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The efficiency of vaccination against cattle necrobacteriosis using an experimental vaccine with a new adjuvant complex

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ABSTRACT

The purpose of the study was to analyze the effectiveness of the adjuvant complex based on saponin, glycerin and chitosan in the experimental series of an inactivated vaccine against cattle necrobacteriosis. 4 groups of animals (30 in each group) were selected for the study, aged 6 to 24 months. The first (1st) group consisted of calves 6 months old, the second (2nd) group consisted of animals 12 months old and the third group – animals 24 months old. The control 4 (fourth) group (non-vaccinated animals) contained 10 animals of each age group – 6, 12 and 24 months old. The animals were vaccinated using a needle-free injector at a dose of 0.4 cm^3 in two points at a distance of 7–10 cm (0.2 cm^3 each) twice at intervals of 4-6 weeks. Revaccinated after 6 months, with a single dose of 0.4 cm^3 (at two points, 0.2 cm^2 each). It was found that the vaccine with the multicomponent adjuvant had low viscosity and hardening temperature, moderate reactivity, high stability during storage, prolonged immunity in vaccinated animals. The immunity in animals was detected 21 days after the first vaccine administration and lasted at least 18 months after the last immunization. The necrobacteriosis incidence in animals of the control non-vaccinated group was 70-80%. During double vaccination, the average level of postvaccinal agglutinating antibodies in serum of experimental groups of animals was 312.08±74.12 – 62.13±69, 07. Humoral response in vaccinated animals provided reliable protection against Fusobacterium necrophorum disease within two years (observation period). Absence of disease symptoms in vaccinated animals during 2 years testified to the high preventive efficiency of the vaccine. The results obtained confirm the validity of the application of multicomponent adjuvant in inactivated sorbed vaccine as a means of specific prevention of animal necrobacteriosis.

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INTRODUCTION

Infectious diseases remain one of the most frequent causes of high animal morbidity and mortality, and vaccination is an effective means of preventing VPDs (Yakobson *et al.*, 1998). The cost of vaccination for any vaccine proven to be effective is about 10 times less than the cost of treating infectious disease. Cattle necrobacteriosis is an infectious disease characterized by purulent-necrotic lesions of the lower limbs, skin, mucous membranes and internal organs (Schetters *et al.*,

1990). For preventive immunization of animals against necrobacteriosis, a large number of vaccines with various adjuvants based on aluminum oxide hydrate, mineral oils, emulsifier and immunostimulants have been developed (Jaworski *et al.*, 2016). The inclusion of adjuvants in vaccines ensures a formation of more pronounced and longer specific immunity (Mammerickx *et al.*, 2010). The vaccines currently used in Russia provide immunity against necrobacteriosis within 4-6 months after the last immunization (Ruggiero and Bartlett, 2019). However, despite the achievements in the selection of adjuvants in the technology of vaccines against necrobacteriosis, plenty of issues require further study and practical solutions (Ungar-Waron *et al.*, 1992, 1999; Konishi *et al.*, 2018). The above is the basis for the development and use of new adjuvants in the production of harmless, highly immunogenic, sorbed vaccines against necrobacteriosis detected in farm animals, to ensure protection against infection through a more intense and prolonged immunity.

MATERIALS AND METHODS

The 'pair' principle was used in the formation of 4 groups of animals, ages from 6 months to 2 years: the 1st group – calves, 6 months of age: 30 heads, the 2nd group – young cattle, 12 months: 30 heads; the 3rd group –animals, 24 months: 30 heads. The control group 4 (fourth) (non-vaccinated animals) contained 10 heads of each age group – 6, 12 and 24 months old. The animals were immunized with the vaccine twice at intervals of 4-6 weeks, 0.4 cm^3 in two points at a distance of 7-10 cm, 0.2 cm^3. Revaccinated after 6 months, with a single dose of 0.4 cm^3 (at two points, 0.2 cm^2 each). Sorbed inactivated vaccine from Fusobacterium necrophorum '0-1' strain with adjuvant based on aluminum hydroxide (Al(OH)3), saponin and glycerin in optimal ratios was used for cattle immunization. The vaccine was prepared by homogenization of inactivated exotoxin sorbed on Al(OH)3 gel from Fusobacterium necrophorum '0-1' strain with adjuvant complex, the viscosity of the obtained emulsions was determined at 40o on the rotary viscometer BP-73, resistance to stratification after centrifugation at 3 thousand rpm for 15 min. Researches of the sorbed vaccine on aggregation resistance, harmlessness and reactivity were carried out by the introduction of 3-5 multiple-dose of the vaccine to white mice and rabbits according to 'Methodical recommendations for studying general toxic action of pharmacological substances' (Khudhair *et al.*, 2016). The immunity intensity was determined by the level of agglutinating antibodies in the serum of vaccinated animals in the agglutination reaction (AR). Blood from animals for the AR was taken after 7, 14, 21 days, as well as 6, 12 and 18 months after vaccination. The animals were observed for 2 years, clinical signs of the disease were taken into account and the results of laboratory research: the bacteriological analysis of purulent-necrotic skin lesions, mucous membranes, ungulates, with the isolation of pure culture of the causative agent necrobacteriosis on hepatic nutrient medium (Kitta-Tarozzi medium) in anaerobic conditions. The prophylactic efficacy of the sorbed vaccine was determined by the absence of symptoms in all groups of vaccinated animals within 2 years after the last immunization against necrobacteriosis (observation period).

RESULTS AND DISCUSSION

Immunogenicity of the vaccine against necrobacteriosis depends not only on the quality and quantity of antigenic material but also on the right choice of adjuvant. Immunogenicity of sorbed preparations increases hundreds of times as the degree of antigen sorption increases, which depends on the ratio of antigen to sorbent in the process of sorption, the presence of non-specific ballast substances of protein and non-protein nature, salt concentration, pH, temperature and time of sorption. In accordance with the set goal, it was necessary to develop a vaccine with immunity duration not less than 12 months after the last immunization, less reactive, with an optimal injection dose and possible intradermal injection method with the use of domestic needle-free injector. The application of the vaccine will allow reducing economic expenses for preventive veterinary measures, to increase the productivity of veterinary workers, develop scientifically grounded recommendations on improvement of the livestock of farm animals (Melnik *et al.*, 2020; Frolova *et al.*, 2020).

The sorbed inactivated vaccine in a dose of 2.0 cm^3 contained 2.0-4.0 mg of Al(OH)3 with inactivated exotoxin isolated from cell suspension of Fusobacterium necrophorum '0-1' strain with a concentration of 3.8-4.0 bln. microbial cells (bln. m.c.); 0.5-1.5 mg saponin, 50.0-60.0 mg glycerin and 2.0 mg chitosan (Table 1).

The results of clinical trials of experimental series of the sorbed vaccine showed harmlessness and high immunogenic activity that met the requirements of TU 9384-032-00482915 to the means of specific prevention against necrobacteriosis. The sorbed vaccine with adjuvant based on saponin, glycerin and chitosan caused the production of agglutinating antibodies in a higher level of 468±64.70 than with-

Table 1: Composition of adjuvant series of a vaccine against necrobacteriosis

Vaccine composition in a dose of 2 cm3	I series	II series	III series	IV series	Average level value of antibodies in rabbit serum in the agglutination reaction (AR)
Al(OH)3	2.0 mg	3.0 mg	4.0 mg	3.0 mg	346±16.54
Saponin	0.5 mg	1.0 mg	1.5 mg	1.0 mg	
Glycerin	60 mg	60 mg	60 mg	50.0 mg	
Chitosan				2.0 mg	468±64.70
Antigen	Inactivated exotoxin from 3.8-4.0 bln. microbial cells (bln m.c.)				

Table 2: Level of postvaccinal antibodies in animals vaccinated with sorbed, inactivated vaccine against necrobacteriosis.

The animal group sorted by age, months	Frequency of vaccine administration	Average antibody level values in AR after vaccination			
		24th day	6 months	12 months	18 months
1st group	1st	312.08±74.12	302.01±14.32	132.16±21.10	69.13±61.07
Calves 6months	2nd	615.22 ±20.32	-	-	-
2nd group	1st	387.31±14.12	326.07±16.40	122.10±10.37	68.66±31.12
young stock 12 months	2nd	643.11 ±20.32	-	-	-
3d group	1st	322.09±50.52	382.56±41.19	132.54±71.20	66.16±11.22
large cattle 24 months	2nd	665.98 ±20.32	-	-	-

out chitosan 346±16.54. During storage at room temperature, at 37°C and 5°C during the whole period of observation (30 days) the vaccine was stable and homogeneous.

When making a sorbed vaccine against necrobacteriosis with a new adjuvant complex, the wishes of veterinary specialists of the northern regions of the Russian Federation were taken into account, who asked to reduce its viscosity, as the previously used vaccine with the usual adjuvant was difficult to inject with a syringe and granulomas were formed in the place of injection, which did not resorbate for a long time. It was established that introduction of the adjuvant complex based on saponin, glycerin and chitosan into the vaccine composition contributed to the reduction of viscosity and possibility of its use in animal immunization in conditions of the northern regions of Russia.

The prophylactic efficacy of experimental series of sorbed inactivated vaccine was assessed by the results of intensity and duration of immune response and by the manifestation of disease symptoms in vaccinated cattle within 2 years after the last vaccination. Immunization was considered effective if the level of agglutinating anti-necrobacterial antibodies in the serum of animals vaccinated with a sorbed inactivated vaccine was not less than 1:16. As a result of improved technology for cleaning and concentration of the antigen, it was possible to reduce the vaccine dose by 10 times. The vaccine instead of the subcutaneous application was injected intracutaneously, 0.4 cm^3 twice at intervals of 4-6 weeks using a needle-free injector BI-7M. The needle-free injection method has a number of significant advantages: labour productivity is significantly increased (200-250 animals are vaccinated by one worker in 1 hour), there is no need to rigidly fix the vaccinated animals, the sterility of the injected drug is preserved, the possibility of re-infection of the vaccinated animals is practically eliminated, the cost-effectiveness is increased.

Average values of postvaccinal antibodies levels in animals vaccinated with sorbed, inactivated necrobacteriosis vaccine are demonstrated in

Table 2.

As it follows from the data obtained, in animals of all groups vaccinated with an inactivated vaccine against necrobacteriosis the postvaccinal antibody level was at the level providing reliable protection against the disease: thus the values of postvaccinal antibody level in the animals were within the following range: 312.08±74.12 – 387.31±14.12.

The safety of vaccinated animals against necrobacteriosis was 98±0.2%. The disease incidence in the control group animals was 70 to 80%.

During the whole observation period, there was no negative impact of the vaccine on animals and their reproductive properties.

CONCLUSIONS

Thus, the inclusion of a new adjuvant complex in the vaccine composition made it possible to offer a practical, highly effective vaccine against animal necrobacteriosis. The tests proved that the vaccine is highly immunogenic, harmless, protects 98±0.2% of vaccinated animals against necrobacteriosis and creates immunity for 18 months. The resistance to necrobacteriosis infection is maintained in animals for at least 18 months, which is 3 times longer compared to previously known vaccines. The revealed properties of the multicomponent adjuvant open up the prospect of its application with the purpose of creation of new vaccines for preventive vaccination and creation of the system of protection of susceptible animals against necrobacteriosis disease.

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Conflict of Interest

The authors declare there are no conflicts of interest regarding this study.

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REFERENCES

Frolova, M. A., Gryn, S. A., Kovaleva, E. I., Albulov, A. I., Melnik, R. N., Eremets, V. I., Popova, V. M., Matveeva, I. N. 2020. Study of radioprotective properties of chitosan at gamma-irradiation. *International Journal of Research in Pharmaceutical Sciences*, 11(2):1734–1737.

Jaworski, J. P., Porta, N. G., Gutierrez, G., Politzki, R. P., Álvarez, I., Galarza, R., Abdala, A., Calvinho, L., Trono, K. G. 2016. Short communication: Relationship between the level of bovine leukemia virus antibody and provirus in blood and milk of cows from a naturally infected herd. *Journal of Dairy Science*, 99(7):5629–5634.

Khudhair, Y. I., Hasso, S. A., Yaseen, N. Y., Al-Shammari, A. M. 2016. Serological and molecular detection of bovine leukemia virus in cattle in Iraq. *Emerging Microbes & Infections*, 5(1):1–6.

Konishi, M., Ishizaki, H., ichiro Kameyama, K., Murakami, K., Yamamoto, T. 2018. The effectiveness of colostral antibodies for preventing bovine leukemia virus (BLV) infection in vitro. *BMC Veterinary Research*, 14(1):419–419.

Mammerickx, M., Portetelle, D., Burny, A. 2010. Experimental Cross-Transmissions of Bovine Leukemia Virus (BLV) between Several Animal Species. *Zentralblatt für Veterinärmedizin Reihe B*, 28(1):69–81.

Melnik, N. V., Eremets, V. I., Neminuschaya, L. A., Klyukina, V. I., Gryn, S. A., Markova, E. V., Smolentsev, . ., Y, S. 2020. The efficiency of probiotics use in the treatment of calves. *International Journal of Research in Pharmaceutical Sciences*, 11(2):1674–1678.

Ruggiero, V. J., Bartlett, P. C. 2019. Control of Bovine Leukemia Virus in Three US Dairy Herds by Culling ELISA-Positive Cows. *Veterinary Medicine International*, 2019:1–6.

Schetters, H., Rohmer, H., Hehlmann, R., Erfle, V. 1990. Quantitative determination of retrovirus-specific immune complexes. *Journal of Immunological Methods*, 134(1):113–119.

Ungar-Waron, H., Brenner, J., Paz, R., Trainin, Z. 1992. Circulating immune complexes in bovine leukemia virus (BLV)-infected cattle. *Veterinary Immunology and Immunopathology*, 34(1-2):173–179.

Ungar-Waron, H., Paz, R., Brenner, J., Yakobson, B., Partosh, N., Trainin, Z. 1999. Experimental infection of calves with bovine leukemia virus (BLV): an applicable model of a retroviral infection. *Veterinary Immunology and Immunopathology*, 67(2):195–201.

Yakobson, B., Brenner, J., Ungar-Waron, H., Trainin, Z. 1998. Short-termed expression of interleukin-12 during experimental BLV infection may direct disease progression to persistent lymphocytosis. *Veterinary Immunology and Immunopathology*, 64(3):207–218.