly administered the Germancap doses of 5.0 cm3 per head as well. at intervals of two weeks. Before and at the 7th, 14th and 30th day after the application of drugs, from calves of both groups, blood was collected for further laboratory studies. The morpho-functional state of the body of the calves before and after introduction of the nanoparticle was assessed by morphological and individual immunological parameters of blood, which were determined according to generally accepted methods and RNGA. The results obtained were statistically

processed using standard computer programs. The difference between the two values was considered probable for p <0,05; 0.01; 0.001 [3, 4]. Research results. In conducting the analysis of the results of our studies, it should be noted that before the time of use of Germancaup and in the study period, the overall clinical status of calves in both groups, both controlled and experimental, remained satisfactory and after the administration of the drug, no different adverse reactions were observed. However, in calves of the experimental group, the use of Germancup led to a significant improvement in appetite, the hair became brilliant, their activity significantly increased. The analysis of laboratory blood tests of calves from the following table 1 indicates that they have a probable growth of total protein in the experimental group at 8.93% (P <0.001) on day 7 after the administration of the drug Germancaup according to the day of the beginning of the experiment, in that time, as in the control group we notice growth of only 1.82%. If we compared the experimental group with respect to the control, then on the 7th day it had a probable increase in the total protein, which was 7.02% (P <0.001). Also, in the experimental group, after 14 and 30 days, the apparent increase in total serum protein intake was observed relative to the administration day at 10.71% and 16.07% (P <0.001). However, in the control group, the values of the total protein content remained within the range corresponding to their content on the day of administration. Having analyzed the content of leukocytes in the blood, it should be noted that during the lunar experimental period in the experimental group also their growth is according to the day of the administration of the drug Germancaup on 7- , 14th and 30th day at 16.67, 14.82 and 22.22%. However, comparing the data of the experimental group with the control, on the 7th, 14th and 30th day, we note the increase in the content of leukocytes by 16.6; 12.73; and 24.53%. Along with this, we also see from Table 1 some changes in the content of phagocytic activity of leukocytes in calves compared with the date of administration of the drug Germancaup until the end of the 30-day period. Thus, with its repeated introduction, the tendency towards a probable growth in percentage ratio is shown to be 4.64; 4.44 and 5.85% of phagocytic leukocyte activity. Also, by analyzing the titre of paratyphoid anti-antibody antibodies in Table 1, it should

be noted that on the seventh day after the administration of the drug Germancaup in the experimental group, it increased by 66.67%, and in the control at 16.67%, but in two weeks it returned to the experimental group to the limits of the previous norm, that is, 1: 500, but in the control group decreased by 16.67%. However, one month after the date of the first administration of the drug in the experimental group, its growth is 2.2 times relative to the day of administration, and in the control group remained within the previous norm.

Thus, from the analysis of this table 1, we see that throughout the experimental period after the double administration of nanoparticle Germancapu in the experimental group in young calves the total protein increased from 8.93 to 16.07%, the content of leukocytes from 14.82 to 22.22%, and phagocytic activity of leukocytes from 4.44 to 5.85%, and next we note the growth of the titer of paratyphoid antibodies among the experimental group in a month relative to the day of administration by 2.2 times.

Table 1

Peculiarities of indices of cellular and humoral immunity components in calves of Ukrainian black-and-white milk (M ± m), n = 5

Time of investigation	Animal groups	Total protein, g / 100ml	Leukocytes	Fagocytic activity of leukocytes,%	Tetra parathephosis
On the day of the administration of the drug	Experimental	5,6±0,06	5,4±0,5	49,5±1,5	1:300
	Chewable	5,6±0,06	5,3±0,4	49,7±1,8	1:300
7th day after the administration of the	Experimental	6,1±0,07***	6,3±0,6	51,9±1,7	1:500
			5,4±0,5	49,6±1,4	1:350

drug	Chewable	5,7±0,04			
Experimental Trial 14th day after the administration of the drug.	Experimental Chewable	6,2±0,05*** 5,6±0,04	6,2±0,3 5,5±0,4	51,7±1,3 49,5±1,1	1:500 1:300
Experimental Chewable on the 30th day after the administration of the Trial Control	Experimental Chewable	6,5±0,04*** 5,6±0,05	6,6±0,6 5,3±0,6	52,5±1,5 49,6±1,3	1:650 1:300

Notes: In this table, the mark * - the difference is likely, compared with the control group of calves, which did not enter immunomodulators (* -P <0,05; ** - P <0,01; ** - P <0.001).

Taking into account the analysis of literature data, we can say that the precursors of immune lymphocytes that do not have the ability to interact with antigen (zero, nonimmunocompetent, lymphocytes) play an important role in increasing the immune potential of calves in the prevention of salmonella, in the course of their development in the central organs of immunity, they are transformed into T- and B-lymphocytes, capable of reacting with certain antigens when they enter the body. Antigen selectively stimulates in peripheral organs of immunity further development and differentiation of only certain clones of T-and B-lymphocytes. It should be noted that in the body of calves, as is known, reactions of humoral type carry B-lymphocytes and cell-T-lymphocytes, and along with this there is a unity of cellular and humoral immunity. However, it is believed that T - Killers are attracted and accumulated in those places where the antigen is located and here they cause inflammation,

or react to the action of lymphokines released by lymphocytes. B-lymphocytes on their surface contain, in addition to their own immunoglobulins of different classes, also receptors to the Fc fragment of IgG and the third component of the complement (C3). Also, at a physiological norm in the body there is a certain ratio of T-lymphocytes to B-cells that take an active part in the production of immunoglobulins of different classes. Thus, for the purpose of more detailed study of the formation of immuno-physiological status in calves against salmonellosis, blood samples were taken on T- and B-lymphocytes, the content of immunoglobulins, and BASK and LASK. Thus, by analyzing the study of T lymphocytes in experimental groups of calves, in comparison with the control, according to the following table 2, we see that already in 7 days after the administration of the drug Germacap their percentage in percentage was significantly increased in the experimental group compared to the control at 5.64% ($P < 0.001$), as well as an increase with respect to the day of drug administration of 3.63%. However, at the same time, we see that there is a slight increase in the number of B-lymphocytes in the experimental group at 7 days relative to the control group by 13,06% ($P < 0,05$), and according to the day of drug administration, the growth was 25,21%.

Also, from the results presented in Table 2, we see the growth in the experimental group relative to the control T-lymphocytes on the 14th day after the start of the experiment at 5.03 and 6.66% at the 30th, and compared with the experimental group before the experiment, then the growth of The 14th day was 2.21% and only 1.26% on the 30th day. At the same time, analyzing Table 2, we see that on the 14th day the calves undergo a decrease in blood T-lymphocytes relative to the 7th day from the beginning of the experiment by 1.37%. Comparing the data of the control group, it is clear that on the 14th day the decrease was only 0.65%, and the difference between the experimental group was 0.72%. Such a decrease in T-lymphocytes at the 14th and 30th day in the experimental group of calves after the start of the trial indicates an elevated immuno-physiological level in the experimental group after repeated intramuscular administration of the nanoparticle of Germancaup. Along with

this, we note the change in the unlikely increase in the number of B-lymphocytes in the whole blood of experimental calves on the 7th and 14th day from the beginning of the experiment relative to the control group by 13.06%; 11,03% (P <0,05) and a slight increase to 3,34% on the 30th day. However, according to the day of the beginning of the experiment, the growth was on the 7th, 14th and 30th day - 25.21, 28.93 and 30.58%. Thus, the study showed that the administration of intramuscular calves of the Germanicap nanoparticle in A dose of 3 cm3 with repeated administration at the same dose in 7 days causes more significant changes in T and B lymphocytes in the experimental group compared with the control group. Along with changes in leukocytes, T-and B-lymphocytes and phagocytic activity are changes in immunoglobulins. Thus, according to Table 2 data, on the 7th day after the administration of the Germanicap nanoparticle, the growth of immunoglobulins in the experimental group was noted as 15.19% relative to the control, however, on the 14th and 30th day the growth decreased accordingly to 14.12% and 12.5 % Also, comparing the experimental group with the day of the experiment on the experiment, we note the growth, which was on the 7th, 14th and 30th day - respectively56.9; 67.24 and 70.69%. By contrast, according to our data in Table 2, the change in blood serum bactericidal activity (BASK) in calves of the experimental group relative to the control on the 7th, 14th and 30th day after the on-site trial an unlikely increase of 13.84% (P <0.05), 8.73 (P <0.01) and 11.79%.

Table №2

Features of indicators of cellular and humoral immunity in calves of Ukrainian black-and-white milk (M ± m), n = 5

Time of study	Animal groups	T lymphocytes%	B-lymphocytes,%	Immunoglobulin content, g / l	BASK blood serum,%	LASC of blood serum,%

On the day of the administration of the drug	Experimental	63,3±1,3	24,2±1,5	5,8±0,5	45,3±1,7	24,1±1,2
	Chewable	63,6±1,4	24,4±1,7	5,9±0,7	45,5±1,9	24,2±1,3
7th day after the administration of the drug	Experimental	65,6±0,9 ***	30,3±1,1 *	9,1±0,8	54,3±1,9 *	25,7±1,3
	Chewable	62,1±0,7	26,8±0,9	7,9±0,9	47,7±1,7	24,9±1,2
Experimental Trial 14th day after the administration of the drug.	Experimental	64,7±1,6	31,2±1,1 *	9,7±1,3	59,8±1,1 **	25,3±1,4
	Chewable	61,6±1,1	28,1±0,7	8,5±1,1	55,0±0,8	24,3±0,9
Experimental Chewable on the 30th day after the administration of the Trial Control	Experimental	64,1±1,3	31,6±1,3	9,9±1,5	64,5±2,3	25,9±1,5
	Chewable	60,1±0,9*	28,7±0,9	8,8±1,3	57,7±2,1	24,8±1,9

Notes: In this table, the mark * - the difference is likely, compared with control group of calves, which did not enter immunomodulators during vaccination (* -P <0,05; ** - P <0.01; *** - P <0.001).

However, in comparison with the experimental group, the growth rate, which was on the 7th, 14th and 30th day - 19.87, 32.01 and 42.38%, was also observed during the experiment. This fact confirms the ability of the blood to self-purify, namely, the presence of highly soluble substances in the blood that

can kill or neutralize microbial cells, including salmonella. Along with this, from this table 2 it is seen that changes in lysozyme activity of blood serum (LASK) in calves of the experimental group, namely, we note its increase in the experimental group as compared with the control group on the 7th, 14th and 30th day relative to the day after staging a trial of 3.21; 4.12 and 4.44%. However, analyzing the experimental group with respect to the day of the experiment on the experiment, we see an increase in the 7th; The 14th and 30th day at 6.64, 4.98 and 7.47%. It should be noted that the analysis of our research on the effect of Germanicap Nanoparticle on the immuno-physiological state of calves in relation to Table 2 shows that during the trial, which lasted a month after two-time intramuscular administration to the experimental group of this preparation at intervals of 7 days, they had blood in The 14th day compared with the control group is a decrease in the number of T-lymphocytes by 0.72%, which indicates an elevated functional state of the systems, their potential ability to react with the antigenic mother Alum to form an adequate immune response, namely, the increased immune status of the organism compared with the control group.

Also, the growth of B-lymphocytes in the experimental group of calves as compared to the control throughout the trial period was noted at 3.34 - 13.06%, but compared to the experimental group with the onset of the experiment, the growth was from 25.21 to 30.58%. Thus, it should be noted the growth of both T- and B-lymphocytes, which indicates that they have a specific ability to create and maintain immunological memory of the pathogens of the disease and other genetically alien agents. At the same time, this fact confirms the increase in the function of humoral immunity and biosynthesis of immunoglobulins of different classes, which is confirmed by the growth of immunoglobulins throughout the trial period from 12.5 to 15.19%. Along with the changes in the body of calves in the growth of T - and B-lymphocytes and immunoglobulins during the experimental period, we note the growth of bactericidal activity of blood at 8.73 - 13.84%, and the growth of lysozyme activity was from 3.21 to 7.47 % This fact of the growth of bactericidal and lysozyme activity of serum confirms the

increase in it of substances of protein nature, which, due to the particularities of directly affect the cells of both the microorganism and the immune system, have bactericidal activity and can lick the cells of microorganisms. This once again confirms the increase of their role in increasing the function of humoral protection of the calves of the body due to the use of nanoparticle Germancaup, taking into account that most of the biometals are present and function in the body in the form of more or less complex biocomplexes.

Conclusion. Nanoparticle Germancap is environmentally safe and has a very high degree of exposure to the body, contributing to increased immunological reactivity and immune potential with the most pronounced tension of immunity when used in early postnatal ontogenesis of calves to gastrointestinal diseases and salmonellosis. It is recommended that it be administered to calves from 10 days of age by intramuscular administration at a dose of 5.0 cm3 per head at an interval of 14 days, indicating its extremely important role in improving their immuno-physiological status and productivity by increasing their growth to 700-850 grams per day.

Bibliography

1. Avdosyeva I.K. Prospects of using nanotechnology gains in veterinary practice / I.K. Avdosyeva, V.G. Kaplunenko, A. G. Pashchenko // Livestock Today. - 2015 - № 7. - P.52 -56.

2. Voloshina N. Prospects for application of colloids of nanoparticles of metals in veterinary medicine / N. Voloshina, V. Kaplunenko, M. Kosinov // Vet. honey. Of Ukraine. - No. 9. - 2008. - P. 32 - 34.

3. Clinical researches of veterinary preparations and fodder additives / I. Y. Kotsyubbas, I. Yu. Bysyuk, V. M. Gorzeyev, O. G. Malik [and others]; for ed. I. Ya. Kotsyubbas. - L .: "SAM" Publishing House Ltd., 2013. - 252 p.

4. Laboratory methods of research in biology, livestock and veterinary medicine: a guide / VV Vlyslo, R. S. Fedoruk, I. B. Ratik and others; for ed. V.V. - Lviv: Spolom, 2012. - 764 p.

5. Lavrov P.Yu. Modern views on immunological reactivity of young animals with salmonella infection / P. Lavrov // Livestocking of Ukraine .- 2011.- No. 6.- P.15 -19.

6. Lebr M. Organic compounds of germanium / M. Lebr, P. Mazerol // Moscow: World, 2009.- P. 124 - 136.

7. Moskalenko VF, Rosenfeld LG, Movchan B.O., Chekman I.S. Nanotechnology, nanomedicine, nanopharmacology: state, prospects of scientific research, introduction into medical practice // 1 national congress "Man and medicine - Ukraine". Kyiv, 2008. - P. 167-168.

8. Paton B. E. Nanoscience and Nanotechnologies: Technical, Medical and Social Aspects / B. E. Paton, V. F. Moskalenko, I. S. Chekman, B. O. Movchan // Bulletin of the National Academy of Sciences of Ukraine. - 2009. - No. 6. - P. 76-80.

9. T. T., Influence of Nanopreparations on Metabolic Processes in Microorganisms / VA T. / Tobacco // Veterinary Biotechnology. - Bull No. 19. - 2011. - 243 p. - Bibliographer: pp. 195 - 201.

10. UlberghZ., Georgina T., Karpov O. Nanotechnology in medicine: the role of colloid-chemical processes // Bulletin of the National Academy of Sciences of Ukraine - 2008. - №8. - p. 28 - 41.

11. Howard P.C. Nanomaterials in FDA regulated products: moving forward with science-based risk assessment // NCTR/ORA Nanotechnology Core Facility (NanoCore), National Center for Toxicological Research, U.S. Food & Drug Administration, Jefferson AR USA. – 28 p.

12. Synthesis and antibacterial properties of silver nanoparticles / C. Baker, A. Pradhan, L. Pakstis [et al.] // J. of Nanoscience and Nanotechnology. - 2005. - Vol. 2, №> 2. - P. 244-247.