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Basics Of Developing A Vaccine For Cattle.

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ABSTRACT

For a significant reduction, and in some cases, the complete elimination of infectious diseases of animals, it is necessary to use biological preparations intended for the specific prevention and diagnosis of diseases. Therefore, the manufacture of such drugs, which have sufficient immunogenicity during the prescribed shelf life, as well as cost-effective and affordable, is the main task of every enterprise in the biological industry.

Keywords: actinobacillus, hydrolysates, the culture of microorganisms, vaccine, bioreactor, prevention, strain, technology.

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INTRODUCTION

Due to the fact that recently there has been a tendency to an increase in the spread of a disease such as cattle actinobacillias, we have proposed a vaccine against this disease for the prevention. According to the international classification of microorganisms, actinobacilli belong to the family Pasteurellaceae, which includes three genera: Pasteurella, Actinobacillus, Haemo pylus [2]. Actinobacillus of cattle in recent years has a tendency to further spread in the North Caucasus, although officially the disease is rarely recorded in accounting and reporting documents. This is apparently due to the fact that veterinary specialists are not well acquainted with the diagnosis of bovine actinobacilliosis and are mistakenly diagnosed as actinomycosis. In addition, in pasteurellosis and hemophillosis in animals, a number of inactivated and live vaccines have been proposed, with the exception of actinobacilli.

Targets and goals. Currently, for the cultivation of various microorganisms, including the causative agent of actinobacillus, nutrient media are used, which are based on hydrolysates from meat, liver, and other protein-containing raw materials. The aim of our work was to find the most optimal growth properties, as well as a relatively inexpensive nutrient medium for the cultivation of actinobacilli. The main task is to master the technology of preparation of an inactivated concentrated hydroxide aluminum vaccine against actinobacillus, taking into account the characteristics of the pathogen itself.

MATERIALS AND METHODS

Actinobacillus is a chronic infectious disease of cattle, less often sheep and other animals. The disease is characterized by purulent lesions of the soft tissues of the head, neck, lymph nodes and blood vessels. The diagnosis of actinobacillus cattle must be made comprehensively, taking into account all diagnostic methods (epizootological data, clinical signs, bacteriological and other studies). The following features are characteristic of epizootological data for actinobacilli: animals aged 14–24 months are most susceptible to the disease, 6–9-month-old calves are less susceptible. So, out of 6,754 animals subjected to clinical examination, 416 patients were identified. The incidence of animals aged 14–18 months was 63.8%, in cows — 29%, calves from 6–9 months — 7.2%. The disease in a stationary dysfunctional economy is most often recorded in the autumn-winter, spring periods. Actinobacillus is manifested in the form of sporadic cases and enzootic. From clinical signs, actinobacilli is characterized by the defeat of the skin, subcutaneous tissue at the site of introduction of the pathogen, most often the pathogen penetrates through the injuries of the mucous membrane of the gums and tongue. In the future, with the development of the disease, the lymphatic vessels of the upper and lower jaws, the submandibular, parotid, and pharyngeal lymph nodes become inflamed. In the early stages of the disease, a softening is observed in the lymph nodes, infiltration with cream-like pus, the lymph node tissue is further destroyed, and the accumulated pus promotes the formation of small, multiple granulomas. Affected abscess lymph node is encapsulated. The inner wall of the capsule is covered with a granular, white-grayish overlay. Inside the connective tissue capsule, there are many partitions that form pockets that communicate with fistulous passages. The contents of such granulomas are milky white or gray in color, thick consistency, odorless. Inflammation of the lymphatic vessels occurs independently or develops following inflammation of the regional lymph nodes. In the course of the lymphatic vessels, specific single or multiple granulomas are formed. The sizes of granulomas vary from one to 15–20 cm. Often they spontaneously enter. The final diagnosis is made after bacteriological tests.

RESULTS AND DISCUSSION

For work, material from healthy cattle was used - lymph nodes of the head and neck (submandibular, parotid, pharyngeal), since actinobacilliosis most often affects the lymphatic system, [1] and the spleen. Based on the selected material, hydrolysates were prepared separately from lymph nodes and spleen. The lymphoid tissue was hydrolyzed by an enzymatic method using the native pancreas of cattle at 43 ° C for 5 days. At the end of the hydrolysis, a biochemical analysis of the hydrolysates was carried out for pH, total and amino nitrogen, peptone, tryptophan, protein. The research results are presented in table 1.

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Table 1: Biochemical parameters of hydrolysates

Hydrolyzed	pH	Amine nitrogen mg%	Peptone,%	Tryptophan, mg /%	Protein,%	Total nitrogen, mg%
The lymph nodes	**7,5**	**658,0**	**1,85**	**92,0**	**0,14**	**784,0**
Spleen	**6,32**	**833,0**	**2,28**	**110,0**	**0,1**	**966,0**

Thus, the medium from hydrolyzed lymph nodes turned out to be poorer in nutritional composition than the medium from the spleen. For the manufacture of the vaccine used deposited strains of the pathogen - strain 95 and 5401 [3], as well as strains of actinobacilli, isolated from animals in the Stavropol region.

The production process of preparing a vaccine against actinobacilli of cattle consisted of the following stages: 1. Working with production strains of the pathogen; 2. The process of selecting and preparing the optimal nutrient medium for the cultivation of strains; 3. Preparation of seed; 4. Cultivation of actinobacilli strains in the reactor to obtain the bacterial mass, determination of the concentration of microbial cells; 5. Inactivation of culture with formalin, addition of adjuvant and antigen concentration; 6. Packaging and packaging of the vaccine; 7. Conducting quality control of the vaccine on certain indicators.

From museum strains of actinobacilli, crops were sown on Hottinger's broth and agar. After establishing the typicality of the cultures, daily production strains from test tubes were sown in 200 cm3 bottles with 100 ml of Hottinger medium. Cultivation was carried out for 18 hours at a temperature of 37 ° C. Grown cultures were examined for growth purity, after which the culture was seeded in a 16-liter bottle with 10 liters of Hottinger broth and a 20% glucose solution was added to its final content in a nutrient medium of 0.4%. Cultivation of mat culture was carried out at a temperature of 37 ° C for 18 hours. Then the resulting matt culture was loaded into a 200-liter bioreactor with 100 liters of sterile nutrient medium. Sowing was carried out in a strict ratio of 10% of the mouthed culture to the nutrient medium, cultivation with the stirrer running (100-120 rpm) for 18 hours. After the time of cultivation, the production cultures were monitored for purity and typical growth by microscopy of smears stained by the Gram method and sowed on MPA, BCH, MBPS and Saburo medium.

Inactivation of the culture was carried out with a 20% formalin solution until its concentration in the culture was 0.4%, only after accumulation of 9 billion microbial bodies in one ml of nutrient medium. After five days, the culture of actinobacilli was sown on the specified nutrient medium to determine the completeness of inactivation. It was found that after three days of cultivation on nutrient media (BCH, MPA, MPB under vaseline oil and Wednesday Saburo) culture growth did not appear. After that, the inactivated culture was sorbed by adding a 3% solution of aluminum hydroxide to a final content of 15%, with a pH value of 5.0-5.4. Then, to concentrate the antigen, 40% of the supernatant was decanted, the pH of the vaccine was set at 7.2-7.6, and sterility testing was performed. For vaccine culture was observed for three days. After sterility was established, the vaccine was packaged in 200 cm^3 vials, which were sealed with rubber stoppers and rolled around with aluminum caps.

To check the quality of the vaccine, five bottles were randomly selected from the obtained series of vaccines and monitored according to the following parameters: appearance; purity and typical growth of the vaccine strain; dose; the number of doses of vaccine in the vial; harmlessness; immunogenic activity. Seeding was carried out on the BCH, MPA, MPB, Wednesday Saburo on sterility. The safety of the vaccine was tested on white mice. Experienced vaccine series were tested in several farms in the Stavropol Territory, which are not good for cattle actinobacillosis, in particular on the collective farm named after M. Chapaeva Kochubeevskogo district of the Stavropol Territory, in the experimental group, the incidence was 8%, while in the control group 36%.

CONCLUSION

The vaccine against actinobacillus is quite technological, and its testing in dysfunctional farms has shown that it is immunogenic.

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