INTRODUCTION

_Clostridium perfringens_ is a gram positive spore forming, anaerobic bacteria widely distributed in the soil and the digestive tract of many domestic animals. Six Types (A, B, C, D, E and F) have been identified on the basis of the toxins produced, with Types B, C and D being the most commonly associated with disease in domestic animals.

Generally _C. perfringens_ organisms are associated with enterotoxemia type of diseases and traditionally _C. perfringens_ type C would be considered in hemorrhagic enteritis cases in cow calf operations, _C. perfringens_ type D in calves after weaning and when on dry feeds and both types C and D may occur in feedlot calves but not usually in adult cattle (Clostridial Diseases Bruce Wren, Veterinarian, Des Moines, Iowa)

Recently, authors are reporting similar diseases associated with _C. perfringens_ type A and various names have been given to these such as “Haemorrhagic Bowell Syndrome”, “Jejunal Haemorrhage syndrome” and “Bovine Clostridial Abomasitis”. This article reviews some aspects of these manifestations of _C. perfringens_ type A
Clostridium perfringens type A

Clostridium perfringens bacteria normally inhabit the digestive tract in small numbers without causing disease. If any toxin is produced, it is in small quantities and passes through the animal without causing problems. If an animal is exposed to a sudden increase in carbohydrates, such as a heavy feeding of milk, lush pastures or supplementary concentrates, resident bacteria may multiply rapidly and C. perfringens produce large amounts of toxin. Toxins are considered to be produced in the sporulating stage of growth as opposed to the logarithmic stage. These toxins may damage the intestines, facilitating the absorption of toxins into the bloodstream with the end result of this toxemia usually being rapid death and it is generally termed enterotoxemia. Diseases caused by C. perfringens type A include histotoxic and enteric diseases and in young calves under circumstances such as above, abomasal tympany, abdominal pain, and haemorrhagic diarrhoea (Berrier, RJ.).

Van Metre and Callan (2005) describe haemorrhagic bowel syndrome (HBS) or jejunal haemorrhage syndrome (JHS) as a newly emerging, highly fatal intestinal disease of adult dairy cows in the United States. Shlegal et al (2012) describe these as characterized by mechanical obstruction of some length of the jejunum, with sloughed mucosa and clotted blood. It is seen in mature cattle of both genders usually averaging 4 years of age and may be associated with the winter season. Schlegal et al (2102) use the term bovine clostridial abomasitis (BCA) to describe the severe abomasitis form of disease. They report that bovine clostridial abomasitis has been reproduced by experimental infection with a C. perfringens BCA isolate in calves which is not necessarily associated with any particular toxin.

Songer G (1999) and Mortimer and Ellis (2001) stated that type A strains of C. perfringens are identified by their ability to produce alpha toxin but do not produce beta, epsilon or iota toxins but that these strains are common, widespread and associated with haemorrhagic enteritis, abomasitis, abomasal haemorrhage and ulceration. Mortimer and Ellis at that stage suggested that there were as yet undetermined groups within Type A that may be associated with specific disease syndromes.

The role of C. perfringens in feedlot sudden death has been controversial and may occur in animals vaccinated against types C and D and Songer states that immunization with C. perfringens types C and D does not give rise to alpha toxin neutralizing antibodies suggesting that type A may in fact play a role. He states further, however, that there is very little concrete information pertaining to pathogenesis of type A enteric infections in cattle but it is still possible that the pathogenesis is mediated by some element other than alpha toxin for example beta2 toxin which is frequently produced by strains from cattle enteritis.

Bozidar Savic et al (2012) investigated enteritis associated with type A in 9 month old calves that showed severe intraluminal haemorrhage in the jejunum suggestive of jejunal haemorrhage syndrome. They stated that some isolates of C. perfringens type A produce b2-toxin which may contribute, along with a-toxin, to the development of haemorrhagic lesions in the small intestine in cases of bovine enterotoxaemia. Isolates of C. perfringens type A have also been suggested as a cause of jejunal hemorrhage syndrome (JHS) in beef and dairy cattle. However, efforts to
experimentally reproduce JHS with this organism have produced varied results implicating the possible presence of other contributing or predisposing factors in the etiology of this syndrome.

Van Metre and Callan describe some strains of *C. perfringens* type A also carry a beta2 toxin gene; these strains are designated as **A+beta2**. Beta2 toxin is also lethal for intestinal cells. The distribution of *C. perfringens* strains that carry this toxin gene is not well described, but they have been identified in the intestine of both healthy and diseased cattle and they hesitate to consider *Cl. perfringens* as the sole pathogen causing HBS. They stated that *Cl. perfringens* proliferates rapidly in the intestine after death, making isolation from necropsy specimens of questionable diagnostic significance. If the lethal toxins of *C. perfringens* can be demonstrated in intestinal contents and/or blood of diseased animals, it is considered more likely that the organism is causing disease, rather than simply acting as a part of the normal gut flora.

*Salmonella* spp, BVD or coccidia which are also diseases capable of inducing intraluminal haemorrhage but according to Bozidar Savic et al (2012), in their case study in calves, the gross and histological lesions did not resemble those typically attributed to the above diseases. They reported a profuse growth of *C. perfringens* type A in all tested samples which suggests that this organism may be implicated in development of the lesions and they suggest that JHS may also occur in calves and may also be associated with *C. perfringens* type A as opposed to the older animal opinions.

Dr Robert Callan (2005) reports that **winter dysentery** is a viral disease resulting in very acute onset of profuse watery diarrhoea in adult cattle occurring typically in the winter months and it has also been observed in beef and feedlot cattle. Winter dysentery is characterized by its highly explosive epidemic nature within a herd. Acute onset of severe watery diarrhoea is characteristic and up to 30-50% of the herd may be affected within just a few days and up to 100% within a week. The faeces are typically dark green, dark brown, or may appear black. Frank blood may be observed in the feces of some cows. Most animals do not demonstrate an elevated temperature once the diarrhoea is present; however, there may be a fever just prior to becoming sick. The cattle are often bright and alert with minimal systemic signs of disease. They generally maintain rumen activity and continue to eat while they are sick. The aetiology is an enteric Corona virus and he suggests that BVD, coccidiosis and salmonella outbreaks may also resemble winter dysentery. *Campylobacter fetus* var. *jejuni*. has also been associated with winter dysentery but while this bacteria is associated with diarrhoea in adult cattle, it is not routinely associated with the explosive outbreaks of diarrhoea classically associated with winter dysentery.

Van Metre and Callan reported on an investigation of HBS focused on characterizing the role of *Aspergillus fumigatus* that can be found in livestock feeds. Genetic material of this fungal agent can be detected in the blood and intestine of animals affected by HBS. The rate of detection of *Aspergillus fumigatus* DNA by polymerase chain reaction in the tissues was compared, with the DNA of this fungal organism being found to be present in the tissues of a significantly greater proportion of cows with HBS than of animals that died of other GI diseases.
Ashworth (2006) reported that Forsberg and Puntenny proposed that a causative agent other than *C. perfringens* was responsible for HBS. The use of real-time SybrGreen quantitative polymerase-chain reaction (PCR) analysis indicated that all HBS cows were infected with *Aspergillus fumigatus* while samples from their control cows were negative. Multiplex PCR analysis of five clostridial toxin genes did not reveal a correlation with HBS and clostridial toxin genes were detected in both HBS and control animals. *Aspergillus spp* has been shown to infect the ruminant gut at multiple sites and to cause intestinal haemorrhage. Haemorrhagic lesions have also been noted in the reticulum, rumen, omasum, and the Peyer’s patches. In essence they reported that *A. fumigatus* correlates closely with HBS and may play an important role in its etiology.

Baines reported on an investigation of two factors associated with JHS presenting as haemorrhaging, severe inflammation, mucosal erosion and large blood clots in the jejunum namely mouldy feed containing *A. fumigatus* and *C. perfringens* type A.

In an earlier study they expanded the disease complex to include mycotoxins and Shiga toxin -producing *Escherichia coli* (STECs) based on a study of JHS cases from five dairy production sites. They reported that this study confirmed earlier reports suggesting that *C. perfringens* type A was not necessarily always involved. The pathologies observed in the beef cattle in this study are the same as those reported in other JHS cases and confirmed that STECs and mycotoxins are part of the disease complex for JHS in beef cattle. Mycotoxigenic fungi are only relevant in that they produce the mycotoxins deposited in the feed.

Meier, in a thesis on the effect of cereal grain type on production and performance and *Cl. perfringens* Colonization in Cattle reported that the link between nutrition and disease has long been established in cattle. She stated that HBS is an emerging disease in cattle and noted that 77% of such cases died or were euthanized and determination of predisposing factors to this disease was considered important in prevention as most cows die suddenly without an opportunity for treatment. An effect of diet was noted in *C. perfringens* shedding in dairy cows. Barley fed cows had lower *C. perfringens* counts than maize fed cows. The increased level of *C. perfringens* in corn fed cows may be a result of increased amounts of starch reaching the jejunum. Also, barley starch is more easily digested by ruminal microbes compared to maize starch, resulting in less starch reaching the jejunum.

It has been proposed that rations rich in energy and protein and low fibre relative to diets fed later in lactation place cows at a greater risk for HBS (Van Metre et al. 2005). Other factors influencing *C. perfringens* shedding may include feed intake behavior, immune status and genetics.

Animals that have larger meals may have higher passage rates through the gut, allowing for more starch to reach the intestine. Immunosuppression may allow for opportunistic growth of *C. perfringens* in the intestine and genetics may predispose individual cows to higher *C. perfringens* faecal shedding and increased risk to contract HBS. She reported that it has been hypothesized that ruminal acidosis may be a predisposing factor to the onset of HBS and that while *C. perfringens* is a normal constituent of the intestinal microflora in
cattle a trend is noted that as cattle age, the level and prevalence of colonization increases. Dairy heifers tended to have low levels of \textit{C. perfringens} colonization. This may be result of age or the high forage ration these animals were fed. It was determined that \textit{C. perfringens} is abundant in feedlot animals that are on high grain rations. As animals are fed increasing levels of concentrate, \textit{C. perfringens} counts and shedding tends to increase. These results are similar to findings of \textit{E. coli} shedding in feedlot cattle.

\textbf{SYMPTOMS}

Disease associated with \textit{C. perfringens} type A is characterized by sudden onset of disease and clinical signs are usually not observed with animals typically found dead or near death and those that have died recently may be found on their side with the head and neck bent backwards. Close observation of animals prior to death may reveal animals that are listless, show signs of colic and haemorrhagic diarrhoea, depending on the amount of toxin produced and the length of illness. Central nervous system signs such as excitement, incoordination, circling, head pressing and convulsions may be noted prior to coma and death. Body temperature is usually normal or subnormal unless associated with convulsions, in which case the body temperature may be one to two degrees above normal.

\textbf{DIAGNOSIS}

Post mortem examination may reveal internal lesions including inflammation of the intestinal wall, diarrhoea with or without blood in the intestine, small areas of haemorrhage on the surface of the intestines and heart, fluid around the heart or rapid degeneration of the kidneys. Diagnosis is based on clinical signs, if noted, post mortem lesions and demonstration of specific toxin in intestinal contents in the case of toxin related conditions. Schlegal states that jejunal hemorrhage syndrome (JHS) is characterized by mechanical obstruction of some length of the jejunum, with sloughed mucosa and clotted blood in mature cattle as opposed to pathology, in the case of BCA, is characterized by acute emphysematous and necrotizing haemorrhagic inflammation of the mucosa of the abomasum, with marked oedema in the lamina propria and submucosa. The disease may produce similar lesions in the rumen, reticulum, and duodenum. In these cases, Gram-positive bacilli typical of \textit{C. perfringens} are often found on the mucosa and in the submucosa and \textit{C. perfringens} is invariably isolated from such cases. Diagnosis of \textit{C. perfringens} type A enteritis is complicated by the fact that \textit{C. perfringens} type A is found in the gastrointestinal tract of healthy animals. \textit{Clostridium perfringens} type A, B, C and D disease are identified by the presence of characteristic pathological and histopathological changes of haemorrhage and necrosis, including gas production and the involvement of the abomasum, as well as the isolation of \textit{C. perfringens} in high numbers from the intestinal contents. Traditionally, the diagnosis of enteric disease caused by \textit{C. perfringens} is based on the detection of specific toxins in the intestinal contents, but no specific toxin has been associated with BCA and Schlegal has suggested that the identification of certain unique virulence determinants in the genomes of isolates may assist in diagnosing the condition.
This disease may also cause softening of the kidney (pulpy kidney) caused by renal oedema, but this is not a reliable sign on necropsy as autolysis may also cause a softened kidney. Animals dead from *C. perfringens* Type D may also have glucose in their urine.

Vetdiagnostix veterinarians have recommended the following sampling technique for best results in attempting to make a diagnosis of *C perfringens* type A toxicosis,

**Samples required from each animal**

- 3 cm section of affected intestine (ileum/jejunum) tied off at each end and wrapped in a sealed plastic envelope to exclude oxygen.
- Liquid intestinal contents collected into a sterile syringe
- Charcoal swab of intestinal lumen.
- 1 cm section of affected intestine (ileum/jejunum) open at both ends and ingesta washed out with 10% buffered formalin, and then placed in 10% buffered formalin.

**Laboratory procedures:**

- Anaerobic cultures (intestine, fluid, charcoal swab)
- *Clostridium perfringens* suspect cultures and intestinal fluid from these positive cases run on the BIO-X enterotoxaemia Elisa kit
- Histopathology

**CONTROL AND PREVENTION**

Due to the acuteness of intoxication and death, treatment is usually not an option and prevention of enterotoxaemia involves both management and vaccination of susceptible animals. Management practices such as gradual feed changes and feeding hay early in the day prior to turning out on lush pastures will decrease the chances of carbohydrate overload. Immunization with a product containing *Clostridium perfringens* Types C and D should be an integral part of a vaccination program. Naive animals should be vaccinated twice, with the second dosage administered three to four weeks after the first or according to manufacturer’s recommendations. Animals entering a feedlot or experiencing diet changes should receive the second vaccination at least two weeks prior to these anticipated events.

Enterotoxaemia may occur in newborn and young animals, in which vaccination prior to exposure is impossible. Prevention of enterotoxaemia in newborns should involve a vaccination program of the dam and dams that have not been vaccinated previously should receive a product containing *C. perfringens* Types C and D toxoid 60 and 30 days prior to giving birth. Previously vaccinated dams should be boostered about 30 days prior to giving birth. Also, transfer of colostrum from the dam to the newborn should be verified.
Due to the possible involvement of fungal toxins and certain bacteria control should include management practices that would reduce those risks. In addition to vaccination, antitoxin that contains antibodies to *C. perfringens* types C and D have been described in a prevention programs. Animals which are at risk, and adequate time is not available for a vaccination program to be instituted (i.e. newborns), can benefit short term from the administration of C and D antitoxin. C and D antitoxin will provide an animal with 10 days to 3 weeks of protection and can also be used in a treatment program, if disease is diagnosed early.

**Conclusion:**

*Clostridium perfringens* associated enteritis cases are a complicated and multifactorial complex of diseases. The finding of a Beta 2 toxin associated with *C perfringens* type A together with many other predisposing or contributing factors but particularly mycotoxins make this an extremely challenging disease complex for veterinarians.

**REFERENCES**

1. Randall J. Berrier, DVM 2001 Colorado Serum Company proudly produces and distributes products containing *Clostridium perfringens* Types C&D Toxoids and C&D Antitoxin. Contact your local distributor or veterinarian and request these and other fine products from Colorado Serum Company.
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QUESTIONS:

1. *Clostridium perfingens* is a
   a. Gram negative intestinal bacteria
   b. Gram negative bacteria found in soil
   c. Fungal organism
   d. Non-pathogenic bacterium
   e. **None of the above**

2. *Clostridium perfringens* type A organisms.
   a. All produce only alpha toxins
   b. Produce beta toxin
   c. Produce epsilon and iota toxin
   d. **Have some strains producing alpha and beta2 toxins**
   e. None of the above

3. *Clostridial perfingens* organisms are associated with
   a. Haemorrhagic Bowell syndrome
   b. Jejunal haemorrhage syndrome
   c. Bovine clostridial abomasitis
   d. Enterotoxaemia
   e. **All of the above**

4. Diseases associated with *Clostridium perfringens* type A are characterized by
   a. Explosive outbreaks of watery diarrhoea
   b. **Acute fatal disease**
   c. Haemorrhage into the caecum
   d. Chronic weight loss
   e. Alert downer animals

5. *Clostridium perfringens* infections should be differentiated from
   a. *Salmonella spp* infections
   b. Diplodiosis (*stenocarpella maydis*) mycotoxicoses
   c. Milk fever
   d. Johne’s disease
   e. Parafilaria infections

6. *Aspergillus fumigatus* is
   a. Not associated with Haemorrhagic bowel syndrome
   b. Associated with the alpha mycotoxin production
   c. **Is associated with HBS and JHS**
   d. Not associated with animal health
e. Not associated with infection of the ruminant gut

7. *Clostridium perfringens* organisms are shed
   a. In lower levels from animals on low roughage rations
   b. Predominantly from dry dairy cows
   c. From acutely dead animals
   d. **More from maize cows than barley fed cows**
   e. None of the above

8. Clostridial diseases may be controlled by
   a. Timely treatment with antibiotics
   b. Vaccination
   c. Use of antitoxin antibodies
   d. A combination of vaccination and management methods
   e. **All of the above**

9. Diagnosis of *Clostridium perfringens* type A disease is
   a. **Largely confirmed by multiple diagnostic tests**
   b. Requires only the identification of gram negative rods on intestinal lumen
   c. Requires only the presence of toxins to be confirmed
   d. Can be positively diagnosed on macroscopic lesions
   e. Can be positively diagnosed without laboratory tests

10. Shiga toxin producing *E coli* organisms are
    a. Not associated with Clostridial diseases
    b. **May be associated with mycotoxins and Clostridium perfringens type A in JHS disease complex**
    c. Not associated with animal disease
    d. Primary factors in ruminal bloat
    e. Associated with winter dysentery