Prevention of the main Clostridial diseases in cattle

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SUMMARY

Clostridial diseases of cattle are an economic and welfare issue worldwide. Clostridia are obligate anaerobic spore-forming gram-positive bacteria able to cause a wide range of pathologies in humans and animals. Pathogenicity is expressed by sporulation in favourable environmental condition with release of toxins. Toxins produced and tissues damaged are generally characteristic for each clostridial. The incidence of clostridiosis is relatively low however the outcome is generally very poor despite the bacteria being sensitive to the most common antibiotic therapies. The generally rapid course of the disease prevents any intervention. Despite a continually developing classification, clostridium that affect cattle can be classified based on their target tissue and pathogenic expression, as neurotoxic, histotoxic and enterotoxic. Scientific knowledge about different clostridial toxins, their aetiopathological mechanisms, risk factors and pathologies involved are generally limited due to the large number of bacteria strains and types involved. Alongside the more studied neurotoxic *C. tetani* and *C. botulinum* for their implications in human medicine, there are lots less known pathogenic strains capable of causing extremely severe clinical patterns in veterinary medicine. In particular regarding enterotoxic clostridia, the incidence of necro-haemorrhagic enteritis and enterotoxaemia is probably wrongly estimated because complete post-mortem investigation is rarely performed and several other reasons can lead to sudden death. The aim of this review is to describe the main clostridial diseases that can affect cattle and some of the possible prevention strategies as controlling major known risk factors and the use of vaccination.

KEY WORDS

Clostridia, clostridiosis, cattle.

INTRODUCTION

Clostridia are obligate anaerobic spore-forming gram-positive bacteria. The genus Clostridium consists of dozens of strains characterized by different pathogenicity, many of which are able to cause illness in humans and other animals. Pathogenicity is expressed not by the presence of clostridial bacteria but by replication; in favourable environmental conditions, sporulation with release of toxins takes place. Clostridial toxins are biologically active proteins that are antigenic in nature. Toxins produced and tissues damaged are generally characteristic for each clostridial[1]. In beef and dairy rearing systems, clostridium-associated diseases are both a welfare and an economic issue.
The incidence of clostridiosis is relatively low however the outcome is generally very poor despite the bacteria being sensitive to the most common antibiotic therapies. The rapid course of the disease, in most cases, prevents any intervention. The aim of this review is to describe the main clostridial diseases that can affect cattle and the prevention strategies that field veterinarians can implement to support the farming system.

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Bovine clostridiosis

The classification of clostridia is a continually developing topic for researchers and is mainly based on the types of toxins produced. From a practical point of view, clostridium that affect cattle can be classified based on their target tissue and pathogenic expression, as neurotoxic, histotoxic and enterotoxic (Table 1).

Neurotoxic clostridia

The main neurotoxic clostridia which affect cattle are *C. tetani* and *C. botulinum*. Tetanus is an acute, often fatal disease of almost all domestic animal species caused by the neurotoxins produced by *Clostridium tetani* in anaerobic conditions. This usually develops after contamination of deep and penetrating wounds. The neurotoxin produced causes the characteristic rigidity and muscle spasms[2-4]. *C. tetani* is present in soil and faeces and there can be several means of entry into the animal. Published literature reports infections of the umbilical cord, infection of wounds caused by barbed wire or pitchfork injuries, injection sites, hoof and interdigital space lesions, oral mucosal wounds caused by coarse forages, vaginal laceration incurred during dystocic calving, uterine prolapse or placental extraction, bedsores or surgical site lesions, dehorning and castration activities. Penicillin or tetracycline treatment to reduce bacteria proliferation in addition to anti-tetanus homologous

Table 1 - Bovine clostridiosis classification.

Type	Strain	Pathology
Neurotoxic clostridia	*C. tetani*	Tetanus
	C. botulinum	Botulism
Histotoxic clostridia	*C. septicum*	Gangrene or tissue infections (muscles or muscles fascia, subcutaneous tissue, liver, abomasum, kidney, etc.)
	C. chauvoei	
	C. novy	
	C. sordellii	
	C. perfringens	
	C. haemolyticum	
Enterotoxic clostridia	*C. sordellii*	Enteritis and Enterotoxaemia
	C. perfringens	
	C. septicum	
	C. difficile	

serum and careful wound/entry site disinfection can improve prognosis. Considering the aetiology, prevention should be based on improving the hygiene of the housing environment and farming/vet tools[5].

Botulism is a neuro-paralytic disease of humans and animals, caused by the neurotoxins produced by *Clostridium botulinum*. *C. botulinum* is a ubiquitous soil-borne pathogen that finds an excellent growing environment in decaying organic matter[6]. In cattle, the signs are generally associated with the ingestion of feed or water contaminated with the remains of carcasses. There are even reported outbreaks linked to contact with poultry litter. Furthermore, *C. botulinum* can directly proliferate in forages without carcasses, in cases of inaccurate collecting and storage procedures (presence of organic refuse, excess soil contamination, high humidity and temperature)[5,7-8]. Botulism usually results in fatality since the neuronal flaccid paralysis cannot be reversed by available therapeutic options except for administration of antitoxin[9]. Its prevention is simply based on good management practice during harvesting and storing feed.

Histotoxic clostridia

Among the different pathologies caused by histotoxic clostridial strains, the two main diseases affecting cattle are 'Blackleg' and 'Malignant oedema'. The differences between these two diseases include the clostridial strains implicated and the entry point into the animal.

Malignant oedema is considered to be an "exogenous" disease because different clostridia, such as *C. septicum*, *C. chauvoei*, *C. novyi*, *C. sordellii*, and *C. perfringens*, from the environment gain access into the tissues after skin or mucosal wounds and development of an anaerobic environment. Main types of trauma that may lead to malignant oedema include, but are not restricted to, intramuscular injections, parturition, shearing, castration, surgery and tail docking[10-11]. Clinically, the involved tissues rapidly develop oedema, characterised by a variable presence of gas, high fever and inappetance. In case of a non-hyperacute form, resulting in sudden death, treatment with penicillin or tetracycline may be effective only if started very early in the disease process and preferably in addition to polyvalent serum administration and surgical curettage of the wound. Despite rapid intervention the prognosis is poor and death typically occurs within 2-5 days after trauma[5].

Blackleg, in contrast, is caused by *C. chauvoei* alone and is considered to be an "endogenous" disease[1,12-14]. Even without a consensus from the scientific world about blackleg pathogenesis, the infection is acquired by the ingestion of *C. chauvoei* spores that are transported from the intestine to the muscles and tissues by macrophages across Peyer's patches. Other hypotheses include entry by oral mucosal wounds due to teething, forestomach traumatic injures by foreign bodies and lesions of the enteric mucosa[15-17]. The spores remain dormant in the target tissues until a traumatic injury induces the anaerobic conditions ideal for their germination, multiplication and toxin production[17-18]. The predisposing traumatic events reported include bumps, blows, mounting behaviour, competition at the feed bunk or at water points, constricted passing in narrow places, goading and transport in general[5]. This infectious disease is acute and globally spread among ruminants, causing significant loss in livestock production[19] as it is generally fatal, being included among the causative agents of sudden death. The disease is typically observed during the warm season, and young cattle aged between 6 to 24 months are mostly affected[18]. *C. chauvoei* is one of the clostridia supporting a fatal hyperacute form counted among the causative agents of sudden death. Moreover, the classical forms are reported with swelling and crepitus of affected skeletal muscles due to a neutrophilic necrotizing myositis[20]. Rarely diagnosed forms include fibrinous pleuritis, pericarditis, epicarditis, meningoencephalitis, severe acute necrotizing enteritis or myositis of sublingual muscles and diaphragm[21-25]. Avoiding soil-contaminated pasture, forages and litter is the most effective preventive strategy especially in those regions characterized by very high annual rainfall that can expose and activate latent spores, after soil excavation or areas with a history of flooding[25-26].

Enterotoxic clostridia

Among enterotoxic clostridia, *Clostridium perfringens* is the major cause of necrotic and haemorrhagic enteritis and enterotoxaemia both in humans and other animal species. In accordance with the other diseases caused by clostridia, although morbidity is rather low, therapy is largely ineffective and mortality is close to 100%, making it an economically important disease[27]. Moreover, it is one of the most widespread bacteria, ubiquitously present in the environment, in soil, food, manure and the normal intestinal microbiota of both humans and animals[28]. *C. perfringens* is classified into five toxinotypes (A, B, C, D and

E), based on their ability to produce different toxins and extracellular enzymes. In addition to producing a combination of the four major toxins (alpha, beta, epsilon and iota toxin), *C. perfringens* strains can produce additional but not less harmful toxins such as enterotoxin and necrotic enteritis B-like toxin[1,29-31]. Despite an ongoing debate among researchers, *C. perfringens* type A strains are the suspected agent in cases of bovine alimentary tract disorders such as clostridial abomasitis and necro-haemorrhagic enteritis. Type D, however, seems to be the aetiological agent of proper "enterotoxaemia"[32]. Indeed, the term "enterotoxaemia" is wrongly applied to generic diseases caused by *C. perfringens*, but should be used only in cases where major signs are caused by systemic actions of the toxins without the presence of important intestinal lesions[1,30-31]. From a practical point of view, since the predisposing factors and outcome of the clinical condition are basically the same, and the correct differential diagnosis does not lead to more valid therapeutic options, the debate is only formal and detailed to the laboratory. Enterotoxic clostridial disease is in fact characterized by a high case fatality rate, sudden deaths, more or less evident lesions of necrotic and haemorrhagic enteritis of the small intestine and, most often, an absence of other clinical signs[28,33-34]. When premonitory signs are noticed by the farmer, cattle death is expected within 5 hours due to necro-haemorrhagic enteritis[35-36]. Those signs are typically lateral recumbency and cold extremities, as consequence of cardiovascular shock. Other signs detected less frequently are colic, respiratory distress, nervous signs, distended abdomen and diarrhoea[25,35-36]. Considering the huge presence of *C. perfringens* in the environment, the development of the disease is linked to the type of bacteria present and the relative pattern of toxins produced, the amount of spores within the enteric tract and the host susceptibility. Several nutritional predisposing factors are in fact described to predispose the enteric environment to the germination and multiplication of *C. perfringens*. Since *C. perfringens* proliferate using the amount of digestible carbohydrates that exceed the digestive and absorptive capacity of the intestinal mucosa, high protein and energy-rich diets predispose to the disease[27-37]. Other dietary issues such as sudden change of feed composition, change of pasture, moving from pasture to burns and high protein concentration in association with low amounts of fibre may alter the microbiota composition promoting clostridial overgrowth[36]. *C. perfringens* also affects preweaned calves. Indeed, necro-haemorrhagic enteritis is more frequently observed in veal calves and suckler calves. The whey present in cow's milk or milk replacer contains high quality, readily available amino acids, potentially predisposing to clostridial overgrowth[27,38-40]. Increasing host susceptibility is not only a matter of nutrition but also stressful environmental conditions, such as regrouping, transport, handling and medical treatments are risk factors for necro-haemorrhagic enteritis[27]. In cases of stress, the consequent intestinal microbiota modification can lead to a less efficient digestive process with more nutrients available for bacterial growth[41-44]. The enteritis slows down intestinal motility and the consequent intestinal stasis diminishes the flushing of bacteria and toxins contributing to further bacterial overgrowth[36,45].

Prevention strategies

Management risk factors

In reviewing the main bovine clostridial diseases, it is evident that prevention of risk factors is fundamental because bacteria are ubiquitous in the environment and that therapy is often in vain due to the very rapid course of disease. The preventive strategies are based on applying good farm management practices in order to avoid animals coming into contact with the pathogens, and limiting their susceptibility to infection through vaccination. Good management practices and high hygiene levels of tools and structures for handling the animals can avoid the risk of tetanus and malignant oedema since wounds or injuries are pre-requisites for the development of disease. Ergonomic structures and effective management could minimize the traumatic events resulting in the onset of blackleg even if complete prevention can only happen by avoiding the presence of Clostridia from the digestive tract. Good management practices in harvesting, storing and feeding the animals can prevent not only blackleg, but even botulism and diseases associated with enterotoxic clostridia. In Table 2 are reported those dietary features able to destabilize the intestinal environment, altering the pH, the transit speed and the balance among the commensal microorganism populations, and thus potentially promoting the ideal conditions for clostridial germination and replication.

To the authors knowledge, no evidence is reported of a direct correlation between other diseases and toxicoses or clostridiosis in cattle. Based on the hypothesis that clostridial enteric colonization is promoted by those factors able to reduce the effectiveness of the digestive process and modulate the intestinal microbiota, it is not possible to exclude that other diseases may promote clostridial germination and proliferation in the gut, for example enteric parasitosis or general diarrhea-based diseases. Furthermore, ruminal acidosis is a condition able to alter intestinal pH and increase tissue permeability in the proximal and distal colon[46]. Moreover, there is clear evidence that mycotoxins initiating intestinal damage are able to promote *C. perfringens* proliferation and the development of necrotic enteritis in poultry[47].

Vaccination

Considering that clostridia-associated diseases are often quickly fatal, other than managing all the risk factors, vaccination is usually the only possible intervention. The available clostridial vaccines are a combination of active compounds against several clostridial strains, often including toxoids of several toxinotypes of *C. perfringens*, mainly type C and D. In addition, toxoids from several other clostridial species are usually present in the same commercial products, such as: *C. chauvoei*, *C. novyi*, *C. sordellii*, *C. septicum* and *C. tetani*[27].

It is an incorrect, common opinion that toxoid-vaccines are not able to stimulate self-antibody production. Regarding blackleg, the study of Araujo et al., (2010)[48] showed that booster shots significantly increased beef calves' serological response at 30 days post-immunization. The higher serum IgG levels against *C. chauvoei* were found in those calves vaccinated at four months of age, followed by a booster dose one month later, and then annually repeated[48]. Furthermore, the interaction between toxins and body defences can explain why calves in veal production systems seems to be more susceptible to necro-haemorrhagic enteritis[27,35-36,40,49]. Calves receiving exclusively milk replacer do not develop an active immunity towards *C. perfringens* alpha toxin, when maternal immunity declines, unlike calves raised for beef production, in which a fluid transition from passive maternal to active immunity is observed[40,50]. Also based on this evidence, the actual scientific opinion gives more impor-

Table 2 - Dietary and feed management issues affecting digestive effectiveness.

Changes in the diet	- Sudden changes in energy and protein dietary content
Lack of fibre	- Forages and concentrates separated - Short cut forages - Diet with low peNDF - Inaccurate TMR charging and/or mixing
Excess fermentation	- Excess starch from barley/wheat/high moisture corn/flaked cereals - Reduced particle size
Protein imbalance	- Imbalance between protein and energy - High soluble protein - Excess of non-protein nitrogen - Not well fermented or stoked silages - High nitrates in water or feed - Inaccurate TMR charging and/or mixing
Feed bunk fermentation	- Unstable silages - Water added to the feed - Warm season and too humid diet - Feed bunk with poor hygiene level
Intake variations	- Lack of feed in the feed bunk for a too long period - Competition - Illness - Water availability and its temperature - Season and burns microclimate
Diet inhomogeneity	- Inaccurate TMR charging and/or mixing - Too short or too long TMR mixing time - Too long cut forages - Absence of binder or appetizer in dry diet

tance to *C. perfringens* alpha toxin in the pathogenesis of necrohaemorrhagic enteritis[51]. Alpha toxin toxoid was not always included in commercial vaccines, or more accurately, not enough focus was put on its preservation and titration. Furthermore, the purification method can affect its presence in vaccines since it is well known that the protective antigenicity of alpha toxin is easily destroyed by formaldehyde inactivation during the vaccines production[52-56].

Clostridial components in vaccines are produced in a reasonably standardized manner by successive passaging of bacterial culture in a medium for growth, in order to obtain the necessary volume of bacteria required for manufacture of the vaccine. At the end of the growth phase the bacteria are inactivated. Bacterial cells and the culture medium are then separated by centrifugation. The resulting supernatant is concentrated and the associated toxins are detoxified to finally constitute the active ingredients of the vaccine. Despite the standard approach to manufacture, the bacterial cells and their toxins are very sensitive to their environment. Small variations (e.g. pH and temperature) within and between manufacturing processes may impact each clostridial component regarding the type of toxins produced and their quantity[57-59].

Implementing the vaccines productive systems and the titration assay can improve the vaccine quality. For example, applying a consolidated shotgun proteomic approach, it was possible to find and quantify the toxoid of alpha toxin in a commercial vaccine (Miloxan - Boehringer-Ingelheim) that had no such characteristic registered. In the Lab of the University of Sacro Cuore of Piacenza, Italy, the following procedure was applied. Total proteins were extracted in phenol, precipitated and then re-suspended in urea/thiourea and protein amount determined by the Bradford colorimetric approach. The same amount of proteins (50 ug) from each sample, was reduced and alkylated, then digested in trypsin overnight (again, the very classical bottom-up approach). Peptides were finally analysed by nano LC-CHIP QTOF tandem mass spectrometry, using a data-dependent approach and label-free quantitation. Peptides were validated at 1% false discovery rate, and protein inference was then done against the proteome downloaded from UniProt (selecting all those proteins having "Clostridium" in organism taxonomy). Briefly, protein inference is based on the matching between the peptide list from our experiments and the peptide list gained from in-silico digestion of the proteome downloaded from UniProt. Single peptide identification was allowed for unique peptides only. Results are reported in Table 3.

CONCLUSIONS

Clostridial diseases of cattle are an economic and welfare issue worldwide. Clostridiosis are non-contagious diseases characterized by low incidence and rapid clinical course that renders therapeutic intervention ineffective. Our knowledge

Table 3 - Quantification of toxoid in a commercial vaccine.

	Toxin	Vaccine tested*	
		Media Total Intensity	% toxin/total intensity
Q46149	Alpha-toxin	5.39E+05	2%
B1R9V5	Beta-toxin	7.25E+06	27%
Q46342	Cytotoxin L	1.57E+07	59%
E7D8R1	Epsilon-toxin (Fragment)	5.23E+05	2%
P04958	Tetanus toxin	1.92E+06	7%
U3YLU7	Toxin B	8.43E+05	3%
	Total intensity (toxins)	2.67E+07	100%

*Miloxan - Boehringer-Ingelheim

about different clostridial toxins, their aetiopathological mechanisms, risk factors and pathologies involved are generally limited due to the large number of bacteria strains and types involved. Furthermore, and in particular regarding enterotoxic clostridia, the incidence of necro-haemorrhagic enteritis and enterotoxaemia is probably wrongly estimated because complete post-mortem investigation is rarely performed and several other reasons can lead to sudden death. Controlling predisposing risk factors and vaccinating, especially young cattle, could minimize this issue. However, it must always be taken into account that vaccine failure can still occur because clostridia are ubiquitous in the environment, some strains are extremely low-dose pathogens, and that, even in a production system where vaccination is regularly implemented, never are all possible antigens and toxoids present in any one commercial product.

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