Diagnostic and therapeutic strategy for *Clostridium perfringens* infection in postpartum dairy cows: a report of 14 cases

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**ABSTRACT**

*Clostridium perfringens* type A infections are economically devastating to dairy farms. In spite of low morbidity, mortality is close to 100%. Although this disease has frequently been reported in calves, it is rarely seen in adult cattle, especially postpartum dairy cows. Sudden onset of symptoms, rapidly followed by death, was successively recorded in 14 postpartum dairy cows. Veterinary treatment was ineffective. The 14 cows fell ill after parturition, either within 24 h (fast invasion) or within 2–3 days (slow invasion). All the cows died shortly after symptom onset. Comprehensive analysis of epidemiology, clinical manifestation, autopsy results, and laboratory tests confirmed the 14 cases as most acute and acute *C. perfringens* type A infections. After the enactment of appropriate preventive and treatment measures, in conjunction with improved feeding management, no further symptoms of infection were observed in the postpartum cows. Our results demonstrated that autopsies and laboratory tests are required for the definitive diagnosis of this disease and highlighted importance of feeding management during the early perinatal period to prevent postpartum diseases in cows. This work also provides a reference for clinical veterinary diagnosis and the treatment of this disease, so as to reduce the economic losses to dairy farms.

**Introduction**

*Clostridium perfringens*, which is part of the normal gastrointestinal microbiota of humans and animals, is ubiquitous in nature; this bacterium is widespread in septic tanks, soil, sewage sludge, wastewater, food, and other sources (Goossens et al. 2017; Yanagimoto and Haramoto 2021; Johnston et al. 2022). *C. perfringens* is Gram-positive, spore-forming, anaerobic bacterium that can cause a variety of severe diseases, such as gas gangrene in humans and enterotoxaemia in animals (Goossens et al. 2016; Rahimoon et al. 2021; Takehara et al. 2021; Zhang et al. 2021). *C. perfringens* isolates have been classified into five toxin types (A, B, C, D, and E) (Lacey et al. 2019). This bacterium produces 15 strong exotoxins; of these, the alpha, beta, epsilon and iota toxin have the strongest pathogenicity (Sepehrifar et al. 2021). These toxins cause various types of tissue damage, including hemolysis, intestinal mucosal necrosis, and neurotoxicity (Navarro et al. 2018). *C. perfringens* infections are often deadly, leading to great economic losses to the dairy farming industry (Sun et al. 2018). *C. perfringens* type A infections represent an economically threat to dairy farms: although morbidity is rather low, mortality is close to 100%. Previous studies have shown that *C. perfringens* type A infections are frequent in calves, but this infection is rarely reported in adult cows, especially in postpartum dairy cows. In this study, we report the diagnosis and treatment of acute *C. perfringens* infections in postpartum dairy cows. This work provides a reference for clinical veterinary diagnostic and therapeutic strategies in response to *C. perfringens* infection, so as to reduce the economic losses to dairy farms.

**Case presentation**

Between late July and early August 2021, 14 postpartum dairy cows at a dairy farm in Heilongjiang Province, China, died after the sudden onset of symptoms. Veterinary treatment was ineffective. The 14 cows became ill after parturition, some within 24 h (fast invasion) and some within 2–3 days (slow invasion). All of the cows died shortly after the onset of symptoms. All the cows were on concrete floors with wood shavings and dried rice husk bedding. The cows were fed TMR (total mixed...
ration), and all feed were ad libitum. The clinical characteristics of the cases (Additional file 1) were both neurological and respiratory. Symptoms appeared rapidly and included lying on the ground, moaning, tooth grinding, muscle shaking, convulsions, opisthotonos, bleating, red foamy liquid discharge from the mouth and nose cavity (Figure 1A), a small volume of brown, soy-sauce-like urine discharged from the vulva (Figure 1B), lateral recumbency with paddling of limbs, and one or two howls followed by sudden death. After death, the abdomen bulged, the tongue protruded out of mouth, conjunctival hyperemia was present, and anal eversion was observed (Figure 1C). This disease only affected soon-to-be postpartum dairy cows (i.e. in the late perinatal period), the morbidity is 12.17% in soon-to-be postpartum dairy cows and the mortality is 100%, but there is no illness or death was recorded in any other individuals of the herd. Cows at this dairy farm were not inoculated against any diseases besides foot-and-mouth.

**Autopsies**

During autopsies, several histopathological abnormalities were observed, including pulmonary congestion, emphysema, widening of interlobular septum, foamy liquid on the section, bleeding and hyperemia in the pulmonary vessels, large volumes of foamy mucus in the larynges and tracheas, severe shedding of the mucous membrane in the stomach, viscous dark-red liquid in the small intestine, and many hemorrhagic spots in the small intestinal mucosa (Figure 1D–I). In addition, some individuals presented with bleeding mesenteric lymph nodes, pericardial effusion, and/or bleeding spots on the surfaces of the heart and epicardium, and the section of the mesenteric lymph nodes was puce. No obvious pathological abnormalities were observed in the liver or spleen of any affected cows.

**Laboratory analyses**

During the autopsies, samples of the heart, liver, spleen, lungs, small intestine (and contents), and trachea were collected for laboratory analysis. Tissue samples were stored in a freezer at −20°C until analysis. The lung, liver, small intestine, small-intestine content, and tracheal secretion samples from the dead cows, as well as blood smear samples taken before death, were Gram stained. Large, Gram-positive bacilli were found in the small intestines and the small-intestine contents. These bacilli were obtusely rounded at both ends and were observed either singly or in groups of two to three connected end-to-end (Figure 2A). No such bacilli were found in the lung, liver, or blood, but abundant leukocytes and granulocytes were observed in these samples. Given the clinical symptoms and the autopsy results, primers for C. perfringens alpha toxin (CPA), bovine rotavirus (BRV), bovine coronavirus (BCV), bovine viral diarrhea virus (BVDV), and bovine adenovirus (BADV) were designed based on the corresponding sequences in GenBank (Table 1). The contents of the small intestines were amplified with qRT-PCR using these primers, following the manufacturer’s instructions (Beijing ComWin Biotech Co., Ltd.,...
Beijing, China). A single band was amplified, consistent with the target fragment of *C. perfringens* type A (Figure 2B).

**Prevention and therapeutic strategy**

Based on epidemiological evidence, clinical manifestations, autopsies, and the laboratory results, the 14 cases were confirmed as acute or most acute *C. perfringens* type A infections. After this diagnosis, prevention and therapeutic strategies were employed, including the emergency inoculation of the dairy cow herd with a vaccine against bovine *C. perfringens* and the adjustment of the calcium-to-phosphorus ratio in the feed. Sick cows were injected with hyperimmune serum against bovine *C. perfringens* and simultaneously received intramuscular injections of penicillin and streptomycin. After the enactment of these preventative and treatment measures, no further instances of sickness in the postpartum cows were recorded.

**Discussion**

*C. perfringens* type A has been implicated in sudden deaths (Zhu et al. 2017); these infections can occur in any season. *C. perfringens* type A is the type most frequently isolated from samples of human, animal, or environmental origin (Qing and Guisheng 2019). The duration of disease varies, from a few minutes or a few hours to three or four days or even longer (Guo 2019). This disease occurs sporadically rather than in concentrated outbreaks (Zheng et al. 2010). The mortality rate of dairy cattle infected with *C. perfringens* type A is 70%–100% (Guo 2019). Most infected cows are high-yield and in good to excellent health. *C. perfringens* type A produces CPA and several other toxins, including CPE and CPB2 (Uzal et al. 2018). Information about pathogenesis of type A enteric infections in ruminants is minimal and often contradictory, but it is generally assumed that most clinical signs and lesions are due to the effects of CPA. Based on clinical symptoms, *C. perfringens* infections are divided into three categories: most acute, acute, and subacute. Here, 11 of the 14 cases had symptoms consistent with the most acute infection, while the symptoms of the remaining three cases were consistent with the acute infection. No cases had subacute symptoms.

The main pathological features of typical *C. perfringens* infections include hemorrhages in the systemic parenchyma and small intestine, as well as petechiae on the heart surface, pericardial effusion, and pulmonary emphysema and/or petechiae. Humans or animals infected with *C. perfringens* also present purple-black livers, with surface petechiae; many petechiae in the intestinal mucosa; dark-red, viscous liquid in the intestine; and swollen, hemorrhagic lymph nodes that are dark-brown in section. Here, the pathological features of the lung, stomach, small intestine, and mesenteric lymph nodes noted in the autopsy were similar to the symptoms typical of *C. perfringens* infection. However, a few obvious differences to typical *C. perfringens* infections were identified: First, only a few of the dead cows presented with pericardial effusion or myocardial hemorrhage. Second, although the livers of the affected cows were purple-black, there were no obvious hepatic petechiae. Finally, while the autopsy revealed severe damage to the lungs, these injuries were similar to the effects of mycoplasma pneumonia, and adhesion between the

**Table 1. Primers sequences used for multi-qRT-PCR.**

| Gene     | Upper primer              | Lower primer                          | Length |
|----------|---------------------------|---------------------------------------|--------|
| CPA      | 5’-GCTTGTTTACTGCGTGGTTGA-3’ | 5’-CTCTGATACTACGTGTAAG-3’             | 402 bp |
| BRV      | 5’-GCTGCAGGAGCTACATGTTGA-3’ | 5’-CTACGCTGGTATCTTGTGAG-3’            | 183 bp |
| BCV      | 5’-GTGGCGTAGATTITCCATGAT-3’ | 5’-ATGCTGTTAACGCGGACAG-3’             | 547 bp |
| BVDV     | 5’-ATGCCTAGTGACGACTGCA-3’  | 5’-TCAACTCAGTGATCGACTC-3’             | 288 bp |
| BADV     | 5’-CTTTTGGAAGCATTTTCTC-3’  | 5’-TGAGAACGAGCATTGATC-3’              | 376 bp |

*C**: *C. perfringens* alpha toxin; BRV: bovine rotavirus; BCV: bovine coronavirus; BVDV: bovine viral diarrhea virus; BADV: bovine adenovirus

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*Figure 2*. The results of the laboratory analyses. A Gram staining of the contents of the small intestine (20×), showing Gram-positive bacilli, obtusely rounded at both ends, appearing singly or in groups of two or three connected end-to-end. B Agarose gel electrophoresis of the qRT-PCR products amplified from the intestinal contents. Lane #1: *C. perfringens* alpha toxin, showing an amplification band at 324-bp, consistent with *C. perfringens* type A. Lane #2: Bovine rotavirus, showing that no DNA was amplified by the qRT-PCR. Lane #3: Bovine coronavirus, showing that no DNA was amplified by the qRT-PCR. Lane #4: Bovine viral diarrhea virus, showing that no DNA was amplified by the qRT-PCR. Lane #5. Bovine adenovirus, showing that no DNA was amplified by the qRT-PCR. Lane M: DNA maker (DL2000).
pleura and lungs was observed in the first few cows to die. These pathological inconsistencies with *C. perfringens* infection affected our initial diagnosis. Based on pulmonary pathology, we diagnosed infectious mycoplasma pneumonia in the first few dead cows. Indeed, due to the hot weather at the time of death, the rapid expansion of corpse abdomen could be considered a natural phenomenon. For this reason, we did not initially consider *C. perfringens* infection. This initial diagnosis error delayed the institution of appropriate prevention and treatment measures for *C. perfringens* infection. In the period of time following the incorrect diagnosis, postpartum dairy cows continued to die suddenly. Finally, these cases were diagnosed as most acute/acute *C. perfringens* type A infection following comprehensive analysis. Because *C. perfringens* type A is a commensal bacterium, it is not possible to detect and diagnose these infections using a single method (e.g., the identification of the alpha toxin in the gastrointestinal tract). Accurate diagnosis can only be made by comprehensively analyzing epidemiology, clinical symptoms, autopsy, bacterial culture, and laboratory tests. In addition, it is worth noting that animal cadavers do not always exhibit all of the pathological features of given disease at autopsy; indeed, only one or two of the main pathological characters are typically exhibited in most cases. Therefore, we cannot mechanically expect textbook diagnostic features; our misdiagnosis of the first few cases reported here is a tragic lesson.

The 14 cases of *C. perfringens* type A infection reported herein occurred only in dairy cows shortly after parturition, and no other cows were affected. This infection may have been restricted to postpartum cows due to the dramatic changes in endocrine function peri- and postpartum, in conjunction with the strain of parturition and the stimulation of lactation. These physiological stressors may have decreased immune resistance or caused imbalances in the intestinal flora. Indeed, the body undergoes a comprehensive adaptive change, which include the endocrine and immune systems, to meet the challenges of parturition and lactation (Ceciliani et al. 2018; Velázquez et al. 2019). In addition, the abrupt change in diet after parturition (i.e., the shift from high fibre roughage in the dry milk period to high-precision carbohydrate-heavy feed postpartum) provided suitable conditions for the survival and reproduction of *C. perfringens*. That is, a sudden increase in dietary carbohydrate has been implicated in *C. perfringens* type A infections (Hou et al. 2022), possibly because undigested carbohydrates in the feed enter the small intestine, creating an imbalance in the microbial flora and enabling the proliferation of *C. perfringens* (Zebeli et al. 2015; Prescott et al. 2016; Wankhade et al. 2017). Alternatively, environmental conditions may have driven the *C. perfringens* type A infections examined here. July and August 2020 were very hot. The parturition barn at the farm in question is densely packed and ventilation is poor, which may have led to severe heat stress in the postpartum dairy cows. In postpartum dairy cows, heat stress reduces voluntary feed intake (VFI), leading to metabolic and nutritional disorders, which exacerbate the negative energy balance postpartum and decrease immunity; as a result, susceptibility to disease may increase (De Rensis et al. 2017). In conjunction with these stressors, lactation pressure may have weakened neutrophil function, lessening the immune response and leaving the cow unable to resist toxin invasion. These four factors may have been the main causes of *C. perfringens* infections in the postpartum dairy cows.

The key aspect of the control strategy for this disease is prevention. This is because bovine *C. perfringens* infections are urgent, rapid-developing, critical diseases with high mortality rates; here, the cows typically died before they could be treated. Regular vaccinations effectively prevent the *C. perfringens* infections, especially in perinatal cows. Here, after were confirmed our diagnosis, a series of comprehensive prevention and control measures were enacted, including emergency vaccinations, adjustment of the calcium-to-phosphorus ratio in the feed, reducing the ratio of postpartum high-precision materials, providing high-quality hay, reducing the feeding density of the cow herd, improving ventilation, and providing spray cooling. These measures adequately controlled the disease, and no additional infections were recorded.

The cases described in this report were confirmed to be most acute and acute *C. perfringens* type A infections based on epidemiology, clinical signs, autopsy, and laboratory tests; the mortality rate after infection was 100%. While the timing of symptom onset varied among cases in previous reports, this disease often occurred in calves in excellent health, often without premonitory signs of illness (Liu et al. 2019). *C. perfringens* type A infections are less frequently reported in adult cows, especially immediately postpartum. Here, we report cases of enterotoxaemia in which the key clinical symptoms (twitching and red foamy liquid discharge from the mouth and nasal cavity) were observed 30 min after the cow suddenly collapsed. Although primary care was effective in those cases, the cows died 24 h after parturition. To our knowledge, these cases are the most acute onset of enterotoxaemia ever reported in dairy cows. Therefore, the results of this case report will act as a reference for clinical veterinarians diagnosing and treating *C. perfringens* type A infections in postpartum cows, reducing the economic losses of dairy farms.

**Conclusions**

This report on the diagnosis and treatment of 14 cases shows that comprehensive assessments, including epidemiology, clinical symptoms, autopsy, and laboratory tests, are necessary for definitive disease diagnoses. Laboratory tests were particularly important. Our results highlighted the importance of feeding management in the early perinatal period for the prevention of postpartum diseases in dairy cows. This work provides a reference for clinical veterinary diagnosis and treatment of *C. perfringens* type A infections, so as to reduce the economic losses of dairy farms.

**Data Availability**

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.
Author Contributions
WL and CHJ were involved in the case analysis and were responsible for writing the manuscript. HS and JJ were involved in the draft preparation and case analysis. ZMX and WL were involved in the case autopsy and analysis. ZD, LS and SY were involved in the laboratory tests. WL and HS were involved in the coordination of the case and were responsible for the interpretation of the results. All the authors read and approved the final version of the manuscript.

Consent to publication
Written consent was obtained from the present owners of the cows for publication of this case report and any accompanying images.

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Disclosure statement
Author Jidong Jin is employed by COFEED FEEDMILL (Changchun) CO, LTD, Changchun, Jilin Province, China. The remaining 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.

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