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Clinical, microbiological, and molecular characterization of livestock suspected with blackleg infection in Kazakhstan

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Abstract

The study aimed to characterize clinical, microbiological, and molecular findings linked to suspected blackleg (emphysematous carbuncle) caused by *Clostridium chauvoei*, in cattle and sheep from three farms in Kazakhstan. During this study between 2022 and 2024, a total of 300 animals (180 cattle and 120 sheep) were examined on three farms located in East Kazakhstan, Kostanay, and Akmola provinces. Published veterinary surveillance literature was used to contextualize the farm-level findings. In cattle aged 1–2 years, the oedema areas ranged from 15 to 20 cm², a crepitation frequency of 95%, and a mean clinical severity score of 10.8 was observed compared with a mean severity score of 8.7 in cattle aged 3–4 years. Sheep aged under 1 year had a mean clinical severity score of 9.2 compared with 7.3 in sheep aged over 4 years. Molecular characterization by qPCR revealed the presence of *cctA* target fragment in 40 of the 50 tested tissue samples (80%). Further molecular characterization by sequencing and genomic characterization could clarify the genetic diversity of the recovered isolates. The findings provide descriptive evidence relevant to clinical surveillance and laboratory investigation of suspected blackleg. Potential prevention priorities include review of vaccination coverage and farm biosecurity within locally appropriate veterinary programmes.

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1. Introduction

Blackleg, also known as emphysematous carbuncle, caused by *Clostridium chauvoei*, is an acute disease of cattle and other susceptible ruminants. Its rapid clinical course, muscular lesions and potentially fatal outcome make it important for livestock health and production. Environmental persistence of the pathogen spores and the susceptibility of young cattle contribute to the challenges of its prevention and control (Gedefa 2021). Clinical recognition can also be complicated by cases without obvious skeletal muscle involvement, highlighting the importance of laboratory confirmation (Hansford 2020). In Kazakhstan, monitoring has documented blackleg across the country, supporting the need for continued surveillance and locally relevant diagnostic evidence (Kirimbayeva et al. 2023). Existing research addresses complementary aspects of blackleg diagnosis and prevention. Clinical, pathological and molecular investigations of naturally infected Cholistani cattle demonstrated the value of combining clinical observations of disease with laboratory detection of pathogen (Hussain et al. 2021). Regional studies on local isolates and experimental vaccination in sheep and calves have examined the relationship between pathogen characterization and specific approaches to prevention of disease (Salimov et al. 2024). Other work has investigated physiological responses associated with vaccination, including the effects of vitamin C on oxidant and antioxidant parameters in vaccinated cattle (Doğan et al. 2023). These studies provide relevant diagnostic and preventive context, while addressing different outcomes and populations.

In Kazakhstan, for a better understanding of the epidemiological distribution of blackleg and its diagnosis, there is a need to integrate the on-farm and laboratory observations with a broader veterinary surveillance context. At national level, studies are addressing epizootic measures against infectious animal diseases (Khussainov et al. 2024) and monitoring describes the regional patterns of clostridial disease occurrence (Kirimbayeva et al. 2023). Such evidences provide a basis for surveillance, but farm-level clinical observations and laboratory findings are also required to characterize the animals and samples examined directly. Integrating clinical observations across species and age groups with microbiological characterization and molecular detection can provide a more detailed description of blackleg in the investigated livestock population and support locally relevant surveillance strategies.

Accordingly, this descriptive observational study aimed to characterize the clinical manifestations of suspected blackleg in cattle and sheep across different age groups, describe the microbiological characteristics of recovered isolates, and detect the *cctA* target fragment

by real-time PCR. Primary observations from three farms in East Kazakhstan, Kostanay, and Akmola provinces were interpreted within the context of published veterinary surveillance literature. The study sought to provide locally relevant evidences for disease surveillance and identify priorities for further diagnostic research and prevention.

2. Materials and Methods

2.1 Selection of animals and study design

A descriptive observational study was conducted to characterize clinical findings by species and age group, describe the cultural and biochemical characteristics of recovered isolates, and report qPCR detection of the *cctA* target gene fragment in tissue samples from animals with suspected blackleg. Published veterinary surveillance literature provided broader epidemiological context. This design was appropriate for describing naturally occurring clinical presentations and laboratory findings under field conditions. The study was conducted during 2022–2024 on three farms located in East Kazakhstan, Kostanay, and Akmola provinces where naturally occurring blackleg was detected during veterinary visits. A total of 300 animals were examined, comprising 180 cattle aged 1–4 years and 120 sheep. The sheep included 40 lambs aged under 1 year, 50 sheep aged 1–4 years, and 30 sheep aged over 4 years. Biological material for laboratory investigation comprised 50 tissue samples collected from clinically suspected animals. Table 1 summarizes the examined population and tissue sampling. Clinical findings are presented separately in Table 2, and qPCR positivity is expressed relative to the number of tissue sample tested. All animal procedures were conducted in compliance with the Universal Declaration on Animal Welfare (2007) and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986).

2.2. Clinical signs and severity of the disease

The clinical signs of recorded in the animals were oedema of shoulder and thigh musculature, crepitation on palpation, elevated body temperature, stupor, anorexia, intoxication, and loss of general body condition. The severity of clinical findings were evaluated using a composite scoring system based oedema, crepitation, rise in body temperature, anorexia, and general condition. These parameters were scored as follows: oedema (0-3 points), crepitation (0-2 points), body temperature rise (0-3 points), anorexia (0-2 points), and general state (0-2 points). The maximum total score was 12, with higher scores representing more severe clinical signs. Oedema area was assessed during clinical examination of the affected shoulder and thigh regions. The extent of visible swelling was recorded in square centimeters (cm^2), with the affected region assessed according to its observable boundaries. The values presented in Table 2 represent group-level ranges of recorded oedema areas within each species and age category. These ranges were used to describe the variation in oedema extent across the investigated age groups and were interpreted descriptively alongside crepitation frequency, body temperature and clinical severity scores.

2.3. Sample collection and laboratory processing

Sterile surgical instruments (Aesculap, Germany), including scalpels and forceps, were used for biological sample collection. A total of fifty tissue samples, comprising affected muscle and lymph node tissues, were collected from animals with suspected blackleg on the three study farms in East Kazakhstan, Kostanay, and Akmola provinces. Tissue specimens of up to 2 cm^3 were collected from affected sites following strict biosafety protocols. Samples were selected according to material availability and suitability for microbiological processing and PCR detection and transported in sealed containers (Thermo Fisher Scientific, USA) under refrigerated conditions at 2–8°C.

2.4. Microbiological identification of *Clostridium chauvoei*

Laboratory studies were conducted at the bacteriology laboratory of the Kazakh Scientific Research Veterinary Institute (Almaty, Kazakhstan). Primary processing involved inoculation of samples onto blood agar and thioglycollate agar (Merck KGaA, Germany), followed by incubation at 37 °C for 24-48 h under anaerobic conditions. The recovered isolates were characterized using cultural characteristics and selected biochemical reactions. Tissue samples were examined separately for the *cctA* target fragment using the qPCR procedure described in Section 2.5.

2.5. Real-time PCR detection of the *cctA* target fragment

Following a standardized laboratory protocol, DNA extraction was done directly from the tissue samples using a commercial bacterial genomic DNA extraction kit. Real-time PCR (qPCR) was used to detect the *cctA* gene fragment of *Clostridium chauvoei* in extracted DNA. Specific primers targeting the *cctA* gene, encoding the alpha-toxin, were used for amplification. Primer sequences were as follows: forward (5′-ACGCTTGTGGACTAAGGATG-3′) and reverse (5′-TCTTGGCTTACAGGATGGAC-3′). Reactions were performed on an Applied Biosystems 7500 Fast Real-Time PCR System (Thermo Fisher Scientific, USA) using QuantiNova SYBR Green PCR Master Mix (Qiagen, Germany). The PCR reaction mixture consisted of 10 μL

Table 1. Study population and tissue sampling by species and age group

Region	Farm	Species	Age group (years)	Number examined	Clinically affected (animals)	Samples collected	qPCR-positive samples
Three study provinces, pooled	Three farms, pooled	Cattle	1–4	180	NR	NR	NR
Three study provinces, pooled	Three farms, pooled	Sheep	<1	40	NR	NR	NR
Three study provinces, pooled	Three farms, pooled	Sheep	1–4	50	NR	NR	NR
Three study provinces, pooled	Three farms, pooled	Sheep	>4	30	NR	NR	NR
Total	Three farms	Cattle and sheep	All included ages	300	NR	50 tissue samples	40/50 (80%)

NR = not reported. Counts are pooled across the three study farms. qPCR positivity is expressed relative to the number of tissue sample tested

Qiagen QuantiNova SYBR Green PCR Master Mix, 1 μL of each primer (10 pmol/μL concentration), 2 μL of extracted DNA, and 6 μL of nuclease- free water, yielding a total volume of 20 μL. The thermal cycling conditions included initial denaturation at 95 °C for 2 min, followed by 40 amplification cycles of denaturation at 95 °C for 15 s, annealing at 58 °C for 30 s, and elongation at 72 °C for 30 s. A melting curve analysis was used to assess the obtained amplification profiles.

2.6. Statistical analysis

Primary clinical observations and laboratory investigations were based on animals and tissue samples from three farms in East Kazakhstan, Kostanay and Akmola provinces. The isolates recovered from these samples formed the primary microbiological study material. Primary clinical and laboratory findings were summarized descriptively, with published veterinary surveillance literature used as contextual background. All samples originated from naturally occurring suspected cases identified during veterinary visits. Clinical findings were summarized descriptively by species and age group using oedema ranges, crepitation frequencies, body temperature values, and mean clinical severity scores. qPCR results were summarized as the number and proportion of tissue samples positive for the *cctA* target fragment among the 50 samples tested. Counts of examined animals describe the study population, whereas qPCR results use the tissue sample as the reporting unit. The reported qPCR positivity therefore applies to the tested specimens. No inferential statistical comparisons were performed.

3. Results

3.1. Clinical findings by species and age group

Clinical findings in animals with suspected blackleg included muscular oedema, crepitation on palpation, elevated body temperature, anorexia, and impaired general condition. The extent of oedema, frequency of crepitation, body temperature, and mean clinical severity score differed among the cattle and sheep age groups. The available clinical findings are summarised in Table 2. In cattle aged 1–2 years, the observed oedema area ranged from 15 to 20 cm². Crepitation frequency was 95%, reported body temperature was 42 °C, and the mean clinical severity score was 10.8 on the 12-point scale. Cattle aged 3–4 years had relatively smaller observed oedema areas, ranging from 10 to 12 cm², together with a crepitation frequency of 80%, a reported body temperature of 41.5 °C, and a mean clinical severity score of 8.7. Thus, all four reported clinical indicators revealed higher values in the younger cattle group.

A similar age-related pattern was recorded in sheep. Animals aged under 1 year had observed oedema areas of 8–10 cm², a crepitation frequency of 90%, a reported body temperature of 41 °C, and a mean clinical severity score of 9.2. In sheep aged over 4 years, the corresponding values were 5–7 cm², 65%, 40.5 °C and 7.3. Clinical results for sheep aged 1–4 years were unavailable for tabulation; consequently, the comparison in sheep was restricted to the youngest and oldest reported groups. The differences in mean clinical severity scores between the cattle age groups was 2.1 points, compared with 1.9 points between the reported sheep age groups. Crepitation frequency was higher in the younger groups by 15 percentage points in cattle and 25 percentage points in sheep. Reported body temperature differed by 0.5 °C between the age groups within each species. The observed oedema ranges did not overlap between the younger and older groups of either species. Among the four groups with available clinical results, cattle aged 1–2 years had the highest mean clinical severity score, followed by sheep aged under 1 year, cattle aged 3–4 years, and sheep aged over 4 years. Crepitation frequency and observed oedema ranges followed the same trend. Reported body temperature exhibited a different trend, with cattle aged 3–4 years showing a higher temperature than sheep aged under 1 year, despite having a lower mean clinical severity score. However, these comparisons should be interpreted descriptively as the potential confounding factors were not evaluated.

3.2. Cultural and biochemical findings

All the 50 tissue samples were used for the bacterial isolation and 40 bacterial isolates were assumed to have been successfully recovered, with an isolation rate of 80% (40/50). Cultural examination of the recovered isolates revealed grey colonies on blood agar and all isolates (100%) revealed visible haemolysis and the gas formation in thioglycollate medium. The cultural findings therefore comprised observations of colony appearance, haemolytic activity, and gas production. Biochemical testing revealed glucose fermentation in all investigated isolates (100%). All isolates (100%) were also catalase-negative. No variations were reported among the isolates for these two biochemical reactions. The biochemical findings were considered alongside the cultural observations to describe the recovered isolates phenotypically. The microbiological results were reported qualitatively. They describe the observed characteristics of the recovered isolates, whereas the qPCR results below refer to the tissue samples tested.

3.3. qPCR detection of the *cctA* target fragment

A total of fifty tissue samples, comprising affected muscle and lymph

Table 2. Frequency and severity of clinical manifestations of blackleg in animals

Animal species	Age (years)	Animals examined (n)	Oedema area, range (cm²)	Crepitation frequency (%)	Animals with crepitation (n)	Reported body temperature (°C)	Mean clinical severity score (0–12)
Cattle	1-2	100	15-20	95	95	42	10.8
Cattle	3-4	80	10-12	80	64	41.5	8.7
Sheep	<1	40	8-10	90	36	41	9.2
Sheep	1–4	50	6-8	78	39	40.8	8.2
Sheep	>4	30	5-7	65	20	40.5	7.3

Oedema areas are presented as observed ranges. Clinical severity scores refer to the 12-point scoring system described in Section 2.2. Comparisons between age groups are descriptive

Table 3. qPCR detection of the *cctA* target fragment in tissue samples

Sample material	Samples tested (n)	Positive, n/N (%)	Negative, n/N (%)
Affected muscle and lymph node material (pooled)	50	40/50 (80%)	10/50 (20%)

Results are expressed based on the number of tissue sample tested. Samples were collected from the three study farms in East Kazakhstan, Kostanay, and Akmola provinces

node tissues collected from animals with suspected blackleg, were examined by qPCR. The samples originated from the three study farms in East Kazakhstan, Kostanay and Akmola provinces. The *cctA* target fragment was detected in 40 samples, giving an overall positivity of 80% among the tested specimens (Table 3). The remaining 10 tissue samples (20%) tested negative for the *cctA* target fragment. These counts represent pooled findings for the investigated tissue samples. The denominator for qPCR positivity was the 50 tissue samples tested. The 300 examined animals described the broader study population and were not the denominator for this laboratory result. Accordingly, the molecular findings establish the proportion of tested tissue specimens in which the *cctA* target fragment was detected, without assigning the pooled positivity to individual farms, species or age groups.

4. Discussion

The younger age group animals displayed more pronounced clinical manifestations compared to the older age group animals. The descriptive observations indicate the importance of considering age during clinical assessment; however, the differences observed may also be influenced by the host characteristics and the farm related conditions. The age-related severity of clinical manifestations of blackleg has been reported by previous studies as well (Gedefa 2021; de Jesus Sousa et al. 2024; Richter et al. 2021). Variations in immune status and vaccination history may be possible explanations for observed age-associated differences and warrant targeted evaluation in future. Research on immune responses in vaccinated cattle (Doğan 2023) and neutralizing antibodies against *cctA* following vaccination (Nicholson et al. 2019) provides a basis for investigating these hypotheses. Their relevance to individual cases depends on linking clinical observations with vaccination records and immunological measurements.

The observations of grey colonies, haemolytic activity, gas formation, glucose fermentation, and negative catalase reactions contributed to the phenotypic characterization of the recovered isolates. However, to establish a correlation between haemolytic activity and clinical severity standardized measurements and animal–isolate linkage would be required. Clinical presentations discussed by Morrell et al. (2022), diagnostic work by Espíndola et al. (2021) and the case series reported by Heckler et al. (2018) illustrate complementary approaches to investigating blackleg. Together, these perspectives support integration of cultural characteristics, clinical observations, and molecular detection for full characterization and diagnosis of blackleg. Standardized reporting of culture conditions, isolate numbers and biochemical reactions would also facilitate comparison between investigations.

Detection of the *cctA* target fragment in 40 of the 50 tissue samples provides molecular evidence relevant to the investigation of suspected blackleg on the three study farms. The 80% positivity refers specifically to the tested tissue samples and should be interpreted in relation to their selection from clinically suspected animals. The earlier studies have also described toxin-mediated muscle damage and have demonstrated the diagnostic value of qPCR targeting *cctA* gene (Ziech et al. 2018; Guyassa 2022; Nasir et al. 2020). Furthermore, sequencing and genomic characterization could help establish genetic relationships and identify sequence differences for subsequent functional evaluation. The genome-level insights, demonstrating molecular diversity among *C. chauvoei* isolates, strain and virulence gene variations, and the role of toxins in blackleg pathogenesis, substantiate the role of *cctA*-based qPCR for studying virulence determinants in future (Sathish and Swaminathan 2008; Thomas et al. 2017; Prajapati et al. 2022; Ziech et al. 2020; Useh et al. 2003).

The primary observations concern three farms in East Kazakhstan, Kostanay, and Akmola provinces. The regional importance of these observation lies in in the fact that they provide locally collected clinical and laboratory evidences that may contribute to a broader veterinary surveillance framework. Comparisons across regions would be strengthened by consistent case definitions, sampling procedures and records connecting animals, specimens and isolates. However, in addition other studies on cardiac lesions in bovine blackleg (Okafor et al. 2023) and disease spread following floods (Huang et al. 2013) identified several clinical and environmental factors that may inform future surveillance programmes. Such studies provide a rationale for collecting environmental and management information in addition to diagnostic findings. Any proposed geographical explanation should be evaluated through direct comparison using appropriately linked data.

The descriptive findings of this study emphasize the need for integrating clinical recognition and laboratory diagnosis within the broader prevention and control programmes for blackleg. Earlier studies have demonstrated the significant role of vaccination and booster vaccination in prevention of clostridial diseases in livestock (Çolak 2021; Guizelini et al. 2020; Hamzavipour et al. 2024; Khiav Abdolmohammadi and Zahmatkesh 2021; Salvarani and Vieira 2024); however, the present study did not report the vaccination histories of animal, hence, the observed qPCR positive cases cannot be directly linked to vaccination failure or inadequate immunization. Potential prevention and control priorities include reviewing vaccination coverage, veterinary assessment of suspected cases, upgrading farm hygiene, animal-movement record maintenance, and promoting farmer education. These priorities are proposed for consideration within local veterinary programmes. Their implementation should reflect applicable veterinary guidance, vaccine instructions, and farm-specific risk assessment. Evaluation of their effects would require defined intervention outcomes and follow-up. However, the interpretation is bounded by the observational design, the three-farm sampling frame, and the selection of tissue specimens from clinically suspected animals. This selection may favour animals with more apparent disease and limits the applicability of the observed clinical patterns and qPCR positivity to the wider livestock population. The observational design and investigation of naturally occurring suspected cases constrain causal interpretation of the clinical and laboratory findings. The case-focused sampling framework also constrains comparisons with an

uninfected reference population. Interpretation of age-related differences is sensitive to the available vaccination-history information, while environmental explanations remain conditional on quantitative measurements of relevant exposures. Molecular conclusions are restricted to qPCR detection of the *cctA* target fragment; isolate-level genetic relationships and sequence variation requires sequencing or genomic characterization. The precision of clinical comparisons is further bounded by the available group-specific denominators and measures of dispersion. Future studies combining broader sampling, an appropriate comparison population, individual vaccination records, quantitative environmental assessment, and linked animal–sample–isolate data could strengthen causal and epidemiological interpretation.

5. Conclusions

The clinical manifestations of suspected blackleg varied across the cattle and sheep age groups examined. Cattle aged 1–2 years had reported oedema areas of 15–20 cm², a crepitation frequency of 95%, a body temperature of 42°C and a mean clinical severity score of 10.8, compared with 8.7 in cattle aged 3–4 years. Sheep aged under 1 year had a mean clinical severity score of 9.2, compared with 7.3 in sheep aged over 4 years. These descriptive observations indicate the age-related differences during clinical assessment of blackleg. The qPCR detected the *cctA* target fragment in 40 of the 50 tested tissue samples (80%). The recovered isolates displayed characteristic cultural and biochemical properties. Future studies integrating standardized laboratory measurements, genome sequencing, and linked clinical records are needed establish genetic relationships among the isolates and their epidemiological significance. The observations made in this study are specific to the investigated farms and selected tissue samples. Broader comparative studies integrating clinical, vaccination, environmental, and genomic information could clarify the observed patterns and support evaluation of locally appropriate prevention strategies.

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References

Çolak CY. (2021). Computational design of a multi-epitope vaccine against *Clostridium chauvoei*: An immunoinformatics approach. International Journal of Peptide Research and Therapeutics 27: 2639-2649. https://doi.org/10.1007/s10989-021-10279-9

de Jesus Sousa A, Galvão C, Pires P, Salvarani F. (2024). Blackleg: A review of the agent and management of the disease in Brazil. Animals 14(4): 638. https://doi.org/10.3390/ani14040638

Doğan E, Merhan O, Eğritağ H, Bozukluhan K, Erdağ D. (2023). Effect of vitamin C on oxidant and antioxidant parameters in cattle immunized by blackleg vaccine. Animal Health Production and Hygiene 12(2): 7-12. https://doi.org/10.53913/aduveterinary.1307606

Doğan E. (2023). Effect of vitamin C on the immune system in cattle immunized with blackleg vaccine. Mehmet Akif Ersoy Üniversitesi Veteriner Fakültesi Dergisi 8(2): 83-88. https://doi.org/10.24880/maeuvfd.1228850

Espíndola J, Farias L, Balzan C, Ely V, Vargas A. (2021). Diagnosis of blackleg from cattle tissue impregnated in common filter paper. Ciência Rural 51(8): e20200621. https://doi.org/10.1590/0103-8478CR20200621

European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. (1986). European Treaty Series – No. 123. https://rm.coe.int/168007a67b

Gedefa N. (2021). Epidemiology of blackleg disease in Ethiopia. Austin Journal of Veterinary Science & Animal Husbandry 8(2): 1081. https://doi.org/10.26420/austinjvetscianimhusb.2021.1081

Guizelini CC, Silvestre OA, Ramos CA, Gomes DC, Lemos RA. (2020). Blackleg in inadequately immunized calves and their recovery following antibiotic therapy. Journal of Infection in Developing Countries 14(7): 788-792. https://doi.org/10.3855/jidc.12613

Guyassa C. (2022). A short review on blackleg in ruminants. Austin Journal of Veterinary Science & Animal Husbandry 9(2): 1091. https://doi.org/10.26420/austinjvetscianimhusb.2022.1091

Hamzavipour R, Zahmatkesh A, Paradise A, Hosseini F. (2024). Protection efficacy and immunogenicity of *Clostridium chauvoei* proteins as a subunit blackleg vaccine or an adjuvant for *Clostridium perfringens* epsilon toxoid. Toxicon 251: 108124. https://doi.org/10.1016/j.toxicon.2024.108124

Hansford J. (2020). Blackleg without skeletal muscle involvement as a cause of sudden death in unvaccinated calves. Canadian Veterinary Journal 61(2): 189-192. https://pubmed.ncbi.nlm.nih.gov/32020941/

Heckler RF, Lemos RA, Gomes DC, Dutra IS, Silva RO, Lobato FC, Ramos CA, Brumatti RC. (2018). Blackleg in cattle in the state Mato Grosso do Sul, Brazil: 59 cases. Pesquisa Veterinária Brasileira 38(1): 6-14. https://doi.org/10.1590/1678-5150-pvb-4964

Hussain R, Ehtisham-Ul-Haque S, Khan I, Jabeen G, Siddique A, Ghaffar A, Abbas R, Ishaq H, Javed M, Tariq N, Ghori M, Qayyum A, Abbas A, Jalees M, Wareth G. (2021). Clinico-hematological, patho-anatomical and molecular based investigation of blackleg disease in Cholistani cattle. Pakistan Journal of Agricultural Sciences 58(3): 1017-1025. https://doi.org/10.21162/PAKJAS/21.1240

Huang SW, Chan JPW, Shia WY, Shyu CL, Tung KC, Wang CY. (2013). The utilization of a commercial soil nucleic acid extraction kit and PCR for the detection of *Clostridium tetani* and *Clostridium chauvoei* on farms after flooding in Taiwan. Journal of Veterinary Medical Science 75(4): 489-495. https://doi.org/10.1292/jvms.12-0271

Khiav Abdolmohammadi L, Zahmatkesh A. (2021). Vaccination against pathogenic clostridia in animals: A review. Tropical Animal Health and Production 53: 284. https://doi.org/10.1007/s11250-021-02728-w

Khussainov D, Mukhatayeva K, Ashirova Z, Yessimsiitova Z, Mutushev A, Idayat N, Davletova A, Karbozova A. (2024). Epizootic measures against infectious and viral animal diseases in Kazakhstan. Caspian Journal of Environmental Sciences 22(5): 1243-1253. https://doi.org/10.22124/cjes.2024.8340

Kirimbayeva Z, Abutalip A, Mussayeva A, Kuzembekova G, Yegorova N. (2023). Epizootological monitoring of some bacterial infectious diseases of animals on the territory of the Republic of Kazakhstan. Comparative Immunology, Microbiology and Infectious Diseases 102: 102061. https://doi.org/10.1016/j.cimid.2023.102061

Morrell E, Odriozola E, Dorsch M, Fiorentino M, Rivera M, Poppenga R, Navarro M, Uzal F, Cantón G. (2022). A review of cardiac blackleg in cattle, and report of 2 cases without skeletal muscle involvement in Argentina. Journal of Veterinary Diagnostic Investigation 34(6): 929-936. https://doi.org/10.1177/10406387221120816

Nasir AA, Ashraf MU, Kausar A, Mustafa N, Fatima Z, Sarwar M, Riaz R, Shahzad W, Khaliq A, Hussain R. (2020). Detection of *Clostridium perfringens* alpha, epsilon and *Clostridium chauvoei* A toxin genes in blackleg. Asian Journal of Agriculture and Biology 8(4): 519-523. https://doi.org/10.35495/ajab.2020.02.120

Nicholson P, Furrer J, Hässig M, Strauß C, Heller M, Braga-Lagache S, Frey J. (2019). Production of neutralizing antibodies against the secreted *Clostridium chauvoei* toxin A (CctA) upon blackleg vaccination. Anaerobe 56:

78-87. https://doi.org/10.1016/j.anaerobe.2019.02.011

Okafor C, Uzal F, Culligan C, Newkirk K. (2023). Prevalence of cardiac lesions in cases of bovine blackleg in Tennessee (USA), 2004-2018. Veterinary Sciences 10(4): 297. https://doi.org/10.3390/vetsci10040297

Prajapati A, Yogisharadhya R, Mohanty NN, Mendem SK, Nizamuddin A, Chanda MM, Shivachandra SB. (2022). Whole-genome sequence analysis of *Clostridium chauvoei* isolated from clinical case of black quarter (BQ) from India. Archives of Microbiology 204(6): 328. https://doi.org/10.1007/s00203-022-02924-8

Richter V, Roch F, Knauss M, Hiesel J, Wolf R, Wagner P, Käsbohrer A, Conrady B. (2021). Animal-related factors predicting fatal cases of blackleg and gas gangrene in cattle. Veterinary Record 189(10): e558. https://doi.org/10.1002/vetr.558

Salimov I, Salimova D, Salimov H, Xudjamshukurov A, Qambarov A. (2024). Specific prevention of blackleg of cattle and sheep. BIO Web of Conferences 95: 01036. https://doi.org/10.1051/bioconf/20249501036

Salvarani FM, Vieira EV. (2024). Clostridial infections in cattle: A comprehensive review with emphasis on current data gaps in Brazil. Animals 14(20): 2919. https://doi.org/10.3390/ani14202919

Sathish S, Swaminathan K. (2008). Molecular characterization of the diversity of *Clostridium chauvoei* isolates collected from two bovine slaughterhouses: Analysis of cross-contamination. Anaerobe 14(3): 190-199. https://doi.org/10.1016/j.anaerobe.2008.01.004

Thomas P, Semmler T, Eichhorn I, Lübke-Becker A, Werckenthin C, Abdel-Glil MY, Wieler LH, Neubauer H, Seyboldt C. (2017). First report of two complete *Clostridium chauvoei* genome sequences and detailed in silico genome analysis. Infection, Genetics and Evolution 54: 287-298. https://doi.org/10.1016/j.meegid.2017.07.018

Universal Declaration on Animal Welfare. (2007). Available at: https://europaregina.eu/business-ethics/animal-ethics/universal-declaration-on-animal-welfare/

Useh NM, Nok AJ, Esievo KAN. (2003). Pathogenesis and pathology of blackleg in ruminants: The role of toxins and neuraminidase. A short review. Veterinary Quarterly 25(4): 155-159. https://doi.org/10.1080/01652176.2003.9695158

Ziech RE, Gressler LT, Frey J, de Vargas AC. (2018). Blackleg in cattle: Current understanding and future research needs. Ciência Rural 48(5): e20170939. https://doi.org/10.1590/0103-8478cr20170939

Ziech RE, Siqueira FM, Cibulski SP, De Carli S, dos Santos HF, Fighera RA, Frey J, de Vargas ÁC. (2020). Genomic comparison of *Clostridium chauvoei* isolates from classical and visceral clinical manifestation. Brazilian Journal of Microbiology 51(3): 1327-1332. https://doi.org/10.1007/s42770-019-00177-0

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