

Specific prevention of emphysematous carbuncle of cattle and sheep

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Abstract. The local strains of the causative agent of emphysematous carbuncle (blackleg) cattle *Ci.chauvoei*. The cultural, morphological, physicochemical, and biological properties of isolated local strains of the causative agent of emphysematous carbuncle (blackleg) were studied. An experimental series of vaccines against animal emphysematous carbuncle (blackleg) was made from selected local strains. The qualitative indicators, immunogenicity and duration and immunity of this vaccine on sheep and calves were studied and a positive result was obtained.

1 Introduction

Providing the republic with meat and dairy products, leather raw materials and other livestock products is one of the main directions of these days. The solution to this problem involves increasing the number of livestock and its productivity. There are a number of negative phenomena that impede the preservation and increase of livestock numbers, including infectious diseases [1, 3].

Emphysematous carbuncle is one of the main infectious diseases among cattle, which significantly causes economic damage to the livestock industry [2].

The infectious agent is a spore-forming microorganism; spores persist in the corpse of a dead animal and in the external environment for more than 10 years without losing their activity (the ability to cause disease) and virulence. In this regard, foci of emphysematous carbuncle persist in nature for a long time and their elimination is difficult. Cattle and sheep of medium and high fatness are more susceptible to this infection.

Emphysematous carbuncle of cattle is an acute disease that lasts 1-2 days, and in some cases the disease can last 4-5 days. Treatment of sick animals generally does not give the desired effect and the animal dies. Preventive measures are one of the main directions in the fight against this infection. To prevent infection, it is necessary to vaccinate all susceptible animals against emphysematous carbuncle. Currently, no vaccines are produced in Uzbekistan to prevent emphysematous carbuncle. In order to prevent emphysematous carbuncle, a vaccine against this infection is purchased from Russia. In the hot conditions

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of Uzbekistan, vaccination with a commercial vaccine does not always give the desired effect, and in some cases, sick animals are found among vaccinated animals [4-5, 7].

Based on the above, the goal was to isolate epizootic strains of emphysematous carbuncle in cattle from regions of the country and to study their cultural, morphological and biological properties. From the isolated epizootic strains, select the most pathogenic strain and, on its basis, prepare an experimental series of vaccine against emphysematous carbuncle in cattle. To study the quality indicators of the vaccine, as well as its protective properties and the duration of specific immunity under experimental conditions on sheep and calves [6-7].

2 Materials and methods

In order to isolate epizootic strains of emphysematous carbuncle in cattle, samples of pathological material (heart with blood, pieces of liver, kidney, muscle from the affected areas) from dead animals sick with emphysematous carbuncle were taken for bacteriological studies. The samples were inoculated onto various nutrient media. The growth of microorganisms is determined by changes in the turbidity of the nutrient medium and the appearance of gas bubbles on the surface of the medium. Smears were prepared from the growth medium, which were Gram stained and examined under a microscope. During the study the morphology of the pathogen, their shape, size, location in smears, motility, and formation of spores and capsules were taken into account. While studying the cultural properties of the isolated strains, attention was paid to the growth pattern of microorganisms in various nutrient media. For determination the pathogenic properties of the pathogen, 4 heads of guinea pigs were infected intramuscularly with a daily culture of each strain from the Kita-Tarotsiya medium at a dose of 0.5 ml. and took into account the number of dead animals in each group.

To determine the dose of LD₁₀₀ of the epizootic strain for sheep, an experiment was carried out on 12 heads of one-year-old sheep, divided into three groups of 4 heads in each group. The first group was infected with a daily culture of *Cl.chauvoei* grown on Kitt Tarotsia medium at a dose of 1.0 ml, the second - 1.5 ml. and the third group at a dose of 2 ml. intramuscularly into the hind limb on the inner hairless side of the thigh.

To determine the infectious dose of LD₁₀₀ for calves, an experiment was carried out on 12 heads of calves 6-7 months of age. The calves were divided into three groups of 4 heads each. The first group was infected with a daily culture of *Cl.chauvoei* grown on Kitt Tarotsia medium at a dose of 1 ml, the second - 2 ml. and the third group at a dose of 3 ml. intramuscularly into the buttock area.

From the isolated epizootic strains, the most pathogenic strain was selected and the "Concentrated GOA formol vaccine against emphysematous carbuncle of cattle and sheep" was prepared from it. To study the protective properties of the vaccine and the duration of post-vaccination immunity in sheep of 6-7 months of age of average fatness and calves of 6-7 months of age of average fatness.

The experiments were carried out on 16 heads of clinically healthy Karakul sheep. The sheep were divided into four groups of 4 heads each; Groups 1 and 2 were experimental, groups 3 and 4 were control. Sheep of the first and second groups were vaccinated with a vaccine prepared from the epizootic strain *Cl.chauvoei*. The vaccine was administered intramuscularly from the inner hairless side of the thigh in a dose of 2 ml. Sheep of the third and fourth groups were injected with 2 ml of sterile saline solution. Sheep of all four groups were under constant observation. No deviations from the norm in clinical parameters were observed in vaccinated and unvaccinated sheep.

16 days and six months after vaccination, sheep of the first and third groups and, respectively, the second and fourth groups were infected with a daily culture of *Cl.chauvoei*

at a lethal dose of LD₁₀₀ for sheep. Infected sheep were kept under constant surveillance and their clinical status was systematically assessed. The carcasses of sheep that died as a result of infection were subjected to a pathological autopsy and all macroscopic changes in the internal organs were taken into account.

To determine the duration of immunity after vaccination in calves, an experiment was conducted on 6 heads of calves, divided into 2 groups of 3 heads each. The first experimental group and the second control group. Calves of the first group were vaccinated with a vaccine prepared from the epizootic strain Cl.chauvoei. The calves in the experimental group were administered the vaccine intramuscularly in an amount of 2 ml. calves in the control group were injected with sterile saline solution in a volume of 2 ml. Calves of both groups were under constant clinical supervision. No deviations from the norm in clinical parameters were observed in vaccinated and unvaccinated calves.

After six months, all calves in the experimental and control groups were infected with a daily culture of Cl.chauvoei grown on Kitt Tarotsia medium at a lethal dose of LD₁₀₀ - 3 ml. Infected calves were under constant clinical observation. The carcasses of dead calves were subjected to a pathological autopsy and the results of macroscopic changes were taken into account.

3 Results and Discussion

As a result of bacteriological examinations of pathological material to isolate the causative agent of emphysematous carbuncle, 12 epizootic strains of Cl.chauvoei were isolated. While studying the morphological properties of the isolated isolates, it was determined that the pathogen is mobile, has peritrichs, in the smear it is a gram-positive, straight thick rod with rounded ends, the length of which is 2-8 μ and the width is 0.6 μ, in the smears the rods are located singly or in pairs.

Based on the cultural properties, it was determined that the pathogen is a strict anaerobe, growing on the meat-peptone liver broth MPLB under petroleum jelly (Kita-Tarotsia medium). The optimal growth temperature was 37.5-38.0 °C. In the liver broth, after 14-18 hours, a uniform turbidity of the medium appeared, which gradually became clearer, as microorganisms began to settle to the bottom of the test tube. During the shak the broth, the sediment easily broke up and formed a uniform, cloudy suspension. Gas bubbles and a specific smell of rancid oil formed on the surface of the Kitt-Tarozzi medium. On glucose-blood agar, the microbe formed delicate, blue-violet, flat colonies with a button-shaped elevation in the center with a zone of hemolysis. The microbe liquefied the gelatin. Pathogenicity was studied from the biological properties of the pathogen (Table 1).

Table 1. Determination of the pathogenicity of epizootic strains of Cl.chauvoei for guinea pigs.

No.	Strains Cl.chauvoei	Number of animals in group	Infectious dose	Death of animals after
1	X-04	4	0.5 ml	8-10 hours
2	I-03	4	0.5 ml	48-72 hours
3	T-04	4	0.5 ml	7-10 hours
4	B-03	4	0.5 ml	48-72 hours
5	P-03	4	0.5 ml	48-72 hours
6	F-05	4	0.5 ml	36-48 hours
7	O-05	4	0.5 ml	36-48 hours
8	T-06	4	0.5 ml	36-48 hours
9	I-06	4	0.5 ml	36-48 hours
10	N-06	4	0.5 ml	36-48 hours
11	T-07	4	0.5 ml	28-30 hours
12	X-08	4	0.5 ml	36-48 hours

As can be seen from Table 1, the pathogenicity of the epizootic strains *Cl.shauvoei* X-04 and T-04 is higher compared to other strains. In guinea pigs with the microorganisms *Cl.shauvoei* X-04 and T-04, the disease occurred in an acute form, with long-term specific signs of emphysematous carbuncle, which were noted 7-10 hours after infection.

To prepare an experimental series of vaccine against emphysematous carbuncle, the most pathogenic epizootic strain *Cl.chauvoei* T-04 was used. The vaccine was prepared from a daily culture of the epizootic strain *Cl.chauvoei* T-04, which was grown in Kitt-Tarozzi medium at 37.5-38.0 °C and inactivated with

0.3% formaldehyde. To precipitate microbes, 15% of 3% GOA was used, and 1% agar-agar was added as a stabilizer.

While determining the immunogenicity of the vaccine and the duration of post-vaccination immunity, the *Cl.chauvoei* X-04 strain was used.

To determine the preventive effectiveness and duration of post-vaccination immunity, the LD100 lethal dose of the pathogen for sheep was previously determined. The results of studying the pathogenicity of the *Cl.chauvoei* X-04 strain are presented in Table 2.

Table 2. Determination of the pathogenic properties of *Cl.chauvoei* X-04 for sheep.

Group numbers	Strain	Number of animals (heads)	Infectious dose (ml)	death within 48-72 hours (heads)	%
1	X-04	4	1.0	2	50
2	X-04	4	1.5	3	75
3	X-04	4	2.0	4	100

As can be seen from Table 2, the LD₁₀₀ *Cl.chauvoei* X-04 for sheep was 2 ml.

The results of research of determination the immunogenicity and duration of post-vaccination immunity of an experimental vaccine sample in sheep are presented in Table 3.

During studies to determine the preventive effectiveness of the vaccine, the condition of the animals was assessed 8-12 hours after infection; the body temperature in sheep of the first (vaccinated) group increased by 0.4-0.6 °C from the physiological norm. The elevated temperature persisted during the first day, then throughout the entire observation period (15 days), it was within the physiological norm. No signs of emphysematous carbuncle were observed in the sheep of the first group; the animals remained healthy throughout the entire observation period.

In sheep of the third group (control), 8 hours after infection, the first signs of an emphysematous carbuncle appeared, which manifested themselves in the form of lameness, difficulty moving, and they tried not to rest on the limb into which the pathogen was injected. The animals were inactive, and food intake decreased sharply. Body temperature increased to 41.4±0.3 °C. Respiratory dynamics were 50-60 times per minute, and the heart rate reached 120 beats per minute. Table 3.

Table 3. Determination of preventive effectiveness and duration of post-vaccination immunity in sheep.

Group numbers	Number of animals	Vaccine dose (ml)	1st infection after 16 days. Infectious dose (ml) (LD ₁₀₀)	2 nd infection after 6 months. Infectious dose (ml) (LD ₁₀₀)	Observation time 15 days	
					Not sick (heads)	Sick and death (heads)
1.	4	2	2	-	4	-
2.	4	2	-	2	4	-
3.	4	-	2	-	-	4
4.	4	-	-	2	-	4

On the second day of the experiment, the sheep were partially immobilized, and obvious lameness was observed. In animals, food intake stopped completely; the animals reacted very weakly to external stimuli. Body temperature reached 41.3 ± 0.3 °C. Respiratory movements were 60-80 times a minute, and the heart rate was 120-140 beats per minute. A warm and painful swelling formed at the injection site, with a crunching sound when pressed. In the afternoon, the sheep's body temperature gradually decreased. On the third day of the experiment, the sheep of the third group died.

During a pathological examination of sheep that died as a result of infection, pathological changes characteristic of an emphysematous carbuncle were identified. At autopsy, samples of pathological material from parenchymal organs were taken from sheep in order to reisolate the pathogen. As a result of culture from pathological material on Kitta-Tarotsia medium, Gram-positive microorganisms CI were isolated. Chauvoei identical in morphological properties to CI. Chauvoei X-04 used to infect sheep.

As a result of studies to determine the duration of post-vaccination immunity in sheep, 8-10 hours after infection, in the experimental group (2), the body temperature in sheep increased by 0.3-0.6 °C from the physiological norm. Increased body temperature was noted in sheep until the second day. In the following days (15 days observation period), it normalized to the physiological norm. In the sheep of the second experimental group, no signs of emphysematous carbuncle were observed and they remained healthy throughout the entire observation period.

Sheep of the fourth control group developed clinical signs of emphysematous carbuncle 8 hours after infection. In sheep, clinical signs were noted in the form of lameness, difficulty moving, and the limb into which the infection was carried out cannot be stepped on. The animals were inactive and did not eat food well. Body temperature increased to 41.1 ± 0.4 °C. Respiratory movements were 60-66 times a minute, and the heart rate reached 110-120 beats a minute. On the second day, the sheep were in a stupor and did not move; when forced to move, lameness was observed in them. The animals refused food and reacted very weakly to external stimuli. Body temperature reached 41.5 ± 0.4 °C. Respiratory movements were 70-80 times a minute, and the heart rate was 120-140 beats per minute. A warm and painful swelling formed at the injection site, and when pressed, crepitus was noted. In the afternoon, the sheep's body temperature gradually began to decrease. On the third day of the experiment, all the sheep of the fourth group died.

During a postmortem examination of sheep that died as a result of infection, changes characteristic of an emphysematous carbuncle were identified. As a result of bacteriological studies, Gram-positive bacilli identical to the causative agent of emphysematous carbuncle CI were isolated from the parenchymal organs of dead sheep in the experimental group. Chauvoei X-04 used for infecting sheep.

The results of studies to determine the duration of post-vaccination immunity in calves are given in Table 5.

Before studying the duration of post-vaccination immunity in calves, the pathogenicity of the pathogen CI. Chauvoei X-04 for calves were studied. Studies on the pathogenic properties of CI. Chauvoei X-04 for calves are presented in Table 4.

Table 4. Studies of the pathogenic properties of CI. Chauvoei X-04 for calves.

Number of group	Strain	Number of animals (heads)	Infectious dose (ml)	death within 48-72 hours (heads)	%
1	X-04	4	1.0	2	50
2	X-04	4	2.0	3	75
3	X-04	4	3.0	4	100

As a result of infection of calves in the experimental group 6 months after immunization, by determining the duration of post-vaccination immunity in calves 8-12

hours after infection, the body temperature in calves in the experimental group increased by 0.5-1.0 °C from the physiological norm. In the calves of the experimental group, the increase in temperature remained until the second day and then throughout the entire experiment (15 days) it was within the physiological norm and did not rise again in Table 5.

Table 5. Determination of the duration of post-vaccination immunity in calves.

Group number	Number of animals	Vaccine dose (ml)	Infection after 6 months. Infectious dose (ml) (LD100)	Observation time 15 days	
				Not sick (heads)	Sick and death (heads)
1.	4	2	3	4	-
2.	4	-	3	-	4

On the first day after infection with Cl. Chauvoei X-04 observed inactivity, depression, increased respiratory movements and heartbeat. In some calves, poor food intake was observed, which subsequently returned to normal. During the observation period, no signs of emphysematous carbuncle were observed in the calves of the experimental group, and they remained healthy.

The calves of the control group showed the first signs of emphysematous carbuncle 8-10 hours after infection. Signs of chromatosis with limited mobility were observed in infected calves of the control group. The hind limb into which the pathogen was injected was not stepped on; it was kept suspended. The animals were reluctant to eat the food. Chills were observed, body temperature increased to 41.7±0.3 °C. Respiratory movements were 48-60 times a minute, and the heart rate reached 90-110 beats a minute. On the second day of the experiment, the calves refused to feed. They stood in one place, without moving, and almost did not react to external irritations. Body temperature reached 42.2±0.3 °C. Breathing was rapid, but superficial, respiratory movements were 60-80 times a minute, the heartbeat was also increased, the pulse was weak, 120-140 beats per minute. A warm and painful swelling formed at the injection site, upon palpation of which crepitus was noted. On the third day, the calves' body temperature gradually decreased. In the second half of the third day, the calves of the control group died.

Calves that died from infection were subjected to a pathological examination and changes characteristic of an emphysematous carbuncle were noted. Samples of pathological material were taken from dead calves for bacteriological examination in order to re-isolate the infectious agent. As a result of bacteriological studies of the pathological material, Gram-positive rods were isolated, located in pairs or singly in the smear, which were identical to the epizootic strain Cl. chauvoei X-04 used to infect calves.

4 Conclusion

For the first time in Uzbekistan, a GOA formol vaccine (full name) was produced, based on the epizootic strain Cl. chauvoei T-04. In experiments on sheep and calves, a high preventive effectiveness of the vaccine was revealed, such as the safety of vaccinated animals after infection with the LD100 epizootic strain

Cl.chauvoei X-04 was 100%. According to the results of experimental work, long-term post-vaccination immunity was revealed in calves and sheep, which persisted for six months.

- For the first time in Uzbekistan, “Concentrated GOA formol vaccine against emphysematous carbuncle of cattle and sheep” was produced from the epizootic strain Cl. Chauvoei T-04.

- In experiments on sheep and calves, a high preventive effectiveness of the vaccine was revealed, such as the safety of vaccinated animals after infection with the LD100 epizootic strain Cl. Chauvoei X-04 was 100%.
- According to the results of experimental work, long-lasting post-vaccination immunity was revealed, which persists for 6 months.

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