ISSN 0008-5286

THE CANADIAN VETERINARY JOURNAL

LA REVUE VÉTÉRINAIRE CANADIENNE

Volume 21 **May-mai 1980** **No. 5**

Clostridium perfringens in Animal Disease: A Review of Current Knowledge

L. NIILO*

SUMMARY

The diseases caused by various types of *Clostridium perfringens* are critically reviewed in the light of current knowledge. Particular emphasis is placed on information concerning these diseases in Canadian livestock.

There are two etiologically clearly-defined acute *C. perfringens* diseases recognized in Canada: hemorrhagic enteritis of the newborn calf, caused by *C. perfringens* type C, and enterotoxemia of sheep, caused by type D. *Clostridium perfringens* type A may play a role as a secondary pathological agent in various disease conditions, such as necrotic enteritis of chickens. It may also cause wound infections and may provide a source for human food poisoning outbreaks.

There appears to be a considerable lack of knowledge regarding the distribution of *C. perfringens* types, their pathogenesis, diagnosis and the incidence of diseases caused by this organism.

RÉSUMÉ

Clostridium perfringens dans les maladies animales: une revue de nos connaissances actuelles

L'auteur présente une revue critique des maladies attribuables aux différents types de *Clostridium perfringens,* à la lumière de nos connaissances actuelles. Il insiste surtout sur les informations relatives aux clostridioses qui affectent le cheptel canadien.

On connaît deux maladies aiguës, définitivement attribuables à *C. perfringens,* au Canada: l'entérite hémorragique du veau naissant, due au type C de *C. perfringens,* et l'entérotoxémie ovine, attribuable à son type D. Le type A de *C. perfringens* joue probablement un rôle, à titre d'agent pathogène secondaire, dans certaines maladies telles que l'entérite nécrotique du poulet. Il peut aussi contaminer les plaies et provoquer des empoisonnements alimentaires, chez l'homme.

Nos connaissances semblent lacunaires en ce qui concerne la distribution des différents types de *C. perfringens,* leur pathogénèse, le diagnostic et l'incidence des maladies qu'ils causent.

INTRODUCTION

Clostridium perfringens usually forms a part of the normal intestinal flora of man and animals and can be found in soil. The pathogenic importance of this organism as the causative agent of gas gangrene was discovered at the end of the last century and since then the organism has been the object of intensive study (29). Besides its involvement in gas gangrene and food poisoning in man, various forms of acute enteritis and fatal enterotoxemias in animals have been attributed to *C. perfringens.* Numerous articles and reviews on the etiology, pathogenesis and clinical aspects of such infections have appeared in the literature. However, much of this information is historical and scattered among various scientific disciplines and publications.

This paper presents a brief critical review of current knowledge regarding *C. perfringens* in veterinary medicine, with particular emphasis on its importance in Canada. It is intended as a guide for practitioners, laboratory diagnosticians and students interested in this subject; also, it may be applicable as introductory material for research workers in related fields. No attempt is made here to provide a comprehensive review of the literature. Such reviews already exist. For those readers who are interested in thorough literature surveys for historical, academic or other reasons the following references are recommended: (9, 24, 29, 39, 51, 56, 62, 64, 69).

Clostridium perfringens TYPES

The *C. perfringens* species is a very heterogeneous group of organisms with respect to their metabolic by-products, toxins and pathogenic potential. For practical classification purposes, the species is divided into five types, from A to E, based on their ability to produce any of the four major lethal toxins (Table I). As these toxins are antigenic, typing is achieved by neutralization of the lethal toxins with type-specific antisera using mice or guinea pigs as test animals. In addition, the various types may produce

TABLE I
MAJOR LETHAL TOXINS OF *C. PERFRINGENS* FOR TYPE DETERMINATION

	Toxins			
Type	Alpha	Beta	Epsilon	Iota
A	++	–	–	–
B	+	++	+	–
C	+	++	–	–
D	+	–	++	–
E	+	–	–	++

++ = Produced as a predominant toxic fraction.
+ = Produced in smaller quantities.
– = Not produced.

*Agriculture Canada, Animal Diseases Research Institute (Western), P.O. Box 640, Lethbridge, Alberta, Canada T1J 3Z4.

other antigenic substances, some of which possess pathogenic properties. Accordingly, varieties are known to exist within types A, B and C which produce certain combinations of antigens or toxins and are associated with clearly defined disease syndromes. The quantity of any of these substances produced varies greatly between individual strains. In classical studies 12 antigenic substances were described, designated by the Greek letters from alpha to nu (7, 56, 65). Sometimes these antigens have been collectively referred to as "toxins" or "toxin components" (29) regardless of their individually demonstrated pathogenicity. Mostly extracellular, they are produced during active growth of the organism. These antigens are listed in Table II, rearranged and grouped according to their practical significance. Group 1 contains the major lethal toxins and minor toxins that are important, either in pathogenesis or for identification. Group 2 contains relatively unimportant substances that may have significance for research purposes. Gamma- and eta-antigens, originally included among the toxins, are not included in this table because these have not been demonstrated with certainty and consistency (24). New substances, discovered since Oakley's original classification (56), are urease, neuraminidase, fibrinolysin (29, 62) and enterotoxin (25). None of these are designated by Greek letters; enterotoxin being a significant toxin discovered in the late 1960s (13, 26) and characterized in early 1970s (25, 63). Also, an early classification included type F which has now been discontinued. The strains with characteristics of type F have now been transferred to type C (65).

Table III summarizes the *C. perfringens* types and varieties associated with disease conditions, correlated with epizootiological distribution. It is evident that the pathology and clinical picture of a disease caused by *C. perfringens* depends largely on the toxins this organism produces. Each toxin has its own pharmacological action which may be cumulative in producing the final effect.

In the following paragraphs the five *C. perfringens* types and the diseases they produce are selectively reviewed on the basis of recently published literature, supplemented by the writer's experience at this Institute.

Type A

This is the most common of all the *C. perfringens* types, the most variable in toxigenic properties and also the most confusing organism in respect to its pathogenicity. In spite of continued intensive study, its role in the pathogenesis of diseases is still not fully understood. In its toxigenic behaviour type A can be subdivided into two varieties (Table III). The "classical" variety, characterized mainly by alpha-toxin production, is associated with gas gangrene, traumatic infections, avian necrotic enteritis and the normal intestinal tract. The enterotoxigenic or human food poisoning variety, characterized by enterotoxin production, is capable of causing human enteritis.

Alpha-toxin production by strains isolated from normal intestinal contents or feces may be so little on laboratory media that it is impossible to type the organism by the toxin neutralization method. Alpha-toxin is an enzyme, chemically known as phospholipase-C (lecithinase-C), which hydrolyzes lecithin into phosphorylcholine and a diglyceride (29). As the membranes of most cells consist of lipoprotein complexes containing lecithin, alpha-toxin leads to their destruction. The resultant biological effect is either hemolysis, necrosis or death, depending on tissues accessible to the toxin. The alpha-toxin, when isolated and purified, has moderate potency [up to 40 ng for median lethal dose (LD_{50})] in mice, which is approximately 1/2000 the potency of *C. tetani* and *C. botulinum* toxins (64). In most culture filtrates its lethal activity in mice may range from zero to 100 minimum lethal doses (MLD) per mL (40, 55, 64). A few exceptional strains grown under favorable, controlled conditions may yield crude toxic filtrates with several hundred MLD/mL (29). It must be emphasized that the values reported by different workers and the methods used for analyses are often diverse due to great technical difficulties involved in such work.

In gas gangrene, the pathological effects of type A are not only due to alpha-toxin alone. The total toxic product, sometimes singularly referred to as "*C. perfringens* toxin" (29), contains several other accessory

TABLE II
C. PERFRINGENS TOXIC OR ANTIGENIC COMPONENTS AND SOME OF THEIR CHARACTERISTICS

Toxin or Antigen	Produced by Types[a]	Major Importance In: Pathogenesis	Major Importance In: Diagnosis	Characteristics
Group 1				
Alpha	A-E	X	X	Lecithinase (calcium-dependent phospholipase-C), hemolytic, necrotizing, lethal
Beta	B,C	X	X	Necrotizing, lethal, trypsin-labile
Delta	(C)		X	Hemolytic
Epsilon	B,D	X	X	Necrotizing, lethal, activated by proteolytic enzymes, increases capillary permeability
Theta	(A-C),D,E		X	Hemolytic, oxygen-labile
Iota	E	X	X	Necrotizing, lethal, activated by proteolytic enzymes
Enterotoxin	(A,C,D)	X	X	Sporulation-dependent enterotoxin, resistant to proteolytic enzymes
Group 2				
Kappa	(A-D),E			Collagenase, necrotizing
Lambda	(B,D)E			Nonspecific proteinase
Mu	(A-E)			Hyaluronidase
Nu	(A-E)			Desoxyribonuclease
Urease, Neuraminidase, Fibrinolysin	(A)?			Enzymes occasionally found. Apparently of no practical significance

[a]Types shown in brackets indicate production by some strains or varieties only.
Gamma- and eta-antigens are deleted from this table.
This table is based on data from several sources (24, 29, 39, 56, 65).

substances which may exert a cumulative effect and exacerbate infection. These minor toxins are: theta-toxin, a hemolysin which destroys erythrocytes and leucocytes, the collagenase (kappa-toxin) which hydrolyses collagen of connective tissue and the hyaluronidase (mu-toxin) which splits mucopolysaccharides and thus promotes spread of toxins in the tissues. These substances (possibly including theta-toxin) are enzymes produced and released during active bacterial growth (29). Theta hemolysin produces the clear zone of hemolysis usually seen on sheep or ox blood-agar media while alpha-toxin is responsible for the incomplete zone of the double hemolytic effect.

Conflicting views exist regarding the clinical effects produced by toxins alone and those attributable to bacterial cell invasion. Typically, a gas gangrene lesion consists of massive destruction of tissue accompanied by severe toxemia and shock. As it has been shown in wide ranging studies (39, 40), gas gangrene can be produced by type A strains which are poor exotoxin producers (27). Isolated toxin fractions consistently fail to produce progressive lesions (29, 39). There is little evidence to suggest that these toxins are exclusively responsible for the pathogenesis of gas gangrene. This would support an alternative explanation such as the invasion and multiplication of the bacteria within the lesion (9). *Clostridium perfringens* is often accompanied by other clostridia and even unrelated bacteria which further confuse the etiological picture of the lesions.

Type A has also been reported as causing enterotoxemia in sheep and calves, sometimes referred to as "sudden death" (51, 64). However, most of these reports are unconfirmed and based largely on isolation of the organism from the intestinal tract and nonspecific clinical signs. This type is easily isolated from tissues, effusions and intestinal tract of cadavers within a few hours after death; it grows rapidly in culture and may mask other organisms, thus giving the impression that it was the only organism present in the tissue examined. Experimentally, it has not been possible to produce acute enterotoxemia in calves and sheep by intraduodenal administration of type A cultures or cell-free toxic filtrates (55). Intravenous injections of crude toxin have proven fatal only if relatively large amounts were given in a short time (55). When slowly administered the toxin was even less effective, probably due to its rapid catabolism and excretion (15). It appears unlikely that copious quantities of alpha-toxin are readily produced in the intestinal tract and rapidly absorbed to cause fatal toxemia. However, the presence in the intestinal tract of a highly toxigenic *C. perfringens* type A may contribute to some other enteric pathology as discussed below.

In poultry, type A has been shown to be capable of causing necrotic enteritis (57). In recent extensive studies necrotic enteritis was produced in young chickens by infusion of type A cultures into the duodenum as well as by dosing with toxic cell-free culture filtrates and by administering live cultures in feed (1, 2, 3, 37). The latter finding seems to support the hypothesis that the toxins rather than the invading organisms are involved in the pathogenesis of this disease. It may be a combination of both factors. In fact, a number of strains isolated from natural avian necrotic enteritis cases in Canada have been found to be potent alpha-toxin producers (50). Histologically, the lesions in chickens have been likened to those of necrotic enteritis of swine (3). In earlier research reports on necrotic enteritis in chickens in Britain, *C. perfringens* type C was thought to be involved (57). Information on the exact mechanism of action of *C. perfringens* and its toxins in necrotic enteritis is not yet available. In Sweden, type A has also been associated with an acute enteric disease of horses (71).

The discovery of the enterotoxigenic variety of *C. perfringens* type A is perhaps the most significant recent research development. The enterotoxin (specifically so named and not to be confused with epsilon-toxin in type D enterotoxemia) is the causative toxin in *C. perfringens* food poisoning. A relatively mild and usually nonfatal human gastroenteritis follows ingestion of food heavily contaminated with this organism and subsequent production of enterotoxin in the intestinal tract (20). The enterotoxin

TABLE III
C. PERFRINGENS TYPES IN PATHOGENIC CONDITIONS[a]

Type	Variety	Found in Canada	Disease or Occurrence	Variety Antigenic Differences
A	1	Yes	Gas gangrene of man and animals. Avian necrotic enteritis. Intestinal commensal. Soil	No enterotoxin
	2	Yes	Food poisoning in man	Produces enterotoxin
B	1	No	Lamb dysentery. Enterotoxemia of foals (Britain)	Produces lambda, mu; no kappa
	2	No	Enterotoxemia of sheep and goats (Iran)	Produces kappa; no lambda, mu
C	1	No	Enterotoxemia ("struck") of sheep (Britain)	Produces delta
	2	Yes	Neonatal hemorrhagic enterotoxemia of calves and foals (Colorado) and lambs (Colorado, Britain)[b]	No delta. Combinations of theta, kappa, mu
	3	No	Enterotoxemia of piglets (Britain, U.S.A.)[c]	
	4	No	Necrotic enteritis of man and fowl (Germany)	Absence of the above
	5	No	Necrotic enteritis of man (Papua New Guinea)	As in variety 2, 3. Produces enterotoxin
D		Yes	Enterotoxemia of sheep, goats and possibly cattle	
E		Yes	Isolated from sheep and cattle, enteritis of rabbits	

[a]This table is based on data of Sterne and Warrack (65), simplified and revised to include later information.
[b]The antigenic position of British strains from lambs is not known; it could be variety 2, 3 or even 1.
[c]It is not clear whether the U.S. strains in pigs are identical to variety 2 or 3.

differs from the classical exotoxins by being produced intracellularly by the sporulating cells of *C. perfringens* and being released upon lysis of these cells (49). The toxin, resistant to digestive enzymes (14, 25, 44), does not necrotize tissues but causes an outpouring of fluid into the lumen of the small intestine, resulting in diarrhea (42, 44). The latest reports indicate that an identical enterotoxin has also been demonstrated in a few strains of *C. perfringens* type C and in one strain of type D (61, 68). In older literature, before the discovery of the enterotoxin, emphasis was erroneously placed on alpha-toxin as the causative agent of food poisoning. Furthermore, the food poisoning strains were serologically typed (17 serotypes) and classified on the basis of their heat resistance (28). It appears probable that the heat resistance of spores was an incidental selection mechanism to resist cooking and thus permit strain survival in food poisoning incidents. It is now known that heat resistant as well as heat sensitive enterotoxigenic strains exist (66). Serotyping serves a purpose in epidemiological investigations of *C. perfringens* food poisoning outbreaks. In spite of its mild clinical manifestations, this illness occurs more frequently than other common causes of bacterial food poisoning or infection, such as *Staphylococcus, Shigella* and *Salmonella* species (4, 36).

The enterotoxigenic *C. perfringens* appears to originate from animals since most of the sources incriminated in food poisoning outbreaks have been meats, particularly beef and poultry (28). The prevalence of these strains in domestic animals appears to vary. In a limited study conducted in Canada (50), 12% of strains isolated from intestinal tract of cattle, sheep and chickens (postmortem cases) were enterotoxigenic. A similar study of normal beef cattle and chickens in California revealed enterotoxigenic *C. perfringens* prevalence rates of 68% and 60% for the two species, respectively (67). There may be a difference in interpretation as the degree of enterotoxin production appears to vary greatly. Although most of the "highly enterotoxigenic" strains in the Canadian study were found associated with enteritis, it is not certain whether or not these strains had any direct role in the pathogenesis of the enteritis, but it appears that they had at least an accessory role. Experimentally, the *C. perfringens* enterotoxin is capable of causing transitory enteritis or enteropathogenic effects on the small intestine of calves, sheep, rabbits and chickens (14, 46, 52, 54). When administered intravenously, the toxin causes a rapid and profound fall in systemic blood pressure, resulting in shock and death in all the animals studied (43, 45, 48). The lethal dose of toxin for calves and sheep when given intravenously is about 13 μg/kg of body weight (47). It appears unlikely that an equivalent dose of enterotoxin is ever absorbed into the systemic circulation if large quantities are produced in the gut under disease conditions. Although the enteropathogenic strains are relatively poor alpha-toxin producers, experiments have shown that they are still capable of causing gas gangrene (27).

Prevention of infections or intoxications caused by type A is difficult and no significant success has been achieved in this field in the last few decades. Vaccination of animals is complicated for at least two important reasons: (i) the organism is so ubiquitous that it is impractical to resort to mass immunization and (ii) the enzymatic nature of the majority of the antigens in this toxin complex is a disadvantage, as enzymes are generally considered weak antigens in comparison to other proteins (29). The enterotoxin is moderately antigenic, but in experiments with parenterally immunized sheep the circulating antibody failed to protect against intraduodenal challenge with either enterotoxin or live cells. Similarly, repeated intestinal challenge (up to nine times) produced diarrheal response without immunity or measurable antibody response (54).

It appears that prevention of diseases caused by *C. perfringens* type A should be directed toward animal husbandry, sanitation, feeding and other related factors.

Type B

According to available reports, *C. perfringens* type B has not been found on the North American continent. It has been reported from Great Britain where it causes lamb dysentery with heavy losses in newborn lambs. It has been involved also in enterotoxemia of foals in that country (64). Lamb dysentery also exists in South Africa (30). Another variety of *C. perfringens* type B, based on its antigenic composition, has been found in enterotoxemia of sheep and goats in Iran (Table III). Type B produces both beta- and epsilon-toxins, but beta-toxin is usually the principal component. Vaccines have been developed to control lamb dysentery in high-risk sheep flocks (30).

Type C

Five antigenic-pathogenic varieties of *C. perfringens* type C have been implicated in various disease conditions (Table III), but their classification is somewhat confusing. The principal lethal toxin in all varieties of this type is beta-toxin. In broth cultures, the toxin potency usually ranges from 500 to 5000 MLD/mL for mice, but some strains may produce barely detectable levels of toxin (55, 64). Beta-toxin is more sensitive to enzymes, elevated temperatures and other environmental factors than the other major lethal toxins of *C. perfringens;* it is readily inactivated by trypsin and other proteolytic enzymes (41, 60). Little is known about the chemistry and pharmacology of this toxin; it has only recently been isolated in pure form (59) and shown to have an LD_{50} of 1.87μg for mice (60).

A variety of type C was shown to cause an acute fatal hemorrhagic enteritis in neonatal lambs and calves in Colorado in the early 1950s (22, 23). Twenty years later the same antigenic variety was found in fatal cases of hemorrhagic enteritis of newborn calves in southern Alberta (53). A consistent feature of this type of enteritis, besides its pathology, is that it appears to be restricted to very young animals, usually one to three days and always less than ten days old. A possible explanation for this age prevalence may be the fact that the digestive system of the newborn with less enzymic content may not permit inactivation of the beta-toxin.

Type C is sometimes found in nonfatal enteric conditions of older calves as well as in healthy adult cows. In Montana, a study of calf scours revealed 80% of the *C. perfringens* isolates to be type C and the same orga-

nism was also found in their dams (38). Many of these isolates were reported to lose their toxigenicity rapidly on laboratory culture. It is not known whether these isolates are antigenically identical to the Colorado variety or comprise a different, less pathogenic variety of type C. Mildly toxigenic type C strains have been reported from New Zealand (8). The role of these strains in the pathogenesis of calf scours is not known. Also, no clear relationship exists between type C associated with severe enteritis of piglets, reported in Minnesota and Iowa (6) and the Colorado variety.

Because of the diversity of pathological conditions, fragmentary information and the varieties of type C involved in countries outside Canada and the USA (Table III), further details will not be discussed here; interested readers should consult appropriate references (7, 12, 16, 17, 31, 64).

Recent advances in research concerning type C have been the purification of beta-toxin, as mentioned earlier. An enterotoxin, in a variety causing necrotic enteritis of man (a severe form of food poisoning) in Papua New Guinea, was shown to be antigenically and biologically identical to the enterotoxin of type A (60).

The use of vaccines and antitoxins as prophylactic measures in high-risk animals have met with varying success, in both cattle and swine (5, 23, 33, 35).

Type D

This is perhaps the best known pathogenic *C. perfringens* type, being widely regarded as the causative organism of fatal enterotoxemia of sheep or "overeating disease". It appears to have a world-wide distribution, but it is not a common intestinal commensal. It produces epsilon-toxin which is almost exclusively responsible for the host pathology and subsequent death. The toxin is produced in the gut by abundantly growing bacterial cells, aided or initiated by some factors of feeding ("overeating") and absorbed into the systemic circulation. The epsilon-toxin is resistant to digestive enzymes; in fact, these enzymes actually convert the freshly secreted less active prototoxin into the fully toxic form.

The epsilon-toxin has been isolated, purified and its pathogenicity studied for a number of years. The pure toxin has an LD_{50} of 1.5 ng in mice (64), but ordinary culture filtrates contain about 15 to 300 MLD/mL (55, 64). The toxin and crude preparations are immunogenic, allowing vaccines (toxoids) to be produced for the control of type D enterotoxemia (34).

Clinically, when large amounts of epsilon-toxin are produced in the gut, its absorption into the systemic circulation increases capillary permeability in many organs and tissues, including intestinal mucosa. This increases its absorption rate and consequently the systemic effects leading to extensive renal damage, hyperglycemia, hypertension and edema in various organs, including brain (9, 18, 19, 32). Detailed studies on the mode of action of epsilon-toxin, conducted on mice, have shown that it is capable of causing widespread damage, after binding to receptor sites on the surface of the endothelial lining of certain blood vessels, of loops of Henle and of distal convoluted tubules of the kidney and to the hepatic sinusoids. The mechanism involved in the uptake of the toxin is apparently mediated by an adenyl cyclase-cyclic adenosine 3′, 5′-monophosphate system (10, 11). A thorough review of enterotoxemia in sheep caused by *C. perfringens* type D has been written by Bullen (9).

Type D has also been isolated from bovine postmortem material and even from normal cattle (51, 64, 70). Such isolations are sometimes made from sheep without clinical disease (9). There have been very few confirmed reports of type D enterotoxemia in cattle showing typical clinical signs, pathology and laboratory findings (21). It is possible to produce type D enterotoxemia in cattle experimentally (55). Such experiments have shown quite distinctive pathological and bacteriological findings (similar to those in sheep) that permit a definitive diagnosis. It appears that naturally occurring acute type D enterotoxemia in cattle is relatively rare.

Recently an enterotoxin immunologically identical to type A enterotoxin has been isolated from one strain of type D (68). This enterotoxin is not to be confused with epsilon-toxin which, by its behavior, may fit the definition of an "enterotoxin". As this development is recent and to avoid further confusion, the main characteristics of the above two toxins are compared in Table IV. It should be emphasized that the accepted specific terms of "enterotoxin" and "enterotoxemia" are not related.

Type E

The principal toxin produced by *C. perfringens* type E is iota-toxin. The characteristics of this toxin are somewhat similar to epsilon-toxin of type D, but they are not antigenically related. The toxin is produced by intact cells and fully activated by proteolytic enzymes. Biological effects of this toxin include necrotizing action and marked increase in capillary permeability (24).

Although type E had been infrequently isolated from domestic animals (51), case reports linking this orga-

TABLE IV
COMPARATIVE CHARACTERISTICS OF *C. PERFRINGENS* EPSILON-TOXIN AND ENTEROTOXIN

Characteristic	Epsilon-toxin	Enterotoxin
Origin	Produced by all strains of types B and D	Produced by some strains of type A, rarely by types C and D
Cellular location	Released by intact cells as a true exotoxin (prototoxin)	Intracellular in sporulating cells only, released by cell lysis
Histotoxic action	Strongly necrotizing	Not necrotizing; induces erythema in skin
Enteric action	Absorbed into systemic circulation	Acts mainly on intestinal mucosa
Clinico-pathological action	Damage to kidneys, brain and other organs; induces edema; results in death	Causes fluid outpouring in gut; results in diarrhea; self-limiting
Recognized disease	Enterotoxemia in sheep	Human food poisoning

nism to a definite disease condition were so rare that doubts were raised about its potential pathogenicity (64). However, a recent report (58) describing a serious outbreak of an enteritis complex in a rabbit colony in Oregon provides quite convincing evidence for the pathogenicity of type E iota-toxin. Although type E organisms were not isolated, the presence of preformed iota-toxin was confirmed by toxin neutralization tests in cecal contents of 23 of 46 rabbits which died of enteritis. The disease was characterized by profuse diarrhea and death within six hours. Dietary factors were suggested as a predisposing cause.

DISCUSSION

Table V contains a summary of *C. perfringens* types associated with diseases of domestic animals in Canada. Simplified and condensed to bare essentials, this table indicates many uncertainties and prompts a number of questions. There appear to be only two etiologically clearly-defined acute diseases: hemorrhagic enteritis of the newborn calf, caused by *C. perfringens* type C and enterotoxemia of sheep, caused by type D. The former disease is age-restricted (neonatal) and the latter has a nutritional predisposition. The remaining diseases are ill-defined and often associated with etiological factors, such as nutrition and management. Information is lacking on disease incidence in various geographical areas of Canada. For example, the acute neonatal hemorrhagic enteritis has been confirmed in calves in southern Alberta, but this condition has not been reported in either calves, lambs, or piglets in the eastern provinces.

Livestock deaths still constitute a significant proportion of economic loss and many cases remain undiagnosed. There are difficulties related to physical diagnostic problems as well as to areas of limited knowledge. The following diagnostic problems may be cited: (i) isolation, demonstration or identification of the causative strain from several different *C perfringens* strains often present in the same organ or carcass, (ii) successful demonstration of pathogenicity or toxigenicity under laboratory conditions and correct interpretation of the results and (iii) clinical recognition of *C. perfringens* infection based on signs and necropsy findings, previous history and geographical location. These diagnostic problems are, in turn, related to research problems because there is a lack of adequate knowledge in these areas. This deficiency in our current knowledge can be alleviated through experimental work at research establishments and by publication of case reports by veterinary practitioners and laboratory diagnosticians. Such knowledge is essential to completely understand the disease processes and to permit development of effective control and/or treatment.

TABLE V
SUMMARY OF INVOLVEMENT OF *C. PERFRINGENS* IN DISEASES OF DOMESTIC ANIMALS IN CANADA

Species	Disease Condition and Organism (Existing or Suspected)
All Species	
All ages	Gas gangrene-like traumatic and wound infections, accidental infections; localized in tissues, e.g., muscles. Any *C. perfringens* type, but mostly type A involved, often in combination with other bacteria; possible auxiliary role in enteric conditions (type A)
Cattle	
Newborn	Acute hemorrhagic enteritis (fatal), type C
Older	Possible auxiliary role in enteric conditions by types A,C,D
Sheep	
Newborn	Acute hemorrhagic enteritis, type C; suspected because of scattered presence of causative organism
Older	Enterotoxemia, type D
Swine	
Piglets	Acute enteritis, type C; suspected because of proximity of cases in Minnesota and Iowa
Horses	
Newborn	Acute hemorrhagic enteritis, type C; suspected because of scattered presence of causative organism
Chickens	Necrotic enteritis, type A; type C possible

REFERENCES

1. AL-SHEIKHLY, F. and R.B. TRUSCOTT. The pathology of necrotic enteritis of chickens following infusion of broth cultures of *Clostridium perfringens* into the duodenum. Avian Dis. 21: 230-240. 1977.
2. AL-SHEIKHLY, F. and R.B. TRUSCOTT. The pathology of necrotic enteritis of chickens following infusion of crude toxins of *Clostridium perfringens* into the duodenum. Avian Dis. 21: 241-255. 1977.
3. AL-SHEIKHLY, F. and R.B. TRUSCOTT. The interaction of *Clostridium perfringens* and its toxins in the production of necrotic enteritis of chickens. Avian Dis. 21: 256-263. 1977.
4. BARKER, W.H., Jr., J.C. SAGERSER, C.V.H. HALL, H.W. ANDERSON and B.J. FRANCIS. Foodborne disease surveillance. Am. J. publ. Hlth 64: 854-859. 1974.
5. BERGELAND, M.E. Pathogenesis and immunity of *Clostridium perfringens* type C enteritis in swine. J. Am. vet. med. Ass. 160: 568-571. 1972.
6. BERGELAND, M.E., T.A. DERMODY and D.K. SORENSEN. Porcine enteritis due to *Clostridium perfringens* type C. I. Epizootiology and diagnosis. Proc. 70th. Meet. U.S. Livestock Sanit. Ass. Buffalo, N.Y. pp. 601-606. 1966.
7. BROOKS, M.E., M. STERNE and G.H. WARRACK. A reassessment of the criteria used for type differentiation of *Clostridium perfringens*. J. Path. Bact. 74: 185-195. 1957.
8. BUDDLE, M.B. "Degraded" strains of *Clostridium welchii* type C isolated from sheep in New Zealand. J. comp. Path. 64: 217-224. 1954.
9. BULLEN, J.J. Role of toxins in host-parasite relationships. *In* Microbial Toxins, Vol. I. S.J. Ajl, S. Kadis and T.C. Montie, Eds. pp. 233-276. New York: Academic Press. 1970.
10. BUXTON, D. The use of an immunoperoxidase technique to investigate by light and electron microscopy the sites of binding of *Clostridium welchii* type D epsilon toxin in mice. J. med. Microbiol. 11: 289-292. 1978.
11. BUXTON, D. Further studies on the mode of action of *Clostridium welchii* type D epsilon toxin. J. med. Microbiol. 11: 293-298. 1978.
12. DICKIE, C.W., D.L. KLINKERMAN and R.J. PETRIE. Enterotoxemia in two foals. J. Am. vet. med. Ass. 173: 306-307. 1978.
13. DUNCAN, C.L. and D.H. STRONG. Experimental production of diarrhea in rabbits with *Clostridium perfringens*. Can. J. Microbiol. 15: 765-770. 1969.
14. DUNCAN, C.L. and D.H. STRONG. Ileal loop fluid accumulation and production of diarrhea in rabbits by cell-free products of *Clostridium perfringens*. J. Bact. 100: 86-94. 1969.
15. ELLNER, P.D. Fate of partially purified C14-labelled toxin of *Clostridium perfringens*. J. Bact. 82: 275-283. 1961.

16. FIELD, H.I. and R.F.W. GOODWIN. The experimental reproduction of enterotoxaemia in piglets. J. Hyg., Camb. 57: 81-91. 1959.
17. FINDLAY, C.R. and D. BUNTAIN. Enterotoxaemia due to *Clostridium welchii* type C in young lambs. Vet. Rec. 82: 224-225. 1968.
18. GARDNER, D.E. Pathology of *Clostridium welchii* type D enterotoxaemia. I. Biochemical and haematological alterations in lambs. J. comp. Path. 83: 499-507. 1973.
19. GARDNER, D.E. Pathology of *Clostridium welchii* type D enterotoxaemia. II. Structural and ultrastructural alterations in the tissues of lambs and mice. J. comp. Path. 83: 509-524. 1973.
20. GENIGEORGIS, C. Public health importance of *Clostridium perfringens*. J. Am. vet. med. Ass. 167: 821-827. 1975.
21. GRINER, L.A., W.W. AICHELMAN and G.D. BROWN. *Clostridium perfringens* type D (epsilon) enterotoxemia in Brown Swiss dairy calves. J. Am. vet. med. Ass. 129: 375-376. 1956.
22. GRINER, L.A. and F.K. BRACKEN. *Clostridium perfringens* (type C) in acute hemorrhagic enteritis of calves. J. Am. vet. med. Ass. 122: 99-102. 1953.
23. GRINER, L.A. and H.W. JOHNSON. *Clostridium perfringens* type C in hemorrhagic enterotoxemia of lambs. J. Am. vet. med. Ass. 125: 125-127. 1954.
24. HAUSCHILD, A.H.W. *Clostridium perfringens* toxins types B, C, D and E. *In* Microbial Toxins, Vol. IIA. S. Kadis, T.C. Montie, and S.J. Ajl, Eds. pp. 159-188. New York: Academic Press. 1971.
25. HAUSCHILD, A.H.W. and R. HILSHEIMER. Purification and characteristics of the enterotoxin of *Clostridium perfringens* type A. Can. J. Microbiol. 17: 1425-1433. 1971.
26. HAUSCHILD, A.H.W., L. NIILO and W.J. DORWARD. *Clostridium perfringens* type A infection of ligated intestinal loops in lambs. Appl. Microbiol. 16: 1235-1239. 1968.
27. HAUSCHILD, A.H.W. and F.S. THATCHER. Experimental gas gangrene with food poisoning *Clostridium perfringens* type A. Can. J. Microbiol. 14: 705-709. 1968.
28. HOBBS, B.C. *Clostridium welchii* as food poisoning organism. J. appl. Bact. 28: 74-82. 1965.
29. ISPOLATOVSKAYA, M.V. Type A *Clostridium perfringens* toxin. *In* Microbial Toxins, Vol. IIA. S. Kadis, T.C. Montie and S.J. Ajl, Eds. pp. 109-158. New York: Academic Press. 1971.
30. JANSEN, B.C. The beta toxin of *Clostridium welchii* type B, Wilsdon, in relation to the production of a vaccine against lamb dysentery. Onderstepoort J. vet. Res. 28: 495-549. 1961.
31. JASMIN, A.M., D.E. COOPERRIDER, C.P. POWELL and J.N. BAUCOM. Enterotoxemia of wildfowl due to *Clostridium perfringens* type C. J. Wildl. Dis. 8: 79-84. 1972.
32. KELLAWAY, C.H., E.R. TRETHEWIE and A.W. TURNER. Neurotoxic and circulatory effects of the toxin of *Cl. welchii* type D. Aust. J. exp. Biol. med. Sci. 18: 225-252. 1940.
33. KENNEDY, K.K., S.J. NORRIS, W.H. BECKENHAUER and A. HOGG. Vaccination of pregnant sows with *Clostridium perfringens* type C toxoid. Vet. Med. small Anim. Clin. 72: 1047-1049. 1977.
34. KENNEDY, K.K., S.J. NORRIS, W.H. BECKENHAUER and R.G. WHITE. Vaccination of cattle and sheep with a combined *Clostridium perfringens* types C and D toxoid. Am. J. vet. Res. 38: 1515-1517. 1977.
35. KENNEDY, K.K., S.J. NORRIS, W.H. BECKENHAUER and R.G. WHITE. Antitoxin response in cattle vaccinated with *Clostridium perfringens* type C toxoid. Vet. Med. small Anim. Clin. 72: 1213-1215. 1977.
36. LOEWENSTEIN, M.S. Epidemiology of *Clostridium perfringens* food poisoning. New. Engl. J. Med. 286: 1026-1028. 1972.
37. LONG, J.R. and R.B. TRUSCOTT. Necrotic enteritis in broiler chickens. III. Reproduction of the disease. Can J. comp. Med. 40: 53-59. 1976.
38. LOZANO, E.A., J.E. CATLIN and W.W. HAWKINS. Incidence of *Clostridium perfringens* in neonatal enteritis of Montana calves. Cornell Vet. 60: 347-359. 1970.
39. MacLENNAN, J.D. The histotoxic clostridial infections of man. Bact. Rev. 26: 177-276. 1962.
40. MOLLBY, R., T. HOLME, C.-E. NORD, C.J. SMYTH and T. WADSTROM. Production of phospholipase C (alpha-toxin), haemolysins and lethal toxins by *Clostridium perfringens* types A to D. J. Gen. Microbiol. 96: 137-144. 1976.
41. NIILO, L. Bovine "enterotoxemia" III. Factors affecting the stability of the toxins of *Clostridium perfringens* types A, C and D. Can. vet. J. 6: 38-42. 1965.
42. NIILO, L. Mechanism of action of the enteropathogenic factor of *Clostridium perfringens* type A. Infection & Immunity 3: 100-106. 1971.
43. NIILO, L. The effect of enterotoxin of *Clostridium welchii (perfringens)* on the systemic blood pressure of sheep. Res. vet. Sci. 13: 503-505. 1972.
44. NIILO, L. Fluid secretory response of bovine Thiry jejunal fistula to enterotoxin of *Clostridium perfringens*. Infection & Immunity 7: 1-4. 1973.
45. NIILO, L. Effect on calves of the intravenous injection of the enterotoxin of *Clostridium welchii* type A. J. comp. Path. 83: 265-269. 1973.
46. NIILO, L. Response of ligated intestinal loops in chickens to the enterotoxin of *Clostridium perfringens*. Appl. Microbiol. 28: 889-891. 1974.
47. NIILO, L. Measurement of biological activities of purified and crude enterotoxin of *Clostridium perfringens*. Infection & Immunity 12: 440-442. 1975.
48. NIILO, L. The effect of enterotoxin of *Clostridium welchii (perfringens)* type A on fowls. Res. vet. Sci. 20; 225-226. 1976.
49. NIILO, L. Enterotoxin formation by *Clostridium perfringens* type A studied by the use of fluorescent antibody. Can. J. Microbiol. 23: 908-915. 1977.
50. NIILO, L. Enterotoxigenic *Clostridium perfringens* type A isolated from intestinal contents of cattle, sheep and chickens. Can. J. comp. Med. 42: 357-363. 1978.
51. NIILO, L. and R.J. AVERY. Bovine "enterotoxemia" I. *Clostridium perfringens* types isolated from animal sources in Alberta and Saskatchewan. Can. vet. J. 4: 31-36. 1963.
52. NIILO, L. and W.J. DORWARD. The effect of enterotoxigenic *Clostridium welchii (perfringens)* type A on the bovine intestine. Res. vet. Sci. 12: 376-378. 1971.
53. NIILO, L., W.N. HARRIES and G.A. JONES. *Clostridium perfringens* type C in hemorrhagic enterotoxemia of neonatal calves in Alberta. Can. vet. J. 15: 224-226. 1974.
54. NIILO, L., A.H.W. HAUSCHILD and W.J. DORWARD. Immunization of sheep against experimental *Clostridium perfringens* type A enteritis. Can. J. Microbiol. 17: 391-395. 1971.
55. NIILO, L., R.E. MOFFATT and R.J. AVERY. Bovine "enterotoxemia" II. Experimental reproduction of the disease. Can. vet. J. 4: 288-298. 1963.
56. OAKLEY, C.L. The toxins of *Clostridium welchii*. Bull. Hygiene 18: 781-806. 1943.
57. PARISH, W.E. Necrotic enteritis in the fowl. II. Examination of the causal *Clostridium welchii*. J. comp. Path. 71: 394-404. 1961.
58. PATTON, N.M., H.T. HOLMES, R.J. RIGGS and P.R. CHEEKE. Enterotoxemia in rabbits. Lab. Anim. Sci. 28: 536-540. 1978.
59. SAKURAI, J. and C.L. DUNCAN. Purification of beta-toxin from *Clostridium perfringens* type C. Infection & Immunity 18: 741-745. 1977.
60. SAKURAI, J. and C.L. DUNCAN. Some properties of beta-toxin produced by *Clostridium perfringens* type C. Infection & Immunity 21: 678-680. 1978.
61. SKJELKVALE, R. and C.L. DUNCAN. Enterotoxin formation by different toxigenic types of *Clostridium perfringens*. Infection & Immunity 11: 563-575. 1975.
62. SMITH, L.DS. Virulence factors of *Clostridium perfringens*. Rev. infect. Dis. 1: 254-260. 1979.
63. STARK, R.L. and C.L. DUNCAN. Purification and biological properties of *Clostridium perfringens* type A enterotoxin. Infection & Immunity 6: 662-673. 1972.
64. STERNE, M. and I. BATTY. Pathogenic Clostridia. London: Butterworth & Co. 1975.
65. STERNE, M. and G.H. WARRACK. The types of *Clostridium perfringens*. J. Path. Bact. 88: 279-283. 1964.
66. SUTTON, R.G.A. and B.C. HOBBS. Food poisoning caused by heat-sensitive *Clostridium welchii*. A report of five recent outbreaks. J. Hyg., Camb. 66: 135-146. 1968.

67. TSAI, C-C., M.J. TORRES-ANJEL and H. RIEMANN. Characteristics of enterotoxigenic *Clostridium perfringens* type A isolated from cattle and chickens. J. Formosan med. Ass. 73: 501-510. 1974.
68. UEMURA, T. and R. SKJELKVALE. An enterotoxin produced by *Clostridium perfringens* type D. Purification by affinity chromatography. Acta path. microbiol. scand. 84: 414-420. 1976.
69. VANCE, H.N. *Clostridium perfringens* as a pathogen of cattle: A literature review. Can. J. comp. Med. 31: 248-250. 1967.
70. VANCE, H.N. A survey of the alimentary tract of cattle for *Clostridium perfringens*. Can. J. comp. Med. 31; 260-264. 1967.
71. WIERUP, M. Equine intestinal clostridiosis. Acta vet. scand. Suppl. 62. 1977.

DIRECTIVES AUX AUTEURS

Nous acceptons de publier des articles originaux et inédits. La rédaction se réserve le droit de faire les corrections nécessaires, mais elle n'effectuera aucun changement important sans consulter l'auteur. Les corrections visent à atteindre une clarté et une concision plus grandes en respectant les coutumes adoptées par la Revue. Pour les textes rédigés en anglais, nous suivons l'orthographe du "Webster's Collegiate Dictionary"; pour les textes français, celui du dictionnaire alphabétique et analogique de la langue française, par Paul Robert.

Manuscrits. La Revue publie des articles en français ou en anglais. Le manuscrit au complet, y compris les renvois, tableaux et illustrations, doit être soumis sur du papier blanc de 21.5 x 28 cm, à lignes numérotées. Le texte doit être dactylographié à double interlignes, avec des marges de 3 cm au moins. Soumettre l'exemplaire original et une copie lisible du manuscrit. On ne doit souligner que les mots qui seront en italique. Les titres, sous-titres, renvois, etc., devraient être conformes à ceux que l'on retrouve dans un numéro récent de la Revue. Toujours copier à doubles interlignes, les notes, renvois et tableaux. Dans le texte écrire en letres les nombres de un à dix, sauf quand le chiffre est suivi d'une abréviation (ex. 9 mg). Ecrire en chiffres arabes les nombres supérieurs à dix. Identifier clairement les tableaux et les bas de vignettes; les dactylographier sur des feuilles séparées apparaissant à la fin du manuscrit.

Résumé. Faire suivre l'article d'un résumé facile à comprendre sans qu'il y ait besoin de se reporter au texte même. Tous les résumés sont traduits en français ou en anglais selon le cas. On invite les auteurs à soumettre eux-mêmes ce résumé dans la langue seconde.

Illustrations. Numéroter toutes les vignettes en *chiffres arabes.* Dans le texte, inscrire le numéro correspondant à chaque vignette. Toujours donner un titre aux vignettes. Fournir des photographies bien contrastées sur papier glacé et les tailler de façon à ne conserver que l'essentiel, i.e. mesurer approximativement 6 *ou* 12 cm largeur. Á l'aide d'un crayon doux; inscrire clairement au dos de chaque illustration: le haut, le numéro approprié et le nom de l'auteur. Tracer les dessins et les graphiques à l'encre de Chine sur papier blanc; inscrire à l'encre et non à la machine les lettres, les chiffres et le texte explicatif. Soumettre les dessins originaux et une série de photographies de format réduit). *Indiquer l'échelle quand il s'agit de photographies et en particulier de photomicrographies.*

Tableaux. Numéroter les tableaux en *chiffres romains* et y référer au moyen de chiffres dans le texte. Toujours donner un titre aussi bref que possible. Réduire le plus possible les entêtes de colonnes; ne pas utiliser de traits verticaux.

Bibliographie. Indiquer clairement les références dans le texte, par des chiffres arabes entre parenthèses, par ex. (2). Les présenter dans l'ordre alphabétique à la fin de l'article, et les dactylographier à *doubles interlignes.*

Quant aux références à des articles de périodiques (2), donner le titre et les pages. Abréger le nom des périodiques selon la liste mondiale des périodiques scientifiques, sauf dans le cas de revues peu connues qu'on citera au complet en indiquant le lieu de publication, l'éditeur et l'année de publication.

2. BRADEN, G.C., A. JONES, F.K. NEAVE, F.H. DODD, R.G. KINGWILL et D.R. WESTGARTH. Les antibiotiques dans des excipients à action lente ou rapide. Vet. Rec. 76: 329-425. 1964.
6. STABLEFORTH, A.W. et I.A. GALLOWAY. Maladies infectieuses des animaux. Volume 2. pp. 16-22. London: Butterworths Scientific Publications. 1959.

Tirés à part. Nous prions les auteurs d'indiquer, le nombre de tirés à part désiré lorsqu'ils retournent les épreuves corrigés.

Études de cas. Le plan peut varier mais, en général, on le divisera ainsi: sujet, anamnèse, signes cliniques, traitement, résultats. quand il y a lieu, ajouter des sous-titres portant sur les examens de laboratoire, la nécropsie et la discussion.

Lorsqu'on mentionne un médicament pour la première fois, utiliser son appellation chimique ou générique et indiquer au bas de la page, la marque de commerce déposée et le nom du fabricant.

La Revue Vétérinaire Canadienne