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Immunogenicity of Vaccine Against Emphysematous Carbuncle of Big Horned Stock, Made From Local Stamm

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Abstract: *Local strain of the causative agent Cl. Chauvoei was isolated, from the affected area (the buttock muscle) of a bull calf, suffering from an emphysema carbuncle. From the isolated strain of the causative agent of the emphysematous carbuncle, GOA formaline-killed vaccine was prepared against the emphysematous carbuncle of animals. The immunogenicity of this vaccine was studied in experiments on sheep and a positive result was obtained.*

Key words: *Vaccine, Carbuncle*

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Introduction

Providing the country with meat, milk, skins and other livestock products is the main problem of today. To solve this problem, it is necessary to increase the number of livestock and increase its productivity. There are a number of negative factors, without which it is very difficult to achieve intentional goals to increase the number of livestock and increase its productivity. Among the diseases of farm animals caused by pathogenic anaerobes, emphysematous carbuncle of cattle, caused by spore-forming bacteria Cl.Chauvoei deserves special attention. Infectious diseases create not only a serious obstacle to the increase in the number of livestock, but also lead to their disease. Emphysematous carbuncle (Emcar) of cattle treats diseases that cause huge economic damage to agriculture. The damage consists of a case with sick animals, forced vaccination in case of illness in unfavorable farms, as well as expenses for burning corpses, disinfection of the territory where the sick animal was kept.

This disease occurs in all parts of the world where livestock is widely developed, including in Uzbekistan. The causative agent of the disease is Cl.Chauvoei, a strict anaerobic, gram-positive mobile bacillus, in the animal body it is distinguished by aggressors. In the corpses and in the external environment, the agent forms spores. Disputes remain indefinitely for a long time in the soil, causing permanent turmoil, a farmyard and pastures. For this reason, the foci of Emcar for a long time are preserved in nature, and they cannot be destroyed. The disease is sensitive to large and small cattle.

Emphysematous carbuncle is an acute infectious disease that occurs in animals between the ages of three months to four years of average and above average fatness. If you think that the illness lasts 1-2 days after the appearance of the first clinical signs, treatment of the disease does not give the desired effect. The basis of measures to combat this disease is prevention. To prevent the disease, it is necessary to vaccinate the weight of susceptible cattle against emphysematous carbuncle.

Until now, there has been no bio preparation in Uzbekistan against the emphysematous carbuncle of animals. To prevent the disease, an average of 4 million doses (8 tons) of vaccine are annually procured from the

Russian Federation. The need of our state in this vaccine is much higher, since there are about 11 million large and over 18 million small cattle in the country. The demand for this vaccine is increasing from year to year.

All this predetermined the goal - to isolate the local strains of the causative agent of the emphysematous carbuncle of Cl.Chauvoei cattle and on its basis to produce a vaccine against the carbuncle of emphysema. Thus, this will provide the country's livestock with an effective vaccine against the emphysematous carbuncle produced from the local strain of the pathogen.

Material and methods of investigation. To isolate the causative agent, blood samples from animals kept together with diseased and dead animals, as well as samples of pathological material (hearts with blood, liver, kidneys, muscle tissue from the affected area) taken from dead animals were brought to the laboratory. They were examined in the usual bacteriological method, in which meat-peptone broth (MPB), meat-peptone-agar (MPA), meat-peptone liver broth - Kitt-Tarozzi medium (MPLB), blood glucose used. To isolate the causative agent, Emcar from the blood samples of animals taken from the fallen animal and the pathological material taken from the dead animals were planted on MPLB nutrient media for vaseline oil. From samples of blood, affected area of muscles and internal organs, the crop was sown directly. Pathological material from dead animals was examined by the usual bacteriological method. To grow the inoculum, they were incubated in a thermostat at a temperature of 37.0-37.5° C. A day later the turbidity of the nutrient medium and the formation of gas on the surface of the medium were detected. Of these, the strokes were prepared on a gram-stained glass and examined by the immersion system of a microscope. The culture-morphological and biological properties of the Emcar pathogens were studied.

A highly pathogenic strain T-04 was selected from isolated strains from which concentrated GOA formaline-killed vaccine was prepared against the emphysematous carbuncle of cattle. To produce the vaccine strain Cl.Chauvoei T-04, MPLB, formalin, aluminum hydroxide, agar-agar, binary solution of sodium hydroxide was used. Guinea pigs weighing 350-450 g were used to determine the immunogenicity of the vaccine, and one-year-old karakul sheep of medium and higher average fatness were used to determine the duration of immunity. The experiments were carried out in the stove of Scientific research institute of Veterinary, observing all measures of septic tank and asepsis.

Own research. As a result of bacteriological studies of blood samples from animals containing dead animals and pathological material taken from dead animals, gram-positive, straight, thick sticks with rounded ends, arranged singly and in pairs, were isolated. In smears, a "hanging drop" was determined that the rods are very mobile. To determine the pathogenicity of the isolated pathogen, daily culture was administered to two guinea pigs at a dose of 0.5 ml in the abdominal muscle. After 36-48 hours, guinea pigs fell with the manifestation of clinical signs of emphysematous carbuncle. With pathological-anatomical autopsy of guinea pigs, an identical pathological picture was observed as in emphysematous carbuncle. From the muscles of the affected area and internal organs of the fallen guinea pigs, the pathogen of the emphysema carbuncle Cl.Chauvoei was re-isolated.

To produce the vaccine, a highly pathogenic local strain of Cl.Chauvoei T-04 was selected from which it was seeded into 1 liter flasks with Kitt-Tarozzi nutrient medium and incubated in a thermostat at 37.0-37.5 ° C for 36 hours. After turbidity of the nutrient media and gassing on their surface, the culture was released from the pieces of liver, and 0.4% of a chemically pure formalin containing 36% of formaldehyde was added to it.

The formalized culture was held at 38.0-39.0° C for 3 days, stirring 2-3 times a day. Three days later, a 3% solution of sterile aluminum hydroxide in an amount of 15% was added to the formalized culture. After thorough mixing, the vaccine was left at room temperature - 18-22° C for 2-3 days until the complete clarification of the supernatant.

A transparent top in an amount of 2/3 of the total volume of the vaccine was aspirated with a siphon and 0.1% of sterile molten agar-agar was heated to 37 °C to the remaining pellet. The pH of the vaccine was adjusted to 7.4 with a two-normal solution of caustic soda.

After thorough mixing, the vaccine was poured into vials, closed with rubber stoppers and rolled with aluminum caps.

Immunogenicity of the vaccine was checked by a single subcutaneous vaccination in the abdominal muscles area to 10 guinea pigs weighing 350-450 g in a dose of 0.4 ml. 18 days after vaccination, 10 vaccinated guinea pigs and 10 guinea pigs of the control group were injected intramuscularly with a daily culture of the highly pathogenic local strain Cl.Chauvoei X-04 at a dose of 20 LD_{50}, predetermined for guinea pig.

As a result of the studies, out of 10 vaccinated guinea pigs, 9 survived for 5 days, and in the control group of 10 non-vaccinated guinea pigs all died and not one survived until the indicated time. The results of the obtained data indicate the high immunogenicity of this vaccine made from a local strain.

To determine the duration of vaccine immunity, an experiment was conducted on 8 heads of sheep, which were divided into four heads into two: a 1-trial and a 2-control group.

The sheep of the experimental group were immunized with our developed GOA formaline-killed vaccine, made from the strain of Cl.Chauvoei T-04. The vaccine was injected intramuscularly at a dose of 2 ml into the inner, hairless side of the thigh. The sheep of both groups were under constant observation. Conditions of maintenance and feeding in animals of both groups were the same. Six months after the vaccination, the sheep of both groups were infected with the daily culture of Cl.Chauvoei X-04 in a dose of 20 LD_{50}, predetermined for sheep. The culture was introduced into the inner, hairless side of the thigh.

Behind the sheep, constant monitoring was established for 30 days. Clinical examination of infected animals was carried out. In the sheep of the experimental group, an increase in body temperature by 0.5-1.2 °C was determined 8 hours after infection, the pulse rate was 80-90 strokes and breathing 30-40 times per minute. The general condition of the animals was slightly depressed; the appetite was weak. This state lasted for 24-36 hours and after that time the condition improved and the clinical indices were equal to physiological norms and it was kept until the end of the experiments. During the observational period among the sheep of the experimental group, clinical signs of emphysematous carbuncle were not observed and they remained healthy.

In the control group, the first clinical signs of the disease appeared 8-10 hours after infection. Clinical signs in the sheep of this group were manifested and proceeded identically. They noted signs of lameness, moved with difficulty, inactive, try not to attack the limb, which introduced the causative agent of the disease. Feed intake is weak. The body temperature was increased from 40 to 40.8° C, the pulse rate was 100-120 beats per minute and the number of respiratory movements was 40-50 times per minute. On the second day of the experiment the lambs of the control group were observed lameness, they almost without movement are standing in one place. Appetite is absent, external irritations are poorly answered. Body temperature increased 40.6-41.3° C. The pulse rate was 130-140 strokes and the number of breaths was 60-70 times per minute. By the evening of the control group in one sheep, the condition deteriorated sharply, the temperature gradually subsided and by the morning of the third day of the experiment she fell. Having shown, the same clinical signs on the third and fourth days fell the remaining infected sheep of the control group.

When commissioning a pathologic-anatomical study of fallen sheep from infection with the causative agent of the emphysematous carbuncle, it was determined that the pathologic-anatomical changes are specific to the emphysematous carbuncle of cattle. During commission research, the identity of the pathologic-anatomical changes in all the sheep of this group was determined.

For the re-isolation of the causative agent of the emphysematous carbuncle, pieces from the internal organs (heart, liver, kidneys, affected muscle) of the exposed sheep were taken. As a result of bacteriological studies, gram-positive, straight, thick sticks with rounded ends, arranged singly and in pairs, were isolated from Kitt-Tarozzi media. These rods were morphologically identical with the causative agent of Cl.Chauvoei disease, with which they infected sheep.

Conclusion. Thus, based on the conducted studies and commission experiments, "Concentrated GOA formaline-killed vaccine against emphysematous carbuncle of big horned stock," made from the local strain Cl.Chauvoei T-04, is a highly immunogenic vaccine that reliably protects animals from disease, with experimental infection at a dose of 20 LD_{50} for 6 months (follow-up period).

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