Blackleg in cattle in the Irkutsk region

Andrei A. Blokhin

Federal Research Center for Virology and Microbiology, Branch in Nizhny Novgorod

Nadezhda N. Toropova

Federal Research Center for Virology and Microbiology, Branch in Nizhny Novgorod

Olga A. Burova

Federal Research Center for Virology and Microbiology, Branch in Nizhny Novgorod

Olga I. Zakharova (✉ ozakharova@ficvim.ru)

Federal Research Center for Virology and Microbiology, Branch in Nizhny Novgorod

Research Article

Keywords: cattle, blackleg, outbreak, pathological and anatomical changes, seasonality

Posted Date: September 14th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-900316/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Blackleg is an acute, toxic, infectious, non-contagious disease of domestic and wild ruminants that occurs while the animals are pastured. This article describes an outbreak of blackleg on a farm in Siberia (Russia). This article provides a detailed description of these cases based on the results of comprehensive diagnostic and epidemiological investigations. The distinctive features (in addition to the characteristic features) were as follows: the outbreak of the disease occurred in early spring when there was abundant snow cover; the disease appeared in both vaccinated and unvaccinated cattle; the characteristic clinical signs were low-grade fever, the absence of crepitus, and the presence of haematomas containing erythrocytes with basophilic granularity.

The evidence suggests that blackleg can present with a variety of conditions and symptoms in cattle.

Main Text

Blackleg is an acute non-contagious, toxic, infectious disease of livestock and wild animals that is characterized by fever, myositis, systemic infection and rapid mortality. The causative agent of the disease is the obligate anaerobic bacillus *Clostridium chauvoei*, which persists in soil in the form of resistant spores^{1,2,3}. This creates convenient conditions for the formation of endemic zones^{4,5}.

Contact of an animal with contaminated soil, water or food leads to the development of the disease⁶. According to the common understanding of the pathogenesis of this disease, *C. chauvoei* spores in contaminated pastures undergo one or more cycles of replication in the intestinal tract without causing the clinical symptoms of the disease^{7,8,9}. When an animal experiences an injury that breaks the barrier of the skin and that open wound becomes contaminated with spores, the spores enter an anaerobic environment and germinate. The vegetative form of the pathogen releases toxins that cause the clinical manifestations of the disease, ultimately leading to the death of the host^{10,11}.

Unvaccinated cattle aged 3 months to 4 years, buffaloes, sheep, goats, elk and reindeer are susceptible to blackleg^{5,12}.

Blackleg is characterized by various disease courses, and the duration and frequency of outbreaks vary^{13,14,15,16}. The disease usually occurs in cattle in the summer and autumn. Poor fodder, including dry grass, increases the risk of the occurrence of an outbreak of blackleg⁵.

Cases in animals are registered in dozens of countries around the world (Kazakhstan, USA (California), Austria, Algeria, Pakistan, Taiwan, Zambia, Belarus)^{17,18,19}.

In the Russian Federation, cases of blackleg have been registered each year in various regions (in the Irkutsk Region in 2011, Kursk Region in 2012, Chuvash Republic in 2013, Trans-Baikal Territory in 2014)²⁰ during grazing seasons, manifesting as sporadic cases or small outbreaks and only rarely as an enzootic disease.

In the acute form, the body temperature of the animal increases to 41-42°C; the animal shows signs of depression, including a refusal to eat and lethargy; and subcutaneous gas oedema forms, especially in the extremities, with swelling and crepitus in the affected muscles^{21,22}. In the hyper-acute form, the clinical signs are usually not observed due to the sudden death of the affected animal⁵.

The characteristic pathological and anatomical changes are acute necrohemorrhagic emphysematous myositis of the skeletal muscles, most often of the thoracic and pelvic belt muscles^{5,17,18}; expressed pulmonary oedema; myocarditis and petechial haemorrhages on the heart; liver enlargement; and venous hyperaemia of the liver and spleen^{17,22,23}. Visceral myonecrosis is less commonly noted and may affect the heart, hyoid muscles and diaphragm^{24,18}. Fibrinous pericarditis occurs very rarely. Emphysematous foetuses with lesions of the skeletal muscles and myocardium similar to those observed on the placenta are found in the uterus in cases of intrauterine infection¹⁰.

The diagnosis has to be confirmed with laboratory tests. The bacteriological diagnosis of blackleg is based on the results of seeding on a nutrient growth medium, microscopy of smears, isolation of pure cultures and bioassays. Blackleg should primarily be differentiated from malignant oedema and anthrax^{25,26,27}.

The control of blackleg is based on symptomatic treatment and vaccination^{28,29,30,31,32}.

The aim of this study was to investigate the possible routes of pathogen entry into the animals, features of the pathogenesis of the disease, clinical symptoms and pathological changes in an outbreak of blackleg that occurred in March 2019 in Siberia. In this article, we analysed the conditions and clinical and epidemic parameters of an outbreak of emphysematous carbuncle that occurred outside the usual season for this disease.

Results

1. Animal welfare and husbandry

The farm is located 800 metres southeast of Zabitui village, Alarsky district, Irkutsk region. As of March 16, 2019, the following animals were present: 108 cattle, 45 horses, and 250 sheep. Different species of animals were kept separate from each other on permanent flooring. The animals did not graze because there was a lot of snow on the pastures.

The animals were fed locally produced hay and crushed grains. Water for household needs and animal consumption was supplied from a borehole. The territory of the farm was established by a solid wooden fence and metal gates. The territory was used for logging and other economic activities. The territory was full of wooden rubbish and metal objects.

We conducted a study that excluded alimentary causes and poisoning with toxins.

2. Epidemiological data

Official reports from the past 10 years yielded no records of blackleg in Zabitui village. This outbreak was the first to be registered in the area.

The outbreak started on March 17, 2019, with sudden death of three cattle on the second day after vaccination against blackleg. The animal carcasses were disposed of the same day by cremation in a specifically designated area.

The epidemic involved pregnant cows, cows with calves and young cattle (n=43). They were vaccinated against blackleg and anthrax two days before the outbreak (15 March). Infections in other species, such as sheep, horses and buffaloes, that were vaccinated against blackleg in December 2018 were not observed.

The outbreak lasted two weeks, from March 17 to April 04, 2019. During this period, 43 head of cattle died (Figure 1).

3. Clinical signs

The following clinical signs were observed in all affected animals: depression-like behaviour, anorexia, and a high body temperature reaching 38.0-39.5 °C. Gross swelling was present on the surfaces of the body, although no crepitus was palpable. In different animals, the oedema was localized to the limbs (Figure 2a), chest, abdomen (Figure 2b), and back (Figure 2c). Paracentesis of the oedema revealed fluid accumulation and haemorrhage. During abdominal auscultation, there were signs of increased gas formation in the intestine.

Several animals showed signs of papillomatosis on the muzzle and vulva, as well as birth trauma. The papilloma and birth trauma were accompanied by the subsequent formation of oedema and compaction of the surrounding subcutaneous tissue and muscles with the formation of dense nodes (Figure 2d).

Abortions and stillbirths were reported in pregnant cows. A total of 3 cows aborted, and all of them subsequently died.

4. Autopsy

Immediately after death, the bodies of the animals released foamy, bloody fluid from the mouth, nose and eyes (Figure 3a).

Necropsies were performed on all the carcasses of the cows and calves, and the following distinct changes were observed at the sites of oedema: haemorrhagic-necrotic myositis with gas formation and serous haemorrhagic infiltration of the loose subcutaneous tissue adjacent to muscles (Figure 3b).

Some animals had serous haemorrhagic lymphadenitis of the pulmonary lymph nodes (48.0%), cardiac haemorrhage (26.5%) (Figure 3c), ruminal tympany with abdominal anaemia and thoracic hyperaemia

(11.1%), haemorrhage in the serous membrane of a scar (26.5%) (Figure 3d), and haemorrhage in the subcutaneous tissue in the area of ribs 5-8 and 10-12 on the left side of the body (2.3%).

One animal had connective tissue adhesions between the peritoneum and the serous membrane of the intestine and between the pulmonary and bone pleura and haemorrhages on the abdominal wall. In the region of the lumbar spine on the abdominal side, there was a haematoma with coagulated blood that was approximately 25 cm long (along the spine) and approximately 13 cm wide. Yellow gelatinous infiltration was found in the muscular tissue of the right hind limb.

The necropsy of the stillborn calves revealed haemorrhages on the spleen, gross haemorrhages in the heart and a lack of any border between the cortical and medulla matter in the kidneys.

5. Laboratory research

During testing, a large amount of gas on the Kitt-Tarozzi medium was observed only on the first day. There was no mortality among the guinea pigs when the bioassay was carried out.

In the preparations of bacterial cultures on the Kitt-Tarozzi medium, gram-positive rods with subterminally located spores were detected (Figures 4a). We confirmed the presence of bacteria in the samples by multiplex PCR.

In a microscopic examination of a blood smear obtained from a haematoma of the scapular shoulder joint that had been present for a week, erythrocytes with basophilic granularity were detected (Figure 4b). At the same time, inclusion bodies were observed in almost 100% of the cells.

Discussion

Blackleg is considered an infection that occurs when the animals are on pasture due to the abundance of dry grass, with summer-autumn seasonality. Rarely, the disease occurs in winter and spring and is caused by non-compliance with sanitary standards and the failure to administer preventive vaccinations^{7,17,28}. This outbreak occurred in the spring, when there was snow cover in Siberia. It occurred on cluttered farmland when there were susceptible cattle in the herd, including pregnant cows. This outbreak occurred due to the presence of inappropriate conditions resulting in trauma.

We suppose that trauma that occurred during the handling necessary for the vaccinations by the veterinarian (46.5% of the animals), mechanical papilloma injuries (41.8% of animals) and trauma that occurred during birth (11.6% of animals) resulted in the entry of the pathogen into the cattle and the subsequent development of blackleg. Consequently, the routes of infection were cutaneous wounds and tears in the mucous membranes of the reproductive tract. This complements the common understanding of the route of infection in cases of blackleg^{5,8,9}.

The clinical picture in the examined animals agreed with previously published data. The characteristic features were depression-like behaviour, refusal to eat, and gross swelling in the extremities, chest,

abdomen and back^{21,22}. Abortions and stillbirths were reported in fecund cows. Distinctive features of the described cases were the body temperature, which increased up to 38-39.5°C, and lack of crepitus under pressure in the area of oedema. Papillomas were the route of infection both while calving (papillomas vulva) and while feeding (papillomas on the muzzle). Tissue damage from papillomas resulted in an open route of infection and the development of the vegetative forms of *C. chauvoei*. Papillomatosis may lead to infection with *C. chauvoei* spores.

Some animals showed the formation of extensive haematomas containing blood with erythrocytes with basophilic granularity. No such cases have been described before. We believe the formation of haematomas was due to the increased vascular permeability and the appearance of basophilic granularity with systemic infection of the body³³.

When the pathological and anatomical investigations were performed on the carcasses of the cows, the following changes were noted: serous haemorrhagic oedema and emphysema of the subcutaneous tissue and skeletal muscles, serous haemorrhagic lymphadenitis of the regional lymph nodes, and hyperaemia of the organs in the chest and head. The presence of haemorrhages on the serous and mucous membranes of the dead animals, as well as haemorrhages on the internal organs of the foetuses, indicates both systemic infection of the animal and intrauterine foetal infection¹⁰.

There are no available reports of some of the pathological and anatomical changes, such as pulmonary atelectasis, abdominal anaemia, connective tissue adhesions between the peritoneum and the serous membrane of the intestine, and adhesions between the lung and bone pleura. These signs may be considered unusual for this infection, and further investigation of the manifestation of the infection under different conditions is needed.

The diagnosis of blackleg was confirmed by laboratory studies. The Kitt-Tarozzi medium yielded characteristic growth with gas formation. Gram-positive C rods were found in smears of cultures obtained from infected and dead animals.

Conclusions

An outbreak of blackleg occurred in Siberia in the early spring when the soil was covered with snow, which is not typical for the seasonality of the blackleg. Some unvaccinated cattle and some cattle that had been vaccinated in the previous two days developed blackleg. The distinctive clinical signs of the described cases were a subfebrile body temperature and the absence of crepitus when the oedema was depressed. In some animals, the formation of extensive haematomas containing blood with erythrocytes with basophilic granularity was noted. Evidence suggests that blackleg can present with a variety of conditions and characteristics in cattle. More research is needed on the various manifestations of blackleg.

Materials And Methods

Area: The field research was carried out from March to April 2019 in Zabitui village, Irkutsk region (E 102.818055; N 53.264713), and the laboratory research was performed in the Federal Research Centre for Virology and Microbiology (Pokrov).

For data collection and evaluation, we used the following methods: owner observations, descriptive epidemiology, clinical diagnostics, pathological examination and bacteriology.

Epidemiological data included the size and type of herd, feeding and housing conditions of the cattle, and list of treatments. We assessed the conditions in which the livestock and the animals were kept during the outbreak based on the results of a survey conducted of the farm's facilities.

The retrospective analysis was based on available official data on blackleg outbreaks in cattle in the Russian Federation, Irkutsk region and Zabitui village over the past 10 years.

Symptoms and pathological changes were described in accordance with the standard guidelines for animal examinations and necropsy with the visual assessment of pathological changes. Necropsy of the carcasses was carried out in the area specifically allocated for the cremation of the remains. Smears of infiltrates sampled from swollen sites were stained with the Romanowsky-Giemsa stain and studied under microscopy at 650X magnification.

The laboratory diagnosis was based on:

- morphology: direct microscopic examination of cultures of *C. chauvoei* after Gram staining;
- microbiological purity: isolation on Kitt-Tarozzi medium and incubation in aerobic and anaerobic conditions;
- multiplex PCR-test.

The strains were identified based on cultural and morphological characteristics using classic tests²⁹. *C. chauvoei* were cultivated under standard anaerobic conditions at 37°C on Kitt-Tarozzi medium.

Samples positive for *C. chauvoei* were analyzed for *C. chauvoei* and *C. septicum* using multiplex PCR techniques³⁴.

Declarations

Author Contributions

Conceptualization, A.A.B.; methodology, A.A.B., and O.I.Z.; validation, O.A.B. and A.A.B.; formal analysis, A.A.B., and O.I.Z.; investigation, O.I.Z., and N.N.T.; data curation, A.A.B.; writing – original draft preparation, O.A.B. O.I.Z., and A.A.B.; writing—review and editing, O.A.B. O.I.Z., and A.A.B.; visualization, O.I.Z.; supervision, A.A.B.; project administration, A.A.B. All authors have read and agree to the published version of the manuscript.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding

The work was supported by the Federal Research Center for Virology and Microbiology for government assignment.

Acknowledgments

None

References

1. Echenique, J. V. Z. *et al.* Blackleg in a free-range brown brocket deer (*Mazama gouazoubira*). *Pesqui. Vet. Bras*, **38**, 2262–2265 (2018).
2. Rychener, L. *et al.* Clostridium chauvoei, an evolutionary dead-end pathogen. *Front. Microbiol.* **8**, (2017).
3. Sathish, S. & Swaminathan, K. Molecular characterization of the diversity of Clostridium chauvoei isolates collected from two bovine slaughterhouses: Analysis of cross-contamination., **14**, 190–199 (2008).
4. Frey, J. & Falquet, L. Patho-genetics of Clostridium chauvoei. *Res. Microbiol.*, **166**, 384–392 (2015).
5. Ziech, R. E., Gressler, L. T., Frey, J. & de Vargas A. C. Blackleg in cattle: Current understanding and future research needs. *Cienc. Rural* **48**, (2018).
6. Datta, S. & Kumar Karmakar, U. *BLACK QUARTER IN A COW: A CASE REPORT Case history and observation.* .
7. Jubb, K. & Palmer's, Cooper, B. J. & Valentine V.A. Pathology of domestic animals. in *Muscle and tendon* 230–233 (2016).
8. Kriek, N. & Odendaal, M. Clostridium chauvoei infections. in *Infectious Diseases of Livestock*. 1856–1862 (Oxford University Press, 2004).
9. Quinn, P. Clostridium species. in *Veterinary Microbiology and Microbial Disease* 233–241 (Wiley-Blackwell 2011).
10. Abreu, C. C. *et al.* Blackleg in cattle: A case report of fetal infection and a literature review. *J. Vet. Diagnostic Investig*, **29**, 612–621 (2017).
11. Useh, N. M., Nok, A. J. & Esievo, K. A. N. Pathogenesis and pathology of blackleg in ruminants: The role of toxins and neuraminidase. A short review. *Vet. Q*, **25**, 155–159 (2003).
12. Scala, C., Duffard, N., Beauchamp, G., Boullier, S. & Locatelli, Y. Antibody Response to Epsilon Toxin of Clostridium Perfringens in Captive Red Deer (Cervus Elaphus) over A 13-Month Period. *J. Zoo*

Wildl. Med, **47**, 38–44 (2016).

13. Macêdo, J. T. S. A. *et al.* Edema maligno em equino causado por *Clostridium chauvoei* Malignant Edema Caused by *Clostridium chauvoei* in a Horse. *Acta Sci. Vet*, **41**, 75–78 (2013).
14. Groseth, P. K., Ersdal, C., Bjelland, A. M. & Stokstad, M. Large outbreak of blackleg in housed cattle. *Veterinary Record* vol. 169 (2011).
15. Raymundo, D. L. *et al.* Clostridial diseases diagnosed in herbivores in Southern Brazil. *Acta Sci. Vet.* **42**, (2014).
16. Seifert, H. S. H. *et al.* Environment, incidence, aetiology, epizootiology and immunoprophylaxis of soil-borne diseases in north-east Mexico. *J. Vet. Med. Ser. B*, **43**, 593–605 (1996).
17. Abreu, C. C. *et al.* Pathology of blackleg in cattle in California, 1991–2015. *J. Vet. Diagnostic Investig*, **30**, 894–901 (2018).
18. Heckler, R. F. *et al.* Blackleg in cattle in the state Mato Grosso do Sul, Brazil: 59 cases. *Pesqui. Vet. Bras*, **38**, 6–14 (2018).
19. Huang, S. W. *et al.* The utilization of a commercial soil nucleic acid extraction kit and PCR for the detection of *Clostridium tetanus* and *Clostridium chauvoei* on farms after flooding in Taiwan. *J. Vet. Med. Sci*, **75**, 489–495 (2013).
20. Country epidemiological reports of Rosselkhoz nadzor. <https://fsvps.gov.ru/fsvps/iac/rf/>.
21. Balakrishnan, G., Ravikumar, G., Roy, P. & Purushothaman, V. Isolation and Identification of *Clostridium chauvoei* from Cattle Suffered from Black Quarter. *J. Pure Appl. Microbiol*, **7**, 2447–2449 (2013).
22. Hussain, R. *et al.* Pathological and clinical investigations of an outbreak of Blackleg disease due to *C. chauvoei* in cattle in Punjab, Pakistan. *J. Infect. Dev. Ctries*, **13**, 786–793 (2019).
23. Uzal, F. A. *et al.* Comparative pathogenesis of enteric clostridial infections in humans and animals., **53**, 11–20 (2018).
24. Casagrande, R. A. *et al.* Histopathological, immunohistochemical and biomolecular diagnosis of myocarditis due to *Clostridium chauvoei* in a bovine. *Ciência Rural. St. Maria, Online*, **45**, 1472–1475 (2015).
25. Garofolo, G., Galante, D., Serrecchia, L., Buonavoglia, D. & Fasanella, A. Development of a real time PCR taqman assay based on the TPI gene for simultaneous identification of *Clostridium chauvoei* and *Clostridium septicum*. *J. Microbiol. Methods*, **84**, 307–311 (2011).
26. Pahlow, S. *et al.* Discrimination of clostridium species using a magnetic bead based hybridization assay. in *Biophotonics: Photonic Solutions for Better Health Care I* Vol. 9129 91292J (SPIE, 2014).
27. Seise, B. *et al.* Clostridium spp. discrimination with a simple bead-based fluorescence assay. *Anal. Methods*, **6**, 2943–2949 (2014).
28. Frey, J., Johansson, A., Bürki, S., Vilei, E. M. & Redhead, K. Cytotoxin CctA, a major virulence factor of *Clostridium chauvoei* conferring protective immunity against myonecrosis. *Vaccine*, **30**, 5500–5505 (2012).

29. Gacem, F., Madadi, M. A., Khecha, N. & Bakour, R. Study of Vaccinal Properties of Clostridium chauvoei Strains Isolated During a Blackleg Outbreak in Cattle in Algeria. *Kafkas Univ. Vet. Fak. Derg*, **21**, 825–829 (2015).
30. Rossi, A. *et al.* Temporal evolution of anti-Clostridium antibody responses in sheep after vaccination with polyvalent clostridial vaccines. *Vet. Immunol. Immunopathol*, **202**, 46–51 (2018).
31. Uzal, F. A. Evidence-Based Medicine Concerning Efficacy of Vaccination Against Clostridium chauvoei Infection in Cattle. *Veterinary Clinics of North America - Food Animal Practice*, **28**, 71–77 (2012).
32. Waldner, C. L., Parker, S. & Campbell, J. R. Vaccine usage in western Canadian cow-calf herds. *Can. Vet. J*, **60**, 414–422 (2019).
33. Harvey, J. W. *Veterinary Hematology: A Diagnostic Guide and Color Atlas*(2012).
34. Assis, R. A. *et al.* PCR multiplex para identificação de isolados de Clostridium chauvoei e Clostridium septicum. *Arq. Bras. Med. Veterinária e Zootec*, **60**, 294–298 (2008).

Figures

Figure 1

Animal deaths from 17 March to 04 April 2019

Figure 2

Distinct clinical signs of blackleg: a - oedema in the scapular shoulder joint of the anterior right limb; b - oedema in the abdominal cavity; c - oedema in the back; d - papilloma trauma on the cow muzzle with subsequent oedema and the formation of nodes in the subcutaneous tissue in the area of the right branch of the lower jaw.

Figure 3

Pathological and anatomical changes in cattle with blackleg: a – foamy, bloody liquid from the eyes; b - haemorrhagic-necrotic myositis; c - gross haemorrhage in the heart; d - haemorrhage in the serous membrane of a scar.

Figure 4

Results of laboratory studies: a - bacteria cultured on Kitt-Tarozzi medium; b - basophilic granularity in cow red blood cells.