Species diversity of Clostridia causing malignant edema in cattle

A V Kapustin, A I Laishevtev, E V Ivanov and A V Danilyuk

Federal State Budget Scientific Institution “Federal Scientific Centre VIEV” (FSC VIEV), Ryazanskiy prospect, 24, 1, Moscow 109428, Russia

E-mail: kapustin_andrei@mail.ru

Abstract. In modern conditions of animal husbandry, when high-tech methods of breeding replace existing approaches to livestock maintenance, timely vaccination is almost the only reliable way to fight clostridiosis. Anaerobic infections are a serious problem for livestock breeding worldwide. The damage is caused by economic losses and animal deaths, as well as by the adverse conditions in the area, contaminated by spores of pathogens and losses from the introduction of quarantine measures and restrictions. Due to the wide spread of pathogens in the environment, the acute or super-acute course of the disease and the severity of damage to body tissues, treatment of animals is almost 100% ineffective compared to specific prevention. However, for the development of an effective immunobiological medication, it is necessary to justify its composition, therefore, it is important to constantly monitor the etiological structure of clostridiosis in cattle.

1. Introduction

Clostridiosis is the name for all diseases of warm-blooded animals caused by various types of anaerobic spore-forming bacteria of the genus Clostridium. In addition to spore formation and oxygen sensitivity, the common feature of Clostridia is the ability to form specific highly active toxins which have a lethal effect on a macroorganism. These toxins, by affecting certain animal tissues and organs, disrupt their functioning, causing the development of specific life-threatening pathological conditions [1, 2].

Clostridia belong to the genus Clostridium, the family Clostridiaceae, the order Clostridiales, the class Clostridia, and the phylum Firmicutes. According to Bergey’s classification, the genus is currently represented by 220 species, divided into five groups according to their ability to ferment gelatin and the location of spores in the cell [3].

The most common disease of anaerobic etiology in cattle is malignant edema. This is an acute wound infection, characterized by fast appearance of edemas and necrosis of the affected tissue with the formation of gases and intoxication of the body. It is impossible to distinguish a separate type of pathogen, which is the main etiological factor of the disease, because, as a rule, they are in association and are represented by the following species: C. perfringens, C. oedematiens (novyi), C. septicum, C. sordellii, C. histolyticum [4, 5, 6]. Also, in some cases, non-pathogenic clostridia appear and form proteolytic enzymes that significantly complicate the course of the disease and cause putrefactive tissue breakdown. Diseases caused by a mixed infection are clinically more severe, faster and, as a rule, are fatal.
Among animals, malignant edema is most often found during mass surgical operations, such as dehorning, docking, castration, and during calving [7]. Depending on the procedure, the infectious process is located in: the uterus in the postpartum period, the head, the scrotum, quite often affections of the abomasum (figure 1) and the small intestine can occur.

![Figure 1. Affection of abomasum in cattle caused by infection of C. septicum.](image)

The disease is almost always acute. If pathogen gets into the wound edema and erythema with pronounced soreness are formed in this area in a few hours. Further, the edema rapidly grows, spreading to the subcutaneous tissue (figure 2).

![Figure 2. Subcutaneous tissue edema caused by Clostridium sordellii.](image)

In addition to edema, crepitus is clearly felt during palpation. The skin is tight, diffusely red or almost black. Pronounced oppression, tachycardia and convulsions occur quite often. In the later stages of the disease, the affected area becomes cold; the pain disappears due to necrosis of the local nerve endings and subnormal temperature. In most cases, death is caused by toxemia and systemic shock within 2-4 days after the appearance of clinical signs [8, 9].
In cows, malignant edema is often associated with difficult calving and is manifested in the form of necrotizing vulvovaginitis, metritis and mastitis. The disease develops within 1 - 3 days after calving, manifests itself in edema of the vulva, perineum, and is accompanied by hyperthermia and oppression. Once the process begins it almost always ends with the death of the animal.

The mammary glands and muscle tissue are also often affected, especially in the area of the shoulder blades and hip muscles (figure 3).

Pathological changes during the gangrenous process are characterized by serious systemic changes and rapid spread of infection throughout the body. Diffuse hemorrhagic gelatinous edema of the subcutaneous tissue and emphysema are observed. A pronounced change in the tissues colour is noted in the affected muscles; in this case some sections may be dark red, and the nearby ones may be of grey or bluish colour and look like boiled meat. There are also serous and subendocardial hemorrhages, enlargement of spleen and/or liver and pulmonary edema, as a result of severe intoxication (figure 4).

In less acute processes caused by *C. septicum*, cattle develop the so-called cellulite, when local damage occurs to separate sections of large skeletal muscles, which are encapsulated later. In addition to it, limited, capsule-covered tissue sections of dark red or cherry colour, with an oily consistency, ranging from 7 to 12 cm in diameter were found in the muscles of some animals. Such changes occur when animals are infected with non-toxic strains of the pathogen or when an infection develops in a vaccinated livestock. The chronic form of the disease may last 30 days, but also ends in death [10].
Exotoxins and spores spread with edematous fluid and exudate to other organs and tissues. Increased intoxication of the animal organism causes the appearance of dystrophic processes in the myocardium, lungs, liver and kidneys. Myocardial paralysis becomes the cause of animal death.

Figure 4. Combined course of malignant edema caused by *C. septicum* and necrotic hepatitis caused by *C. novyi*.

The most frequently detected cases are of gangrenous affection of thigh muscles, accompanied by the formation of extensive edema, crepitus of the tissue under pressure, followed by their melting, the presence of exudate, gas bubbles, a specific smell [11, 12].

Malignant edema is a common infectious disease in livestock breeding, with a specific clinicopathological manifestation. A peculiarity of the infection is the fact that several types of clostridia can simultaneously be the causative agent of the pathology [13]. Wide species diversity requires the use of appropriate measures for the control and prevention of anaerobic infections. However, since the disease is characterized by rapid death of animals, antibiotic therapy is often not able to demonstrate the necessary therapeutic effect. That is why, the use of means of specific prevention of clostridiosis is a more effective means of fighting the considered group of infections [13, 14, 15].

The purpose of the research was to study the species diversity of clostridia causing malignant edema in cattle on the territory of the Russian Federation.

2. Materials and methods

The etiological significance of various types of clostridia in the structure of malignant edema in cattle was assessed by analyzing the results of studies of the pathological material taken from dead or slaughtered animals with clinical signs of anaerobic infections. The material for the study was taken in the livestock enterprises infamous for clostridiosis cases in various regions of the Russian Federation during an epizootological examination, which includes a clinical examination of animals, diagnostic slaughter, and autopsy with sampling for subsequent bacteriological study.

The studies were carried out between 2007 and 2019 in livestock enterprises infamous for clostridiosis cases in Moscow, Ryazan, Kirov, Belgorod, Saratov, Irkutsk, Lipetsk, Orel, Leningrad, Tver, Ulyanovsk and Chelyabinsk regions, the Republic of Mordovia and Kalmykia, Stavropol and Krasnodar Krai of the Russian Federation (16 regions). A total of 2913 samples of pathological material from 1002 animals were studied, from which 714 isolates of clostridia were differentiated.

The bacteriological study on the presence of anaerobes was carried out by sowing material on agarized and liquid nutrient media, which were cultured in an anaerostat at 37 °C for 48-72 hours.
Schaedler Agar and/or meat-peptone liver agar with the addition of blood and glucose were used as agar media. Sowing was carried out by the method of imprint smears, or suspensions of the study material were preliminarily prepared. At the same time as sowing on agarized media was carried out, sowing on Kitt-Tarozzi medium was performed to accumulate the pathogen.

The identification of clostridia was also carried out by the biological method, infecting guinea pigs with a prepared suspension of material, which made it possible to quickly confirm the presence of clostridia and get rid of extraneous microflora.

Identification of Clostridium spp. isolates was carried out by studying the cultural and morphological properties during the growth in liquid and solid nutrient media, as well as by studying biochemical, toxigenic and pathogenic properties of cultures. Biochemical properties were determined with the use of media from diagnostic test-systems for the identification of clinically significant anaerobic microorganisms and using the method of time-of-flight mass spectrometry MALDI-TOF.

For bacteriological study, sections of affected tissues, pieces of parenchymal organs (liver, spleen, and kidneys), blood from heart, and a section of the small intestine with contents were selected.

Samples delivered to the laboratory were examined microscopically and, if the presence of large gram-positive rods in smears was detected, sowing on nutrient media was performed. Growth in the Kitt-Tarozzi medium was accompanied by dense turbidity and gas formation. At the same time, large gram-positive spore-containing rods were found in Gram stained smears. In the case of growth in the media of abundant facultatively anaerobic microflora that interferes with the release of anaerobes, the sowings were kept in a dark place at room temperature for 10-14 days, after which they were warmed up in a water bath at 90 °C for 10 min, as a result, while the spore forms of anaerobes survived, and extraneous microflora perished. The sediment was resown on Kitt-Tarozzi medium for accumulation, and then on meat-peptone liver agar in order to obtain a pure culture of pathogens.

3. Results and discussion

As a result of the research, it was possible to establish the species spectrum of clostridia, somehow related to the occurrence of malignant edema in cattle. In addition to the widespread species described by various researchers, there are rare species, including those that were not previously described in Russia as pathogens of anaerobic infections in ruminants. Thus, during the bacteriological study of pathological material from cattle, C. septicum is found in 34.5% of cases, C. perfringens type A - 23.25%, C. perfringens type C - 14.25%, C. perfringens type D - 6.5%, C. oedematium - 2.5%, C. sordellii - 6.5%. All other species (C. sporogenes, C. tetani, C. baratii, C. bifermentans, C. difficile, C. hastiforme, C. histolyticum, C. innocuum, C. sporogenes, C. tertium) are found in the range of 1-2%.

4. Conclusion

Clostridiosis is the most urgent problem of modern dairy and beef cattle breeding. Due to the rapid development of the infectious process, the high toxicity of pathogens and the extensive damage to body tissues, treatment of the disease is often ineffective and leads to the disposal of highly productive animals. Specific prevention is the only reliable way to prevent anaerobic infections. A great number of immunobiological medications with a wide antigenic composition have been developed and are applied in the world for this purpose. In our country, for a long time this problem was not paid necessary attention to, therefore, vaccines for the prevention of anaerobic etiology in cattle have not been developed. The only widely used medication for the prevention of anaerobic infections in cattle has been a vaccine against emphysematous carbuncle, which is not enough in modern conditions.

The spread of anaerobic infections makes it necessary to create new tools for their specific prevention, considering modern epizootic data and the etiological structure. In order to solve this problem, a study of 2913 samples of sectional material obtained from 32 cattle breeding enterprises in various regions of the Russian Federation was conducted. As a result of the study, it was found that productive livestock clostridiosis occurs mainly in the form of malignant edema, in which the following species predominate: C. septicum - found in 34.5% of cases, C. perfringens type A - 23.25%, C. perfringens type C - 14.25%, C. perfringens type D - 6.5%, C. oedematium - 2.5%, C. sordellii - 6.5%.
The obtained data should be considered when developing vaccines for cattle against anaerobic infections.

Acknowledgements
Work is done within the approved state task and the plan of researches to Federal State Budget Institution «Federal Scientific Centre VIEV» (FSC VIEV) for 2019-2021 without attraction of additional sources of financing.

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