Epizootological data of cattle infection caused by bacillus cereus on a dairy farm of the Kemerovo region

Anastasia Metleva[1], *Oksana* Smolovskaya

Kuzbass State Agricultural Academy, Kemerovo, Russian Federation

Abstract. Diseases caused by Bacillus cereus of non-food origin are becoming relevant. This article describes a case of an epizootic situation associated with the spread of B. cereus through cattle feed and its ubiquitous distribution to all environmental objects and animal biotopes on the farm. B. cereus can cause not only food poisoning but also a generalized form of the infectious process, characterized by severe intestinal stagnation with a thickened and edematous wall, hemorrhages on the mucosal surface, catarrhal enteritis, systemic lymphadenopathy, degeneration of muscle fibers, interstitial pneumonia with pulmonary edema and pericardial effusion, meningeal and splenic hyperemia, glomerulonephritis and renal liver failure. Bacillus cereus was the predominant microorganism in quantitative terms with the specified pathoanatomical picture. Microorganisms of the Enterococcuscea, Staphylococcuscea families were found together with B. cereus. B. cereus was found in biological material (cervical mucus, nasal discharge) from live animals and in environmental objects. The removal of contaminated feed from the diet of animals led to a decrease in the death of livestock. It was concluded based on this that the main factor in the transmission of the pathogen was the food supply.

Keywords: generalized infection, transmission factors, feed, environmental objects.

1 Introduction

There is an increasing interest in *B. cereus* as the causative agent of infectious diseases in the last 20 years due to the increase in the number of diseases associated with this microorganism [1].

According to the database https://www.gbif.org/, the maximum number of *B. cereus* cultures found and registered was observed in 2016. Molecular-genetic research aimed at detecting this microorganism in various research objects, including collections of microorganisms from various institutes around the world, began in 1981, which is associated with the beginning of the use of genome research by the polymerase chain reaction method and the entry of microbiology into the era of genetic research. However, after 2016 the pool of research began to subside (Fig. 1).

[1] Corresponding author: zveryski@mail.ru

Fig.1. Distribution of the number of studied *B.cereus* cultures over the years according to the website https://www.gbif.org/.

When studying the distribution of *Bacillus spp.* in the world, according to the site https://www.gbif.org/, it was found that the most common microorganisms of this family are found in the Altai Territory. In the Kemerovo region outbreaks of diseases caused by *B. cereus* were not registered by the veterinary service, scientific communities in the field of biology, veterinary medicine and medicine. Despite this, finds of various *B. cereus* strains were recorded in the neighboring Altai Territory (Fig. 2) [2].

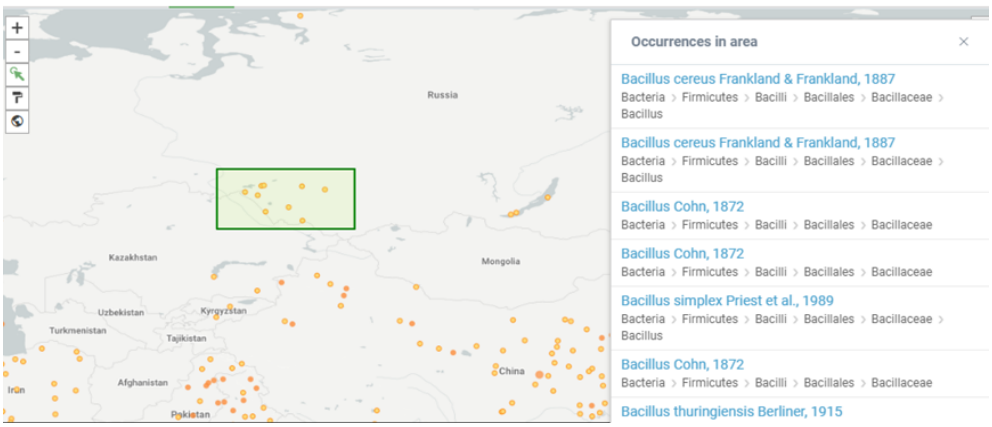

Fig. 2. Distribution of B. cereus strains on the world map according to the website https://www.gbif.org/.

B. cereus actively manifests itself as a causative agent of food poisoning in humans and food poisoning in animals. Information about B. cereus as a causative agent of non-gastrointestinal diseases has appeared more often in the literature recently [3]. *B. cereus* is becoming known as an independent etiological factor in the infectious process among immunosuppressed livestock. These diseases include: meningitis and brain abscesses, endophthalmitis, pneumonia and gas gangrene-like skin infection, wound infection, endocarditis, urinary tract infection, and liver infection [4]. Non-gastrointestinal infections caused by *B. cereus* are not as common as food poisoning but can occur when the body's resistance decreases. There are few reports about virulent *B. cereus* in animals in the literature [5, 6]. For example, cases of sporadic necrotizing placentitis caused by *B. cereus* in cows have been described, resulting in abortion and death of the mother due to the production of necrotizing toxins [7, 8]. Also, cases of mastitis and

inflammatory diseases of the pelvic organs in cows and goats caused by *B.cereus* are described [9, 10].

B. cereus causes severe diarrhea, gastric erosion and ulcerative syndrome, as well as hemorrhagic inflammation in the lungs and immunosuppression in poultry when associated with other pathogens [6] Associative forms of infections caused by both B. cereus and other opportunistic microorganisms are characterized by severe infection which is often fatal. So bacterial endophthalmitis caused by Staphylococcus aureus, Enterococcus faecalis and B.cereus is characterized by increased contagiousness, the most striking clinical picture with an earlier onset of the disease manifestation stage than the infection caused by the first two types of bacteria. In addition, generalization of the infectious process caused by B. cereus was observed, while S. aureus and E. faecalis were found only in some organs [11].

B. cereus is inherently resistant. It survives under in vitro conditions when cultivated on various media and under various temperature conditions. B. cereus spores are found and persist for a long time in soil, dust, water and the environment, on equipment for filtering and ventilating polluted air, on the hands of personnel, alcohol-based handwashing solutions, etc. Therefore, very often, the detection of B. cereus in clinical material (blood, wound exudate, sputum, etc.) is attributed to contamination of clinical samples with secondary microflora at the sampling stage [12].

The main link in the pathogenetic process in bacillary infection is the production of toxins. Hemolysin has hemolytic, cytotoxic and dermonecrotic effects and induces vascular permeability. Cytotoxin K has dermonecrotic, cytotoxic and hemolytic effects. Phospholipases such as lecithinase and phosphatidylinositol play an important role in respiratory tract infections leading to tissue necrosis and hemorrhage [13].

Pathogenic properties can be determined at the stage of cultivation on dense nutrient media. There is a correlation between swarming growth of colonies on dense nutrient medium and hemolysin secretion in B. cereus. In addition, flagellum-dependent motility patterns (i.e. swimming and swarming) and biofilm production unlock the pathogenic potential of B. cereus as these factors promote the colonization process of host tissues. The most toxigenic strains had swarming growth, while the avirulent strains grew in isolated colonies [14].

Large concentrations of necrotizing toxins are formed during spore germination after B. cereus adhesion to epithelial cells and polymorphonuclear leukocytes. Adhesion to the epithelial cells of the small intestine occurs due to the hydrophobic surface structures of the cell wall, protruding crystalline formations of the surface protein layer and the S-layer [15].

2 Materials and Method

The initiation of the study was the complaint of the owner of a livestock farm about sporadic cases of animals' death. Biological material from living animals and pathological material from dead animals were taken for the study. Samples from environmental objects, feed (silage) from a livestock farm in the Kemerovo region from April to May 2022 were examined.

Samples of biological material were taken from the cervical canal in the amount of 20 pieces; nasal cavity – 15 pcs.; pathological discharge from the differents parts of udder affected by mastitis – 10 pcs.; feed – 3 pcs.; swabs from the walls of livestock buildings – 20 pcs.; pathological material from dead animals – 8 pcs.

Sample selection. Sampling from the cervical canal was carried out with a sterile probe with a swap by the visocervical method with a sterile vaginal speculum. The swab was moistened in the liquid just before taking the flush. After sampling, the swap was placed in a sterile saline solution in a test tube to deliver the sample to the laboratory.

A sample from the nasal cavity was taken with a sterile swab. The animal was fixed by the nasal septum by an assistant in sterile gloves. The swap was passed along the walls of the nasal

passages under eye control in order to avoid injury to the animals. Delivery to the laboratory was carried out in a test tube with 5 ml of sterile saline.

When sampling swabs from the surface of livestock buildings, a sterile swab moistened with sterile saline solution added to each test tube in an amount of 5.0 ml was used.

Feed (silage) was taken in point samples from different areas, which were mixed into a combined sample weighing 0.5 kg and delivered to the laboratory in a sterile container.

The sampling of the different parts of udder removed from the affected mastitis was carried out in sterile gloves after clean, dry teats were treated with 70% alcohol. The milking of the first streams was carried out in a specially designated container, and sampling was carried out in a sterile container, avoiding its contact with the nipples.

Pathological material from a dead animal was taken with sterile instruments into sterile containers.

The time of delivery of swabs to the laboratory did not exceed 6 hours from the moment of sampling. The study was carried out using the bacteriological method for determining microorganisms of various groups. For this purpose:

- samples from the cervical canal and nasal cavity were carried out through a series of dilutions in physiological saline. 1 ml of the initial dilution 1:5 was placed in a test tube with 9 ml of sterile saline, obtaining a dilution of 1:10. After mixing, 1 ml was transferred from the first tube to the second tube, making a 1:100 dilution and so on until a dilution of 1:100000000 was obtained. Then, each dilution was inoculated onto dense selective media: Endo medium, Ploskirev's agar, blood agar (incubated at 37°C for 18-24 hours), Enterococcogar, yolk-milk-salt agar (incubated at 37°C for 24-48 hours), medium Saburo (incubated at 30^0C for up to 5 days), lactobacgar and Bifidum semi-liquid medium (incubated at 37^0C for up to 5 days under anaerobic conditions);

- samples with mastitis udder, environmental objects were inoculated into meat-peptone broth with a sterile bacteriological loop, incubated at 37°C for 18-24 hours. After incubation, inoculation was carried out on dense selective media: Endo medium, Ploskirev's agar, blood agar (incubated at 37°C for 18-24 hours), Enterococcogar, yolk-milk-salt agar (incubated at 37^0C for 24-48 hours), Saburo medium (incubated at 30^0C for up to 5 days);

- samples of pathological material were inoculated with a sterile Pasteur pipette into meat-peptone broth and incubated at 37°C for 18-24 hours. After incubation, inoculation was carried out on dense selective media: Endo medium, Ploskirev's agar, blood agar (incubated at 37°C for 18-24 hours), Enterococcogar, yolk-milk-salt agar (incubated at 37°C for 24-48 hours), Saburo medium (incubated at 30^0C for up to 5 days);

- for the study of feed, 1 g of feed taken from the average sample was placed in a sterile test tube, 9 ml of saline was added and thoroughly shaken (1: 10). Subsequent dilutions (up to 1:1,000,000) were prepared from the obtained suspension. After that, inoculations were made from the upper layer of the liquid.

To account for microbial contamination, 1 ml of each dilution was added to sterile bacteriological dishes and 10 ml of sterile, melted and cooled to a temperature of 44-45°C beef-peptone agar was poured. Carefully inoculated material was distributed in agar. After solidification of the medium, the cups were placed in a thermostat at a temperature of 37°C for 24–48 hours. After incubation, grown colonies were counted only in dishes containing no more than 300 colonies. The results obtained by counting colonies were multiplied by dilutions, summed up and the number of microbes in 1 g of feed was determined.

In parallel, 1 g of feed was inoculated with a sterile Pasteur pipette into meat-peptone broth and incubated at 37°C for 18–24 hours. After incubation, inoculation was carried out on dense selective media: Endo medium, Ploskirev's agar, blood agar (incubated at 37°C for 18-24 hours), Enterococcogar, yolk-milk-salt agar (incubated at 37°C for 24-48 hours), Saburo medium (incubated at 30^0C for up to 5 days).

After incubation, biochemical typing of microorganisms was carried out on Hiss's differential diagnostic media to determine saccharolytic properties, reducing and peptonizing properties were determined on sterile skimmed milk with methylene blue.

3 Results

A livestock farm located in the north of the Kemerovo region reported an unusual increased mortality of 23 cattle aged from 2 to 4 years from January to April 2022. Symptoms appeared suddenly and included shortness of breath and myositis, which quickly led to the death of the animals. Autopsies showed severe intestinal stasis with a thickened and edematous wall, hemorrhages on the mucosal surface, catarrhal enteritis, systemic lymphadenopathy, degeneration of muscle fibers, interstitial pneumonia with pulmonary edema, pericardial effusion, meningeal and splenic hyperemia, glomerulonephritis and renal liver failure.

The following microbiological profile was established as a result of microbiological studies of biological material:

- Bacillus cereus (44.4%) was found in swabs from the nose and cervical canal, in the separated parts of the udder affected by mastitis;
- Enterococcus faecalis (5.5%) with mastitis;
- Staphylococcus saprophyticus (11.1%) was cultivated for mastitis and rhinitis;
- Staphylococcus epidermidis (5.5%) in swabs from the cervical canal;
- Pseudomonas tremae (5.5%) was found in mastitis;
- Staphylococcus aureus (5.5%) was found in mastitis;
- Streptococcus spp. (11.1;%) installed in mastitis and in the cervical canal
- Micrococcus cohnii (11.1%) is installed in the cervical canal.

Pathological material from dead animals is presented:

- B.cereus (42.8%);
- Enterococcus faecium (25%);
- S.epidermidis (25%).

From the objects of the external environment revealed:

- B.cereus (57.14%), incl. from feed;
- E.faecalis (14.2%);
- P.tremae (28.5%) (Fig. 3).

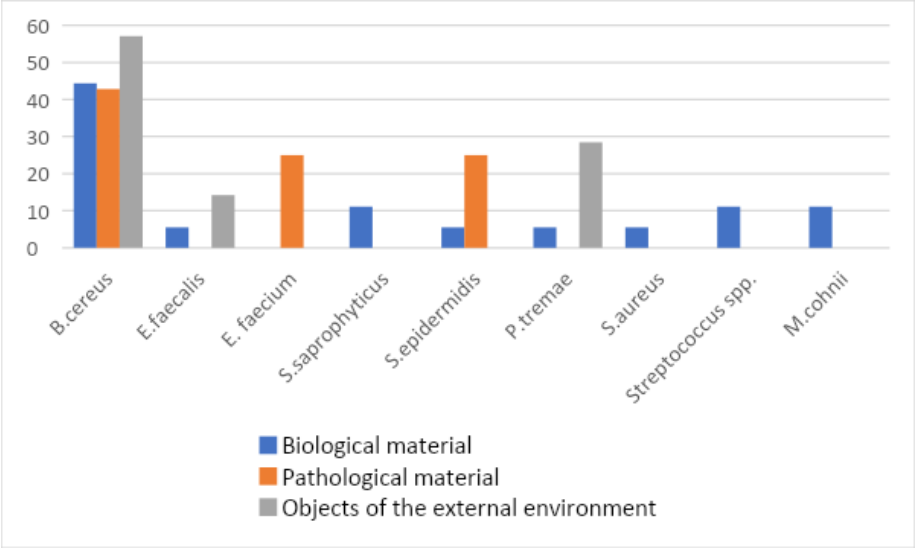

Fig. 3. The proportion of microorganisms in the studied objects (Compiled by the authors).

Growth of B.cereus on blood agar as wrinkled irregular large colonies surrounded by a zone of α-hemolysis is presented (Fig. 4).

Fig.4. Growth of B.cereus on dense nutrient media (Compiled by the authors).

When stained according Gram, gram-positive thick short rods with a subterminal spore, equal in diameter to vegetative cells, presented in the form of straight or slightly curved thin bacilli with square ends, singly or in short chains, were identified (Fig. 5).

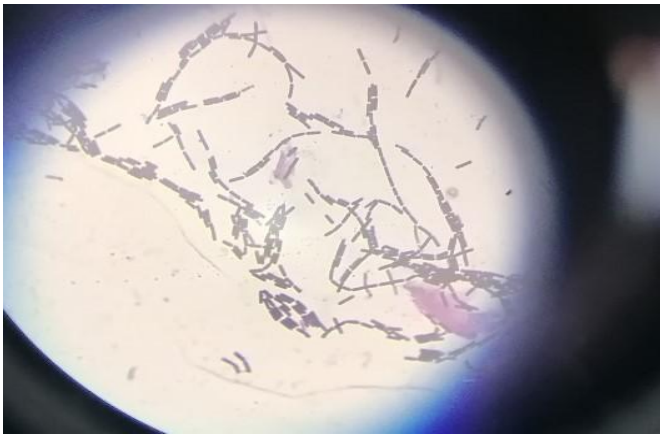

Fig. 5. B.cereus in smears from cultures isolated from biological material (Compiled by the authors).

An analysis of additional B. cereus virulence factors, which can contribute to the development of the infectious process was carried out under laboratory conditions. It included the presence of mobility, hemolytic activity and the presence of phospholipases. B. cereus colonies are dull grey and opaque with a rough matte surface when grown under aerobic conditions on 5% blood agar at 37°C. The edges of the colonies are uneven, growing in swarming from the site of the initial growth of bacteria. In some cases, smooth colonies develop either alone or in the middle of rough colonies.

Animal feed isolates were phenotypically similar to cultures from biological and pathological materials, thus indirectly confirming that the same strain of B. cereus is associated with an outbreak of infection transmitted through the alimentary route through feed.

4 Discussion

The severity of the outbreak and the number of sick animals indicate the need to ensure high sanitary and hygienic measures in the field of veterinary medicine. Although information confirming the importance of B. cereus in the infectious pathology of animals is scarce, the situation described represents a sporadic epizootic outbreak of infection.

The case reported in this article indicates the ability of B. cereus to cause severe systemic infections in cattle. Although the isolate has not been tested in live animals to reproduce the disease, the information obtained provides evidence that the dietary route of infection with B. cereus can lead to death in cattle through multiple organ damage.

After the removal of the contaminated feed, outbreaks with similar clinical symptoms in cows ceased on the farm and a causal relationship was made between the contamination of the feed base with B. cereus and the onset of infection caused by this microorganism. We can assume that the present cases were caused by the alimentary route of infection by ingestion of a virulent strain of B. cereus.

It can be assumed that the generalization of B. cereus infection is the result of a multifactorial process, including microbial motility, adhesion factors and the production of degrading enzymes and various toxins with the simultaneous presence of other types of opportunistic microorganisms.

Conclusion. The fact of a non-gastrointestinal form of cattle infection caused by B.cereus, transmitted through the food supply by alimentary transmission, has been established. The disease is characterized by gastroenteritis, myositis, pneumonia, diseases of the liver and kidneys. B.cereus is found not only in pathological material from dead animals but also in swabs from the cervical canal, nasal cavity, as well as from environmental objects of livestock complexes. The work carried out indicates the need for regular microbiological quality control of feed, feed additives and quality control of environmental disinfection, which is an important requirement for the safety and welfare of livestock in order to eliminate any possible risk of infection, even if the susceptible animal is not considered susceptible to contamination by alimentary transmission through the feed base.

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