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Inactivated Liposomal Vaccine Against Bovine Infectious Rhinotracheitis And Parainfluenza-3.

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ABSTRACT

The searching of new immunostimulants for enhancing the immunogenicity of vaccines is a foreground trend nowadays. The conducted researches are a scientific rationale for the development of a liposomal vaccine and the evaluation of its prophylactic efficacy against infectious rhinotracheitis and parainfluenza-3 in cattle. Vaccine batcheshad a manifested immune stimulating effect, and compatibility of antigens and liposomal structures. Biological preparation components are available and economically acceptable. The obtained vaccine samples were tested for the item of sterility, innocuity, antigenic and immunogenic activity. Laboratory control of experimental batchesshowed that they are sterile, innocuous, did not cause clinical scoredeviations from the physiologically normal states oflaboratory animals, as well as pathological changes in organs and tissues. Vaccines had a manifested antigenic activity, induced the formation of specific antibodies. Antibody titers have steadily increased to the 180 days of research.The liposomal vaccine was discovered to induce post-vaccination reactions of a local or general character in laboratory animals even while administered with increased 15-times immunizing dose. The evaluation of the immunological status of laboratory rabbits vaccinated with liposomal vaccine was carried out by determining the number of T and B lymphocytes by means of the E-rosetting method. It is discovered that the preparations contribute to activate cellular immunity in vaccinated animals. Production tests were carried out on an experimental batch of inactivated liposomal vaccine against IRTand PI-3 cattle in one month calves. The results of the experiments showed that our developed biological preparation has a high antigenic activity and can be used to prevent mixed respiratory infections in young cattle.

Keywords: liposomal vaccine, virus,infectious rhinotracheitis,parainfluenza-3, calves, rabbits.

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INTRODUCTION

Respiratory diseases of cattle are widespread in many countries around the world. Thus, the relative number of cattle herds that are unfavorable for infectious rhinotracheitis is 10% in France, 60% in Spain, 63% in Belgium, 70% in the Netherlands, as well as in Russia. The most significant of the groups are infectious rhinotracheitis (IRT), parainfluenza-3 (PI-3) and bovine viral diarrhea(BVD) [16].

Under modern industrial technology conditions, the decreasing of the body resistance is the main cause of the animal respiratory diseases. [23, 61, 64, 86, 93].

In modern industrial cattle breeding, vaccination is the most effective way to prevent respiratory infections in cattle. Therefore, the searching of the most immunogenic vaccine and the developing of improvement of the vaccine effectiveness are one of the main objectives of veterinary medicine.

Based on the above, the purpose of our researches was the development of manufacturing technology and control of liposomal inactivated vaccine against infectious bovine rhinotracheitis and parainfluenza-3 in cattle and determination of preventive efficiency under industrial environments.

MATERIALS AND METHODS

Experimental and industrial tests were carried out in accordance with the requirements for the experiment on the analogues selection, control setting, adherencing to identical feeding conditions, animal management and controlling results.

Viruses: While producing the vaccine batches, the antigens of the parainfluenza-3 virus — «PTK-45/86» strain and herpesvirus type 1 (IRT) in cattle — the strain «TK-A (RNIEV)-B2» were used.

Serological and immunological researches:

-antihemagglutinins to the PI-3 virus in the blood serum of the laboratory animals were determined in the hemagglutinationinhibition test (HGIT) using the set of instruments for diagnosis of FSI “Kursk Biofactory– BIOK company”. Serum dilution of 1: 32 and above was considered a diagnostic titer.
- antibodies to the infectious rhinotracheitis virus were detected in the neutralization reaction (PH) on the finite cell line MDBK using the vaccine strain «TK-A (RNIEV) -B2» of herpesvirus type I in cattle with infectious activity 6.0 lgTCE 50 / ml. Serum activity of 1: 4 and more was taken as a diagnostic titer.

Liposome secretion: The main component for producing liposomes - phosphatidylcholines (lecithin) - was obtained from chicken yolk [3].94% of ether and acetone were used as the organic solvent. In order to complete the formation of bilayer vesicles in the manufacture of liposomes the phospholipid solution was constantly stirred on a magnetic stirrer.

UM-4 apparatus by the Unitracompany (bath volume 4 dm^3, frequency 25 kHz) with a low intensity ultrasound exposure was used for ultrasonic treatment of viral antigens. The ultrasonic bath was pre-filled with distilled water (intermediary agent) into which the vials with the mixture were placed. Ultrasonic treatment was carried out for 10-15 minutes at room temperature.

A rotary evaporator (RVO-64, Czechoslovakia) was used to evaporate and obtain a lipid film. During evaporation the vial was constantly in a temperature-controlled water bath and rotated (40° C at 4 ± 2 mm Hg). It is important to control the process so that the organic solvent does not boil up.

The ultra structure of liposomal vesicles was studied under a JEM 100 CX-2 electron microscope («Jeol» Japan) using the negative contrast method.

The development of optimal antigen ratios with liposomal structures. Experienced vaccine series were prepared by mixing in ratios of 1: 1; 1: 5; 1:10 formaldehyde inactivated herpesvirus type 1 antigens and parainfluenza-3 virus with liposomal structures. The mixing procedure was performed during the isolation of liposomes.

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Antigenic activity of the drug was studied by subcutaneous administration to white mice. Blood from animals for serological studies was taken before vaccination, as well as after 14, 28 and 60 days from the beginning of the experiment.The serum of mice was investigated in HIRfor the presence of antibodies to the virus PI-3 in cattle.

RESULTS AND DISCUSSION

Laboratory control of experimental samples of sterility vaccine was carried out by plating on nutrient media of the BEB, BEAA, BELB and Sabouraud, innocuity - on white mice and antigenic activity - on rabbits.

The control results showed that all experimental samples of the vaccine are sterile, innocent and possess high antigenic activity. Clinical observations conducted after vaccination did not reveal a local and general response to the introduced biologics in the experimental animals.

In order to make comprehensive assessmentof the T- and B- systems of the immune systems when using an inactivated liposomal vaccine against IRT and cattle PI-3 3 groups of rabbits were formed with 8 animals in each. Animals of the first group were injected with inactivated liposomal vaccine at a dose of 2.0 cm^3intramuscularly, two times, with an interval of 14 days.The second group of rabbits was immunized with the associated inactivated emulsion vaccine against PI-3 and IRT in cattle in a similar dose and frequency.

The third group of rabbits is the control one (intact).

Blood sampling from animals for immunological studies was carried out before immunization in 45 and 60 days since thebeginning of the experiment.

The number of T-and B-lymphocytes was determined by means of E-rosettingmethod. This method is based on the indication of surface specific receptors for various lymphocyte subpopulations, which form an outlet pattern by binding red blood cells. The lymphocyte joined 3-5 red blood cells is used as a rosette.

The results of experimental studies showed that the tested vaccines were innocentfor rabbits and had marked antigenic activity (see table).

Table: Studying of cellular immunity in vaccinated rabbits (M ± m), n = 8

№	Group	Research duration		
		beforeimmunization	45 days	60 days
T-lymphocytes, %				
1	1 Group	33,38±0,45	41,5±0,45***	41,38±0,40***
2	2 Group	32,13±0,47	39,75±0,63***	38,0±0,29**
3	3 Group(control)	34,00±0,49	35,25±0,27	36,5±0,45
B-lymphocytes, %				
1	1 Group	20,50±0,49	36,18±0,29***	39,5±0,25***
2	2 Group	28,00±0,29	33,09±0,23***	34,54±0,18***
3	3Group(control)	21,5±0,17	21,06±0,29	22,13±0,32

Note: **=p≤0,01;***= p≤0,001

It was discovered that while applying the inactivated liposomal vaccine, there was a gradual rise of T-lymphocytes quantity from 33.38% to 41.5%, i.e. to 8.12%.

The maximum number of T-lymphocytes was detected 45 days after the experimental animals were injected with an experimental liposomal vaccine, followed by a slight decrease by the 60th day.

The administration of the associated inactivated emulsion vaccine against IRT and PI-3 contributed to the increase of T-lymphocytes quantity by the 45th day of research from 32.13% to 39.75%, i.e. - to 7.62%.

According to the results of studies in groups where experimental liposomal vaccine was administered, a significant increase of T-lymphocytes quantity was observed.

It is important to note that when using the associated vaccine with liposomal structures, the increase of B-lymphocytes quantity was detected twiceafter two months the first injections of the drugs were done. While studying the blood sampling on the 45th and 60th day after the administration of the associated inactivated vaccine, the increase of B-lymphocytes quantity to 1.2 times respectivelywas observed.

The administration of an experimental vaccine to rabbits caused theriseof T- and B-cell immunity during the first month after the first injection, with its gradual decrease by the fourth month. Studies show that the combined use of vaccines with liposomal structures significantly increases the intensity of cellular immunity.

Thus, the results of studies have shown that the liposomal vaccine has a high antigenic activity and enhances the immunological rearrangement of the animal organisms, as evidenced by the increase in the number of blood T- and B- lymphocytes.

Production tests of associated inactivated liposomal vaccine against IRT and PI-3 in cattle and associated inactivated emulsion vaccine against IRT and PI-3 in cattle were performed under farm conditions.

Laboratory control of the vaccine sample tested showed that they are sterile, innocent and have high antigenic activity concerning the laboratory animals.

20 calves of 30 days oldwere used in the experiment, which were divided into 2 groups with 10 heads in each. Animals of both groups were vaccinated with a dose of 1.0 cm^3 intramuscularly, two times, with an interval of 14 days.

The first group of experimental calves was vaccinated with an associated inactivated liposomal vaccine against parainfluenza-3 and infectious bovine rhinotracheitis in cattle, and the second group with an associated inactivated emulsion vaccine against PI-3 and cattle IRT.

The studying of the antigenic activity of vaccines was based on the detection of post-vaccination antibodies to parainfluenza-3 virus in the reaction of inhibition of hemagglutinationin the blood serum of experimental calves and to herpesvirus type 1 of cattle in the neutralization reaction. For this purpose blood sampling from animals for serological studies was carried out before vaccination, 14 days later (revaccination), as well as 45, 60, 180 days after the administration of biopreparations.

Analysis of serological studiesresults presented in Table 1 and 2 showed a significant increase in antibody titers to viral antigens on the 14th day after vaccination, which reached a maximum level by the 60th day from the beginning of immunization.

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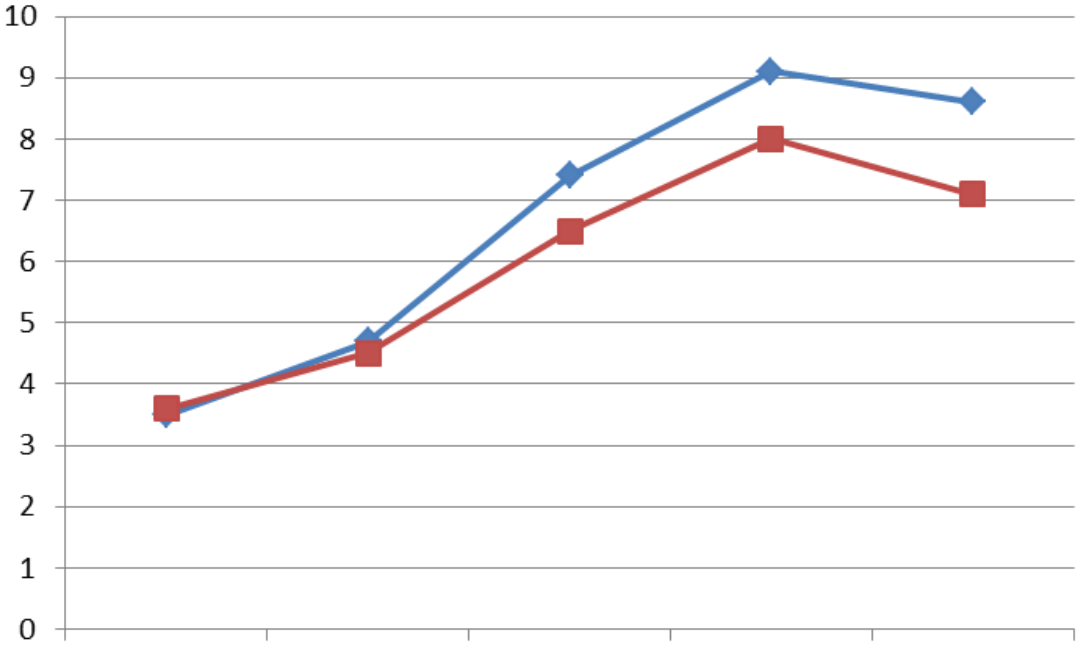

Table 1: Dynamics of specific antibodies accumulation to parainfluenza-3 virus in HIRin vaccinated calves

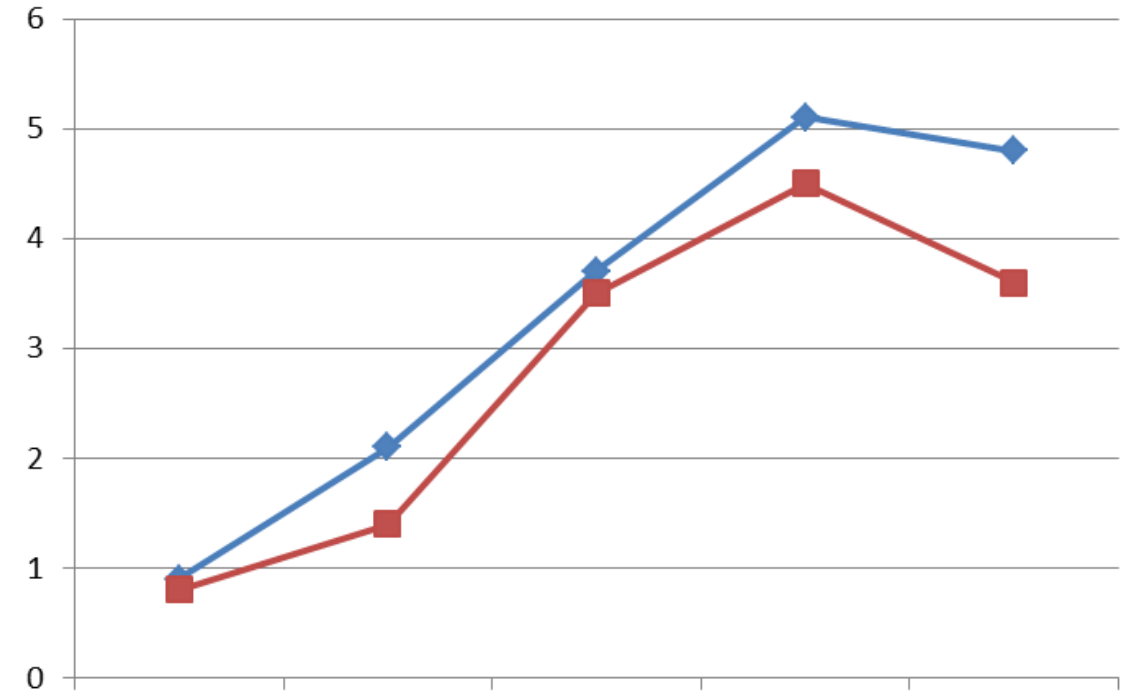

Table 2: Dynamics of specific antibodies accumulation to the infectious bovine rhinotracheitis virus in PH in vaccinated animals

While investigating of blood serum in experimental calves the maximum level of antibody titers to parainfluenza-3 virus (9.0-10.0 $\log_2$) and to herpesvirus type I cattle (4.0-6.0 $\log_2$) was detected two months after vaccination. By the 180th day from the beginning of the experiment they slightly were decreasing.

It should be noted that the average antibody titers to viral antigens on the 60th day of the study were 0.5-1.1 $\log_2$ higher while the administratingthe experimental liposomal vaccine against infectious rhinotracheitis and parainfluenza-3 in cattle compared with the emulsion variant of the biological preparation.

Associated liposomal vaccine against PI-3 and cattle IRT, twice injected to calves in the amount of 1.0 cm^3, facilitates to a significant increase of titer of specific antibodies to the PI-3 virus in HIRup to 180 days after vaccination by 1.5 log_2 and an IRT virus in PH at 1.2 log_2, respectively.

Thus, developed batch of associated inactivated liposomal vaccine against IRT and PI-3 in cattle has a high antigenic activity and can be recommended for the prevention of viral respiratory infections in cattle.

CONCLUSION

The associated inactivated liposomal vaccine against infectious rhinotracheitis and parainfluenza-3 bovine was developed for the first time with the optimal ratio (1:1) of viral antigen and liposomal structures established.

The well-developed method of obtaining liposomal corpuscles made it possible to effectively incorporate the antigens of the PI-3 virus and IRT in cattle. Experimental associated inactivated liposomal vaccine causes an increase in T- and B-cell immunity. Meanwhile the liposomal structure facilitates the activation of cellular immunity within 180 days. The associated liposomal vaccine for PI-3 and IRT in cattle, administered twice in the quantity of 1.0 cm^3 to calves, contributes to a significant increase of antibody titer to PI-3 in the hemagglutination inhibition reaction to 180 days after vaccination by 1.5 log_2 and to the IRT in the neutralization reaction at 1.2 log_2, respectively.

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