

## Clostridial diseases of small ruminants

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**Abstract** – Members of the genus *Clostridium* are extraordinarily diverse in their natural habitats, and, when introduced to animal hosts, a few produce acute and often fatal disease. In sheep and goats, as in many other species of domestic animals, pathogenesis is often mediated by one or more of the many toxic proteins produced by these organisms. Prevention and control strategies are frequently based upon amelioration, by immunoprophylaxis, of the effects of these molecules. In spite of their recognition for many years, clostridial diseases still present challenges to veterinary practitioners, diagnosticians and animal producers worldwide. © Inra/Elsevier, Paris

**myonecrosis / enteritis / enterotoxemia / neuromuscular disease / bacterial toxins**

**Résumé** – Clostridioses chez les petits ruminants. Les membres du genre *Clostridium* sont extraordinairement divers dans leur habitat naturel, et, lorsqu'ils ont pénétré chez leur hôte, certains provoquent une maladie aiguë et souvent mortelle. Chez les moutons et les chèvres, comme dans beaucoup d'autres espèces animales domestiques, une ou plusieurs des nombreuses protéines toxiques produites par ces microorganismes est souvent à l'origine de la pathogenèse. La prévention et les stratégies de contrôle sont fréquemment basées sur l'amélioration, par immunoprophylaxie, des effets de ces molécules. Malgré leur reconnaissance depuis de nombreuses années, les clostridioses présentent toujours des défis aux praticiens vétérinaires, aux personnes qui diagnostiquent les maladies, et aux producteurs, dans le monde entier. © Inra/Elsevier, Paris

**myonécrose / entérite / entérotoxémie / maladie neuromusculaire / toxine bactérienne**

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## 1. INTRODUCTION

Clostridia are widely recognized as pathogens of humans, domestic animals and wildlife (*tables I and II*). The ready availability of inexpensive, efficacious immunoprophylactic products has not eliminated clostridial infections. Proven and putative virulence attributes mediate the pathogenesis of many types of infections in myriad hosts. This review of clostridial disease in small ruminants will cover muscle and soft tissue infections, intoxications and toxicoinfections, and enteric infections. Other reviews (Smith, 1979; McDonel, 1980) provide a broader context.

## 2. MUSCLE AND SOFT TISSUE INFECTIONS

### 2.1. *Clostridium perfringens*

*Clostridium perfringens* is the most important cause of clostridial disease in domestic animals (*table II*). As many as 17 exotoxins of *C. perfringens* are described in the literature (McDonel, 1986;

Hatheway, 1990; Rood and Cole, 1991), but a definitive role in pathogenesis has been demonstrated for only a few. The species is divided into types on the basis of production of the four major toxins,  $\alpha$ ,  $\beta$ ,  $\epsilon$  and  $\iota$  (*table III*), as determined by in vivo protection tests in guinea pigs or mice (Walker, 1990).  $\alpha$  toxin is hemolytic, necrotizing and potentially lethal (Rood and Cole, 1991), causing cytotoxicity through hydrolysis of sphingomyelin and other membrane phospholipids (Elder and Miles, 1957; Smith, 1979). Genes with significant sequence homology to *cpa*, the  $\alpha$  toxin gene (Titball et al., 1989), can be found in other clostridia (Titball et al., 1993b).

Mucosal necrosis and, possibly, central nervous system (CNS) lesions are caused by  $\beta$  toxin (Jolivet-Reynaud et al., 1986). The  $\beta$  toxin gene (*cpb*) has significant sequence homology with  $\alpha$  toxin,  $\gamma$  toxin and leukocidin of *Staphylococcus aureus* (Hunter et al., 1993). The mouse minimum lethal dose (MLD) is about 500 ng per kg IV (Sakurai and Fujii, 1987). Proteolysis of  $\epsilon$  prototoxin (McDonel, 1986) converts it to  $\epsilon$  toxin, resulting in a > 1 000-fold increase in toxicity (Bhown and Habeeb, 1977).  $\epsilon$  toxin

**Table I.** Clostridia as agents of disease in small ruminants.

Organism	Major toxin(s)	Disease(s)	Species affected
<i>C. septicum</i>	$\alpha$	abomasitis (sheep) malignant edema (cattle, sheep)	sheep, cattle
<i>C. chauvoei</i>	$\alpha, \beta$	blackleg	sheep, cattle
<i>C. novyi</i>	$\alpha, \beta$	Wound infections (‘bighead’), infectious necrotic hepatitis	sheep, goats
<i>C. haemolyticum</i>	$\beta$	bacillary hemoglobinuria	cattle
<i>C. botulinum</i>	botulinum toxins A, B, C <sub>1</sub> , D, E, F, G C <sub>2</sub> (ADP-ribosylating)	botulism	sheep, cattle
<i>C. tetani</i>	tetanus toxin tetanolysin	tetanus	sheep, cattle, goats

**Table II.** Diseases produced by toxigenic types of *C. perfringens*.

Toxin type	Major toxins	Diseases
A	$\alpha$	myonecrosis, food poisoning, necrotic enteritis in fowl, enterotoxemia in cattle and lambs, necrotizing enterocolitis in piglets; possibly equine colitis, canine hemorrhagic gastroenteritis
B	$\alpha, \beta, \epsilon$	dysentery in newborn lambs, chronic enteritis in older lambs (‘pine’), hemorrhagic enteritis in neonatal calves and foals, hemorrhagic enterotoxemia in adult sheep
C	$\alpha, \beta$	enteritis necroticans (pigbel) in humans, necrotic enteritis in fowl, hemorrhagic or necrotic enterotoxemia in neonatal pigs, lambs, calves, goats, foals, acute enterotoxemia (‘struck’) in adult sheep
D	$\alpha, \epsilon$	enterotoxemia in lambs (‘pulpy kidney’) and calves, enterocolitis in neonatal and adult goats, possibly enterotoxemia in adult cattle
E	$\alpha, \iota$	enterotoxemia likely in calves and lambs, enteritis in rabbits; host range and disease type unclear
A–E	enterotoxin	canine and porcine enteritis; possibly bovine and equine enteritis

Constructed from MacLennan, 1962; McDonel, 1980; Niilo, 1980; Rood and Cole, 1991.

**Table III.** Activities of the major toxins of *C. perfringens*.

Toxin	Activity
$\alpha$	phospholipase/sphingomyelinase C, hemolytic, lethal, necrotizing
$\beta$	induces inflammation, necrosis of intestinal mucosa, lethal
$\epsilon$	protease-activated protoxin; increases intestinal permeability; central nervous system toxicity; lethal
$\iota$	Ia ADP-ribosylates actin, Ib mediates binding; dermonecrotic, lethal

is necrotizing (Buxton, 1978), and the mouse MLD is about 300 ng (Sakurai and Fujii, 1987). Component Ia of  $\iota$  toxin ADP-ribosylates globular skeletal muscle and nonmuscle actin (Stiles and Wilkins, 1986a, b; Vanderkerckhove et al., 1987); binding to sensitive cells and entry to the cytosol is mediated by Ib (Stiles and Wilkins, 1986b; Considine and Simpson, 1991; Rood and Cole, 1991). Physiologic effects include increased vascular permeability, dermonecrosis and lethality (Bosworth, 1943; Craig and Miles, 1961).  $\iota$  toxin is similar in structure and activity to *C. spiroforme* toxin and C2 toxin of *C. botulinum* types C and D (Perelle et al., 1993). It is antigenically similar to an ADP ribosyltransferase produced by *C. difficile* (Popoff et al., 1988).

While not considered a major toxin in the classical sense, enterotoxin (CPE) is often an important virulence attribute of *C. perfringens* (McDonel, 1986; McClane et al., 1988; Granum and Stewart, 1993). CPE production and sporulation are coregulated, and toxin is released when the vegetative cell is lysed. Proteolytic cleavage of CPE is followed by binding (via a C-terminal domain) and cytotoxicity (via a N-terminal domain). Pore formation results in altered permeability, inhibition of macromolecular synthesis, cytoskeletal disintegration and lysis (Hulkower et al., 1989; McClane and Wnek, 1990). CPE and its gene (*cpe*) can occur in strains of all

five toxigenic types (Cole, 1995; Songer and Meer, 1996; Meer and Songer, 1997).

Direct proof (i.e. in vivo studies with isogenic mutants) of a role in pathogenesis is lacking for all toxins of *C. perfringens*, with the exception of  $\alpha$  and  $\theta$  toxins in histotoxic infections (Awad et al., 1995). Results of studies of the direct effects in vivo of purified toxins, or of vaccination/challenge studies, are compelling, and there is little question that the other major, and probably minor, toxins are important in pathogenesis.

Strains of type A are important causes of wound contamination, anaerobic cellulitis and gas gangrene (Hatheway, 1990), and  $\alpha$  toxin plays a central role in pathogenesis (Awad et al., 1995). Antibodies against native  $\alpha$  toxin and against a genetically truncated C-terminal portion of the molecule (amino acids 247–370) protect mice against challenge with toxin or multiple LD<sub>50</sub> of *C. perfringens* (Titball et al., 1993a; Williamson and Titball, 1993).

Type A, as well as other types of *C. perfringens*, cause enteric disease in sheep, and are discussed below.

The key diagnostic components are evaluation of clinical signs and lesions and bacteriologic culture; detection of toxins is also important, but is rarely practised in many parts of the world (Walker, 1990; Carter and Chengappa, 1991). Cytotoxicity assays (Berry et al., 1988; Mahony

et al., 1989) and immunoassays (McClane and Strouse, 1984; Harmon and Kautter, 1986; McClane and Snyder, 1987; Berry et al., 1988; Cudjoe et al., 1991) for CPE have been reported, as have immunoelectrophoresis (Henderson, 1984), latex agglutination (Martin and Naylor, 1994), immunodiffusion (Beh and Buttery, 1978), and ELISA (Naylor et al., 1987; Martin et al., 1988; El-Idrissi and Ward, 1992; Holdsworth and Parratt, 1994) for enterotoxemia-associated toxins. Toxin detection alone does not confirm the existence of disease and failure to demonstrate toxins (particularly  $\beta$  toxin in gut contents), can be expected owing to protease degradation.

Gene probes and PCR assays have been reported (Havard et al., 1992; Saito et al., 1992; Fach and Guillou, 1993; Daube et al., 1994; Kokai-Kun et al., 1994; Songer and Meer, 1996; Meer and Songer, 1997). In one study of more than 750 strains from bovine enterotoxemia, all hybridized with probes for  $\alpha$  toxin and sialidase genes, most with a probe for the  $\theta$  toxin gene, a few with the probe for the *cpe*, and none with probes for *cpb*, the  $\epsilon$  toxin gene (*etx*) and the  $\tau$  toxin genes (*iap* and *ibp*) (Daube et al., 1994). PCR primers derived from the sequences of *cpa*, *cpb*, *etx*, *iap* and *ibp* have been successfully used to amplify toxin genes (Fach et al., 1993; Songer and Meer, 1996; Meer and Songer, 1997).

## 2.2. *Clostridium septicum*

*Clostridium septicum* is commonly found in soil and in the feces of domestic animals (Princewill, 1985). Flukes can carry spores into the livers of sheep (Petrov et al., 1985), and iatrogenic infections occur (Harwood, 1984; Mullaney et al., 1984), most commonly in horses. Wound infections by *C. septicum* are often called malignant edema, and usually follow direct contamination of a traumatic

wound, including those incurred through castration or docking. Umbilical infections are not uncommon in sheep (Timoney et al., 1988).

Hemorrhage, edema and necrosis develop rapidly as the infection spreads along muscular fascial planes. Early lesions are initially painful and warm, with pitting edema, but with time, the tissue becomes crepitant and cold. Death follows, often in less than 24 h.

Toxic or potentially toxic products of *C. septicum* include  $\alpha$  toxin [oxygen-stable hemolysin (Ballard et al., 1992)],  $\beta$  toxin (DNase, leukocidin),  $\gamma$  toxin [hyaluronidase (Princewill and Oakley, 1976)],  $\delta$  toxin (oxygen-labile hemolysin), a neuraminidase and hemagglutinin (Gadalla and Collee, 1968), a chitinase (Clarke and Tracey, 1956) and sialidase (Zenz et al., 1993). Unambiguous statements about a role in pathogenesis can be made only for  $\alpha$  toxin. Purified  $\alpha$  toxin is a cationic protein of about 48 kDa, which is activated by proteolytic removal of a 4-kDa carboxy-terminal fragment (Ballard et al., 1993). The MLD is about 10  $\mu$ g per kg (Ballard et al., 1993) and death following *C. septicum* challenge is delayed in  $\alpha$  toxin-immunized mice. Although a role for potential virulence attributes other than  $\alpha$  toxin has not been proven (Hatheway, 1990), it seems likely that, in combination, they increase capillary permeability and cause myonecrosis and systemic toxicity (Riddell et al., 1993).

The brief clinical course dictates a preference for prevention rather than treatment. Antibody responses to somatic and toxin antigens (Hjerpe, 1990; Gyles, 1993) yield lifelong immunity (Green et al., 1987). Diagnosis of malignant edema is based upon clinical signs, gross and microscopic findings at post mortem, Gram-stains of direct smears and bacteriologic culture (Carter, 1984). *C. chauvoei* infection should be ruled out (Carter and Chengappa, 1991) by use of a rapid method

such as a fluorescent antibody test (Batty and Walker, 1963).

### 2.3. *Clostridium chauvoei*

*Clostridium chauvoei* causes blackleg, an emphysematous, necrotizing myositis (table 1), which, in sheep, most often resembles malignant edema or gas gangrene. Affected animals may develop high fever, anorexia, depression and lameness, with crepitant lesions, but sudden death is common. The central areas of lesions are dry and emphysematous, while the periphery is often edematous, hemorrhagic and necrotic. Evidence of leukocytic infiltration is negligible (Timoney et al., 1988; Gyles, 1993). The roles of  $\alpha$  toxin, which is necrotizing, hemolytic and lethal, and  $\beta$  toxin, a DNase which may be responsible for degeneration of muscle cell nuclei (Ramachandran, 1969), have not been precisely defined.

Protection follows vaccination, and apparently arises from the immune response to a heat-labile, soluble antigen (Verpoort et al., 1966). Equine hyperimmune serum and penicillin can be used for therapy and prophylaxis.

### 2.4. *Clostridium novyi*

*Clostridium novyi* type C is nontoxigenic and avirulent, but strains of type A cause gas gangrene in humans and wound infections in animals, and the hallmark lesion is edema. 'Bighead' with rapidly spreading edema of the head, neck and cranial thorax, occurs in young rams following invasion by *C. novyi* type A of subcutaneous tissues damaged by fighting (Sterne and Batty, 1975).

Infectious necrotic hepatitis ('black disease') of sheep and cattle is the result of *C. novyi* type B infection. Dormant spores germinate in liver tissue, often damaged

by fluke migration, and dissemination of  $\alpha$  toxin yields systemic effects with acute or peracute death (Elder and Miles, 1957). Its cardio-, neuro- histo- and hepatotoxic effects produce edema, serosal effusion and focal hepatic necrosis (Elder and Miles, 1957; Aikat and Dible, 1960; Cotran, 1967; Rutter and Collee, 1967). The name 'black disease' derives from the characteristic darkening of the underside of the skin due to venous congestion. Roles of other toxins, including  $\beta$  (lecithinase),  $\gamma$  (necrotizing phospholipase D),  $\delta$  (oxygen-labile hemolysin) and  $\epsilon$  (lipase), are uncertain. *C. novyi* type D, often referred to as *C. haemolyticum*, causes bacillary hemoglobinuria of cattle (Smith and Williams, 1984).

Typically, there is no effective treatment for *C. novyi* infections, but effective prophylaxis with bacterin:toxoids or toxoids can be achieved (Timoney et al., 1988).

## 3. ENTERIC DISEASES

### 3.1. *Clostridium perfringens*

*Clostridium perfringens* type A causes enterotoxemia, or yellow lamb disease, which occurs primarily in the western US (McGowan et al., 1958). Depression, anemia, icterus and hemoglobinuria, are followed by death after a clinical course of 6–12 h, and large numbers of *C. perfringens* are found in intestinal contents. A similar condition occurs in goats (Russell, 1970), and type A probably also causes tympany, sometimes accompanied by hemorrhagic, necrotic abomasitis in calves. Gram-positive bacilli are demonstrable on the mucosa and in the submucosa and  $\alpha$  toxin is found in intestinal contents (Roeder et al., 1988). Intravascular hemolysis, capillary endothelial damage, platelet aggregation, shock and cardiac effects in

natural infections are predictable systemic actions of a hemolytic toxin (Stevens et al., 1988; Timoney et al., 1988). Chymotrypsin resistance of  $\alpha$  toxin from enterotoxemia isolates may allow accumulation in the gut and entry to circulation (Ginter et al., 1995).

*C. perfringens* type B is frequently isolated from cases of dysentery in newborn lambs (table II) and hemorrhagic enteritis in goats (Frank, 1956). Disease is more common in the UK, South Africa and the Middle East than in the US (Timoney et al., 1988). In lambs, inappetence, abdominal pain and bloody diarrhea are followed by recumbency and coma. Lesions consist primarily of hemorrhagic enteritis, with evidence of enterotoxemia (Frank, 1956). Chronic disease in older lambs ('pine') is characterized by chronic abdominal pain without diarrhea. Pathogenesis of type B infections may be due to additive or synergistic effects of  $\alpha$ ,  $\beta$  and  $\epsilon$  toxins.

Neonates of most species are highly susceptible to infection by *C. perfringens* type C (MacKinnon, 1989) (table II), and colonization in advance of normal intestinal flora or alteration of flora by dietary changes are significant factors in pathogenesis (Timoney et al., 1988). In lambs, type C infection resembles lamb dysentery, and may be accompanied by nervous signs, including tetany and opisthotonus. Peracute death, occasionally without other clinical signs, is not uncommon, but the clinical course may also extend to several days. Young ewes and other adult sheep can also develop type C enterotoxemia, a condition known as 'struck', in which the clinical disease occurs so rapidly that it often suggests that the animal has been struck by lightning. Mucosal damage, perhaps caused by poor quality feed, facilitates abomasal and small intestinal multiplication of organisms, with resulting mucosal necrosis. Fluid accumulation in the peritoneum and thoracic cavity sug-

gest toxemia, and enteric lesions, dysentery and diarrhea are often absent (Sterne and Thomson, 1963). Similarities of *cpb*, the  $\beta$  toxin gene, to the genes for staphylococcal  $\alpha$  and  $\gamma$  toxins and leukocidin (Hunter et al., 1993), strengthen suggestions that  $\beta$  toxin may affect the CNS (Jolivet-Reynaud et al., 1986; McDonel, 1986). However, hemorrhagic enterotoxemia has not been reproduced in lambs by inoculation with cell-free culture supernatant fluid (Niilo, 1986).

Enterotoxemia ('overeating') in sheep of all ages except newborns is caused by *C. perfringens* type D (table II) (Timoney et al., 1988). Lambs 3–10 weeks old, suckling heavily lactating ewes, are commonly affected, as are feedlot animals up to 10 months of age. Upsets in the gut flora, following sudden changes to a rich diet, continuous feeding of concentrates (Popoff, 1984), and the presence of excess dietary starch in the small intestine are often involved.  $\epsilon$  toxin facilitates its own absorption (Niilo, 1993), resulting in toxemia with little or no enteritis. Some animals display dullness, retraction of the head, opisthotonus and convulsions (Niilo, 1993; Popoff, 1984), but sudden death is common. Degeneration and necrosis in the CNS is typical (Buxton and Morgan, 1976), and focal encephalomalacia is a chronic neurological manifestation of non-fatal disease (Griner, 1961; Buxton and Morgan, 1976). The extent of incoordination and convulsions is directly related to the severity of lesions (Griner, 1961). Peritoneal and pericardial effusions are typical in sheep, and glycosuria is pathognomonic (Gardner, 1973; Niilo, 1993). The common name 'pulpy kidney' derives from the post mortem autolysis of hyperemic, toxin-damaged tissue.

Goats develop catarrhal, fibrinous, or hemorrhagic enterocolitis. The condition is often chronic, and pulpy kidney is absent (von Rotz et al., 1984; Blackwell and Butler, 1992).

*C. perfringens* type E is an apparently uncommon cause of enterotoxemia of lambs (table II), and recent isolates have been obtained from calves with hemorrhagic enteritis, in the western and mid-western US (Meer and Songer, 1997). However, type E remains of uncertain overall importance in animal disease.

An increasing body of evidence suggests a role for enterotoxigenic strains, particularly of type A, in the etiology of diarrheal conditions in several animal species (Estrada-Correa and Taylor, 1989; Niilo, 1993). In one study, CPE production was observed in 12 % of isolates from cattle, sheep and chickens with enteritis (Niilo, 1978), and in another, genotyping revealed that about 5 % of isolates are enterotoxigenic, with most of these being type A (Songer and Meer, 1996; Meer and Songer, 1997).

CPE is weakly immunogenic when administered via the intestinal tract. Disease gives rise to serum antibodies in sheep and other domestic species, but antibodies produced following parenteral inoculation are not protective (Niilo and Cho, 1985; Estrada-Correa and Taylor, 1989). The best target for immunoprophylaxis may be the toxin's membrane binding event (Hanna et al., 1989; Mietzner et al., 1992).

Immunoprophylaxis is a control measure of paramount importance, due to the rapid and frequently fatal course of disease caused by the various types of *C. perfringens*. Lambs born to ewes vaccinated against types B, C or D are protected against dysentery (Smith and Matsuoka, 1959; Kennedy et al., 1977; Odendaal et al., 1989), and may be immunized at 3 days of age (Kennedy et al., 1977). Enterocolitis, but not toxemia, may occur in vaccinated goats (Blackwell et al., 1991; Blackwell and Butler, 1992).

### 3.2. *Clostridium septicum*

*Clostridium septicum* also causes enteric infections (Schamber et al., 1986) (table I). The organism penetrates the lining of the abomasum of sheep, producing braxy, a fatal bacteremia (Saunders, 1986). Mortality rates are high in yearling sheep in the UK, Norway and Iceland, and cases have been reported in Europe, Australia (Ellis et al., 1983) and the US. The pathogenesis of *C. septicum*-infection is not well understood, but impaired mucosal function may follow ingestion of frozen feed. The organism then multiplies and disseminates, producing local lesions and toxemia (Saunders, 1986; Schamber et al., 1986). Edema, hemorrhage, and necrosis occur in the abomasum and proximal small intestine (Ellis et al., 1983). The pathogenesis is not well-understood, but  $\alpha$  toxin is probably of primary importance.

## 4. NEUROTOXIC DISEASES

### 4.1. *Clostridium botulinum*

Botulism, caused by *C. botulinum*, is an intoxication with any of seven neurotoxins, which results in neuroparalysis (Smith and Sugiyama, 1988; Rocke, 1993) (table I). Botulinum toxins share the ability to block acetylcholine release from cholinergic nerve endings (Simpson, 1981), but are serologically distinct.  $C_2$  toxin is not neurotoxic, but has ADP-ribosylating activity similar to  $\iota$ , toxins of *C. perfringens* and *C. spiroforme* (Ohishi 1983; Simpson, 1989).

Singular names, including loin disease and lamziekte (cattle), limberneck and western duck sickness (waterfowl), and spinal typhus and shaker foal syndrome (horses), have been applied to the various conditions affecting animals. The disease in sheep can arise from many sources

(Smith and Sugiyama, 1988). Phosphorus deficiency may encourage pica, leading to consumption of botulinum toxin-containing carcasses, and death due to botulism. Clinical signs include anorexia, incoordination, ataxia, difficulty in swallowing and excessive salivation. Flaccid paralysis, affecting the respiratory system, eventually causes death of the animal.

Dogma states that enough toxin to immunize is more than enough to kill (Timoney et al., 1988; Rocke, 1993), but toxoids of botulinum toxin are used for immunoprophylaxis (Jansen et al., 1976; Johnston and Whitlock, 1987; Smith and Sugiyama, 1988). Polyvalent antitoxins can be effective for therapy.

#### 4.2. *Clostridium tetani*

Tetanus, caused by *C. tetani*, usually follows contamination of a wound or the umbilicus by soil, but nonaseptic surgery, docking and castrating, and ear tagging may also be initiating factors. The wound may be trivial, but necrosis is usually required to provide conditions of lowered oxygen and allow germination of spores. The incubation period varies with the toxinogenicity of the strain, rate of transfer of toxin to the target tissues, and the relative susceptibility of the host, and may range from 24 h to 2 weeks (Kryzhanovsky, 1981; Wellhöner, 1982). Toxin is transported retrograde, moving intraaxonally via the peripheral motor nerve endings. It binds to presynaptic axonal terminals, resulting in hyperactivity of motor neurons. Clinical signs include muscular tremor and increased stimulus response, impaired muscle function in the head and neck, and difficulty in chewing and swallowing due to trismus. Spasms give way to permanent rigidity, respiration becomes increasingly difficult, and death follows in a few days to less than 2 weeks, with a

case fatality rate of at least 50 % (Timoney et al., 1988).

Strains of *C. tetani* which do not produce tetanus toxin are avirulent, and widespread vaccination with toxoid has dramatically lessened the impact of tetanus on animal production. Passive immunity acquired from the dam protects for 2–3 months. Attention to apparent wounds, and administration of penicillin to halt production of toxin and antitoxin to neutralize preformed toxin, are useful therapeutics.

The past decade has brought many advances in the understanding of the nature and mechanism of action of tetanus and botulinum neurotoxins. These molecules are two-chain polypeptides of  $M_r$  150 000. Blockade of neurotransmitter release in the CNS (glycine and GABA by tetanus toxin and acetylcholine by botulinum toxins) causes spastic (in tetanus) or flaccid (in botulism) paralysis. Binding to specific receptors on nerve terminals is followed by internalization of toxin into neurons (Poulain et al., 1996). The light chain of the toxin molecule, which has zinc-dependent endopeptidase activity (de Paiva et al., 1993; Li et al., 1994), is translocated from the endosomal compartment to the cytosol, where it specifically attacks one of three synaptic proteins (VAMP/synaptobrevin, syntaxin, or SNAP-25) which mediate exocytosis of neurotransmitters (Cornille et al., 1997; Galli et al., 1994). A second, recently reported, inhibitory mechanism does not involve proteolysis, but may result from activation of neuronal transglutaminases (Deloye et al., 1997; Poulain, 1994).

#### 5. CONCLUSION

Control by vaccination has decreased the incidence, and perhaps also the visibility, of clostridial diseases in domestic animals. This seems particularly true of

enteric diseases. Renewed interest in mechanisms of pathogenesis has yielded new information about clostridia, and particularly in the mode of action of their toxins. Genetic systems in clostridia are still relatively primitive, but great progress in development of shuttle vectors, methods for transformation and other genetic manipulations holds the promise of rapid advances in the immediate future. Continued accumulation of knowledge on the role of toxins in clostridial diseases will probably yield improved prophylaxis as a practical end result.

The growing concern with undesirable post-vaccination effects, such as injection site reactions leading to trimming at slaughter, has stimulated the veterinary biologic industries to seek a new paradigm for the preparation and delivery of immunoprophylactic products. Recombinant proteins, delivered by conventional means, by application of 'slow-release' media, or by in vivo expression from attenuated bacterial delivery systems, will likely be a focus of major effort in this arena.

Improved methods for diagnosis also stand to have an impact on the future incidence of clostridial diseases. Some of these new methods will be based upon immunologic detection of organisms or toxins, others will involve detection of specific microbial genes, and, with new knowledge on the specific activity of clostridial toxins, still others may be founded on detection of toxin activities (e.g. endopeptidase activity of tetanus and botulinum neurotoxins). It is important to provide animal producers, veterinary practitioners and diagnosticians with better tools for day-to-day management of disease cases, whether sporadic or epidemic. It may be more important to consistently apply sensitive and specific diagnostic methods, and to take advantage of opportunities to communicate results of such testing to the animal health community,

providing a better sense of the importance of various clostridial diseases.

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