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The Challenges of Diagnosis and Control of Enterotoxaemia Caused by *Clostridium perfringens* in Small Ruminants

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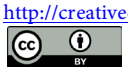

Abstract

Enterotoxaemia is one of the important pathologies caused by *Clostridium perfringens*, which produces intestinal and systemic disease in goats, sheep and other animals. These Gram-positive anaerobic bacteria are normally resident in the intestinal tract of ruminants but during favourable conditions, proliferate uncontrollably and release toxins which produce disease in the host. Different strains of *C. perfringens* are responsible for several clinical syndromes, including lamb dysentery, pulpy kidney disease and struck. However, the pathology and pathogenesis of caprine enterotoxaemia is not well understood, with limited studies available in goats. Caprine enterotoxaemia can be controlled with the better understanding of its risk factors and pathogenesis. The diagnosis of enterotoxaemia in animals is complex and often requires group of tests than one single test for better specificity and sensitivity. Tentative diagnosis of enterotoxaemia in sheep and goats is based on the history, clinical signs and gross lesions during post-mortem examination of animals; however, confirmatory diagnosis of enterotoxaemia requires different laboratory diagnostic tools. Toxin detection of *C. perfringens* in case of enterotoxaemia is furthermost accepted benchmark in establishing a definitive diagnosis of enterotoxaemia in intestinal contents. Measuring urine glucose or observing Gram-stained smears of intestinal mucosa can be used as supplementary tests. However, it is also imperative that enterotoxaemia cannot be ruled out in the event of negativity of aforementioned diagnostic tests. Hence, definitive diagnosis of enterotoxaemia in goats can be achieved with

the use of molecular techniques (PCR, ELISA and immune-fluorescence) coupled with toxin detection in intestine or biological assays including mouse inoculation test (MIT). In case of goats, vaccine efficacy is poor which may be due to need of high to moderate level of serum antibodies to protect against both systemic and enteric effects because intestinal form of disease is partially independent of the circulating anti-toxin antibodies. Thus, for the prevention and control of enterotoxaemia in goats and sheep, these aspects must be considered to develop more holistic control measures.

Keywords

Clostridium perfringens, Control, Diagnosis, Enterotoxaemia, Goat, Prevention, Sheep, Small Ruminants

1. Introduction

The word "enterotoxaemia" denotes toxaemia of intestinal origin that occurs when toxins produced in the intestines are absorbed into the bloodstream. Enterotoxaemia can be caused by various microorganisms, but the term is most frequently used in relation to the absorption of toxins produced by species of the genus *Clostridium* [1] [2]. The genus *Clostridium* comprises more than 100 species, several of which are pathogenic, producing exotoxins that act locally or systemically on the host's cells and tissues. Although several species of *Clostridium* can cause enterotoxaemia in different hosts, the syndrome as come to be associated with the species *Clostridium perfringens* in ruminants [2]. Sheep enterotoxaemia caused by *C. perfringens* is well documented; however, the situation is less clear in goats on account of limited studies carried in the goats. It causes sudden death in goats of all age groups [3]. The excess toxin production is synonymous with the disease and its absorption into systemic circulation produces widespread effects [4]. But it is also recognized that some toxins of *C. perfringens* produced in intestine act locally [5]. In goat, histopathological changes are not consistently found as in case sheep, and the confirmatory diagnosis of enterotoxaemia is mostly based only on the detection of epsilon toxin in the intestinal contents at significant level. Toxinotyping based PCR can, in many cases, provide the final piece of information needed to establish a diagnosis.

Enterotoxaemia can be controlled only with better understanding of its risk factors and pathogenesis. The current available vaccine provides short-lived protective titres in goats than sheep [6] and needs booster doses very frequently every three to four months interval throughout their life for full-proof protection from the disease [7]. Moreover, the main contention for protection should encompass effective diagnosis of the *C. perfringens* toxinotypes during the worst case scenario of vaccination failures or reduced effectiveness of immunization against ET in goats.

2. Aetiology

Clostridium perfringens is a Gram-positive, anaerobic rod-shaped bacterium, successively named *Bacterium welchii* and *Clostridium welchii* in honour of William Henry Welch, who first isolated it from a human cadaver died of an aortic aneurism in 1891. The species name *perfringens* ("breaking through" in Latin), proposed by Veillon and Zuber [8] in 1898 (*Bacillus perfringens*), replaced *welchii* in the fifth edition of Bergey's Manual of Determinative Bacteriology. *C. perfringens* is probably the best-known and most widespread anaerobic pathogen throughout the world. It is a normal component of the intestinal flora of healthy warm-blooded animals and human beings [9]. Like all bacterial species, *C. perfringens* can be subdivided into strains according to the results of different typing methods. PCR based Genotyping is generally used for toxin genotyping of *C. perfringens* [10].

C. perfringens can produce up to 30 potential toxins, and as before now, strains are traditionally classified into five categories (A, B, C, D and E) according to the production of four major toxins (α, β, ι and ε) [9]. However, this classification system has been recently revised by including two more major toxins, namely enterotoxin (CPE) and necrotic B-like toxin (NetB) [11] (**Table 1**). This revised classification has introduced two new toxinotypes *i.e.* enterotoxin (CPE) producing *C. perfringens* type F and necrotic enteritis B-like toxin (NetB) producing *C. perfringens* type G. NetB was identified as a principle toxin behind the pathophysiology of necrotic enteritis in chickens [12]. In addition to these typing toxins, the bacterium is able to produce a number of other toxins such as β_2, δ, θ, ν, λ, k (collagenase), μ (N acetyl galactosaminidase), sialidase, various haemolysins, enterotoxin and TpeL [13] [14].

The principal cause of caprine enterotoxaemia is *Clostridium perfringens* type D [15] [16] [17] [18] whereas *C. perfringens* type A is most commonly isolated type in ovine enterotoxaemia [19]-[24]. The bacterium is resident in the ruminant digestive tract, generally in low numbers. It is passed in faeces and may persist in soil, though it perishes more quickly in soil than other *Clostridium* spp. The organism has a quick generation time, allowing for rapid proliferation in the intestine under favourable conditions and release of toxins that causes disease in the host.

Table 1. Revised classification scheme of *C. perfringens* [11].

C. perfrin-gens type	Major Toxins					
	α (CPA)	β (CPB)	ϵ (ETX)	ι (ITX)	CPE	NetB
A	+	−	−	−	−	−
B	+	+	+	−	−	−
C	+	+	−	−	+/−	−
D	+	−	+	−	+/−	−
E	+	−	−	+	+/−	−
F	+	−	−	−	+	−
G	+	−	−	−	−	+

3. Virulence Factors

C. perfringens produces an array of toxins and enzymes which are encoded by 23 virulence genes [25]. The inherent strategies followed by this organism helps it to survive in host as well as in outer environment. This includes its ability to survive in the presence of oxygen, *i.e.* aero-tolerance [26], a very short generation time of 12 - 17 min at 37˚C [27] and the spore-forming power, which plays a crucial role in the transmission and persistence of organism in environment and host [28]. The characteristics of various toxins and enzymes were depicted in Table 2.

4. Predisposing Factors

Usually *C. perfringens* are found in intestine and in soil in fairly low numbers. Investigation of intestinal tract materials from healthy goats had shown 2.94% incidence of *C. perfringens* type D [29]. This was further supported by a recent study on healthy goats in which type A, C and D isolate were detected respectively in 75.6%, 0.4% and 0.4% of total faecal samples [30]. However, certain favorable conditions lead to the excess growth of *C. perfringens.* Excess carbohydrate-rich or pulverized feed or sudden change in diet can cause decrease in peristaltic movement, produce anaerobic environment, causing rapid and excessive proliferation of organisms and production of lethal toxins in the intestine [9]. The disease may occur in goats of all ages with animals in good body condition appears to be most susceptible [15]. Interestingly, several well understood predisposing factors in sheep cannot be strictly applied to goats, such as the disease usually occurs in single lambs of high milk producing ewes but type of birth is not a predisposing factor in goats [31]. Further, enterotoxaemia causes the maximum loss in growing lambs fed on concentrate rations in feed lots, but this management condition is rarely encountered among goats [32]. Factors which slow down peristalsis and retard the movements of the intestine, such as sudden exposure to grain and garden greens, large increase in quantity of milk consumed without gradually increasing the amount over several days [31], a heavy worm burden (both tapeworms and lung worms), changes from poor to lush pasture, feeding of bread or other bakery goods and feeding of a bran/molasses mash are mentioned as the predisposing factors in goats. But some outbreaks of enterotoxaemia type D have been earlier reported [33] in goats under extensive grazing systems without known diet change. Recently, the association of several risk factors viz. age, deworming, season, overcrowding and carbohydrate rich diet with occurrence of enterotoxaemia in sheep were studied [24]. Out of these, carbohydrate rich diet and overcrowding are found highly associated with elevated *in-vivo* bacterial growth with odds ratio of 5.44 and 2.26, respectively.

5. Epidemiology

Enterotoxaemia is a common economically important devastating disease of sheep and goats throughout the world [34] [35], and is probably the most important

Table 2. Characteristics of toxins and enzymes of *C. perfringens.*

Toxin/ Enzyme	Location of gene	Molecular mass (kDa)	LD_{50} (mice)	Main targets	Biological activity of toxin	References
Alpha toxin (CPA)	Chromosome	43	3 µg	Phosphatidylcholine, Sphingomyelin	• Essential for growth & dissemination of infection • Hemolysis via phospholipase & sphingomyelinase activity • Inhibition of neutrophil & erythroid differentiation • Vasoconstriction via activation of arachidonic acid cascade	[58]-[63]
Beta toxin (CPB)	Plasmid	35	<400 ng	Intestinal epithelial, Endothelial cells, sensory neurons	• Necrosis of intestinal mucosal epithelium and vascular endothelium • Oedema, dermonecrosis & plasma extravasation though release of substance P from toxin-stimulated sensory neurons • Cytotoxic to platelets	[64] [65] [66] [67]
Epsilon toxin (ETX)	Plasmid	33	70 - 100 ng	Endothelial cells, mucosal tight junctions, lymphocytes, all vital organs including brain	• Increased permeability of intestine & blood vessels • Degeneration of distal tubule cells in kidney • Cerebral oedema & necrosis via damage to BBB • Demyelination by causing necrosis of oligodendrocytes • Release of glutamate by stimulating target cells in cerebellum & hippocampus • Cytotoxic to lymphocytes	[5] [54] [68]-[74]
Iota toxin (ITX)	Plasmid	Ia-48 Ib-72	40 µg	Cell cytoskeleton (actin)	• Increased permeability & necrosis of intestinal epithelial cells by actin depolymerization	[75] [76] [77]
Enterotoxin (CPE)	Chromosome or plasmid	35	81 µg	Claudins of tight junctions	• Loss of contact between intestinal epithelial cells	[78] [79] [80] [81]
Necrotic enteritis B-like toxin (NetB)	Plasmid	33	-	Enterocytes	• Destruction of extracellular matrix, lamina propria and intercellular junctions	[82]
Beta-2 toxin (CPB2)	Plasmid	28	160 µg	Enterocytes, endothelial cells	• Dermonecrosis, oedema & enterotoxic	[83]
Perfringolysin O (PFO)/Theta toxin	Chromosome	54	15 µg	Enterocytes, endothelial cells, leucocytes	• Act synergistically with CPA & ETX • Disruption of endothelial integrity • Leukocytotoxic at high dose	[84] [85] [86]
Toxin *C. perfringens* large cytotoxin (TpeL)	Plasmid	191	600 µg	GTPase Ras	• Inactivation of Ras protein with resultant disruption of intercellular junctions & increased cell barrier permeability	[87] [88]
Lamba toxin	Plasmid	36	-	Endothelial cells	• Increases vascular permeability • Activates prototoxin forms of ETX & ITX	[89] [90]
Delta toxin	Plasmid	32	-	Different blood cells, GM2 ganglioside on cell membranes	• Cytotoxic to various eukaryotic cells such as macrophages, platelets, erythrocytes including various cell lines	[91]
Necrotizing enteritis toxin F (NetF)	Plasmid	34.3	-	Sialic acid on cell membranes	• Cytotoxic to various cell lines such as equine ovarian, porcine kidney 15, Vero cell line etc.	[92] [93]
Sialidase (NanH, NanI, NanJ)	Chromosome	43, 77, 129	-	Sialic acid linkages	• Alters host cell surface to promote bacterial attachment & colonization • Increased ETX binding & cytotoxicity	[52] [53] [94]

cause of sudden death in small ruminants of different ages [3]. The suckling lambs between 3 and 10 weeks of age are highly susceptible [36]. The ovine enterotoxemia was reported from different countries such as India [37], Switzerland [38], Italy [39], United Kingdom [40], Turkey [41].

The caprine enterotoxaemia has been reported in Argentina, Australia, Brazil, Canada, Germany, Great Britain, Greece, France, India, Iran, South Africa, Sri Lanka, Switzerland and in the United States [42] [43] (**Figure 1**). The prevalence rates of enterotoxaemia ranging between 24.13% and 100% were reported from different countries around the world [44]. In an extensive prevalence study conducted in Iran, the prevalence rate of enterotoxaemia in sheep was 0.14% (128/87,802) and case fatality rate ranged between 0% and 80% with an average of 40% [45]. In general, morbidity rate does not exceed 10% of the herd but nearly kills 100% of the affected animals owing to its high lethality [46]. Another study states 31% and 22% prevalence of *C. perfringens* in sheep and goats, respectively [47]. A total of fourteen outbreaks in Tamil Nadu state of India are reported through active and passive surveillance during the period of two years (June 2007 to May 2009) [48]. In a recent study, incidence enterotoxaemia in goat kids was found to be 15.13% (n = 238) and 27.38% (n = 84) in diarrhoeic faecal and intestinal loop samples, respectively (Singh, 2017), whereas in post-weaned goats the incidence was 16.07% (n = 168) and 22.75% (n = 189) in diarrhoeic faecal and intestinal loop samples, respectively [43].

6. Pathogenesis and Pathogenicity

C. perfringens is a normal commensal of digestive tract [49]. They reside in the gut without producing much damage as low bacterial number produces small quantity of toxins that too removed quickly from gut by normal peristalsis. Apart from this, gut microenvironment poses certain barriers against bacterial proliferation and colonization. These include competition with other commensals such as *Escherichia coli* for common niche, inhibition of proliferation of *C. perfringens* by secondary bile acids, Deoxycholate [50] and inhibition of growth and toxin production by *Lactobacillus* spp. [51]. However, certain virulence factors and environmental conditions favor bacterial proliferation and colonization. Sialidases (NanI, NanH, NanJ) secreted by organism seems to be important in initial establishment of infection. They alters host cell surface and promote bacterial attachment [52] [53].

Sudden ingestion of carbohydrate-rich feeds permits more undigested starch to pass through the rumen to the abomasum and intestine where it serves as a nutrient substrate for rapid proliferation of the organism (**Figure 2**). Excess carbohydrate intake may also predispose to reduced motility. This proliferation of *C. perfringens* type D in conjunction with reduced peristalsis enhances the concentration and pathogenic potential of the epsilon toxin produced by the organism. Based on *in vitro* findings, it is postulated that the absence of glucose in small intestine, due to failure of starch digestion, can stimulate ETX production

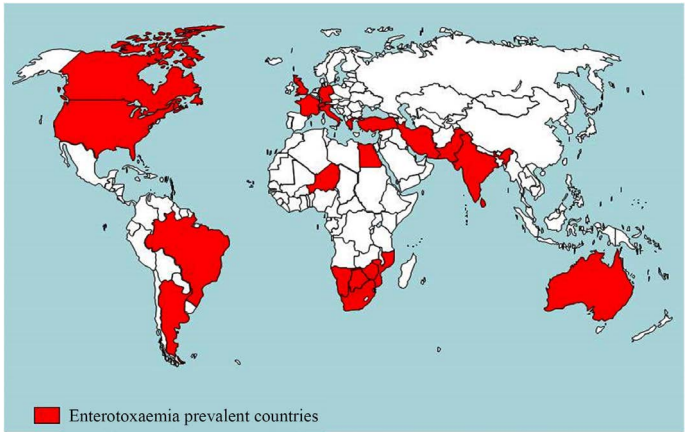

Figure 1. Prevalence of enterotoxaemia among small ruminants in different countries of world.

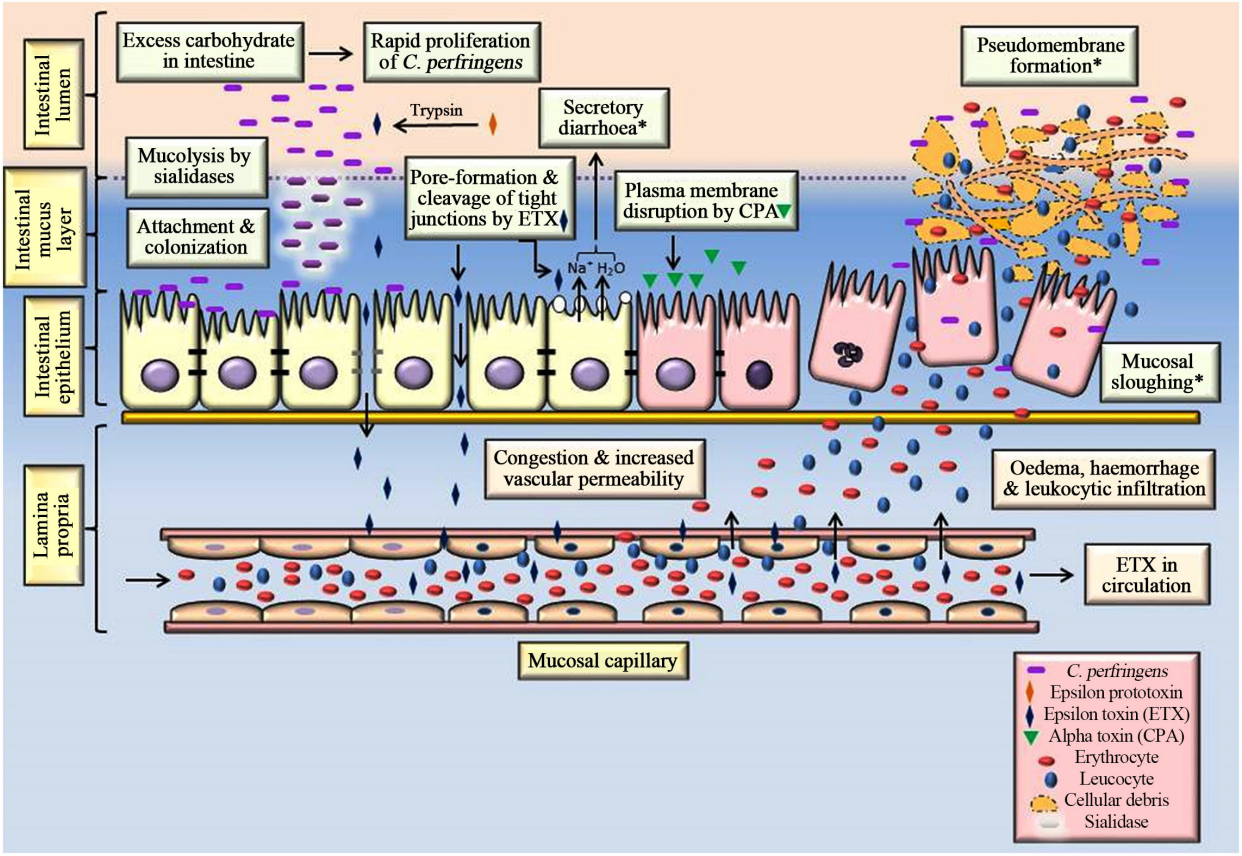

Figure 2. Pathogenesis of *C. perfringens* type D enterotoxaemia in small ruminants. Presence of excess carbohydrate in intestine provide good medium for rapid proliferation of *C. perfringens* type D organisms with production of alpha toxin (CPA), epsilon prototoxin, enzymes (sialidases) and other minor toxin like beta2 toxin (depending on the isolate). Host trypsin in intestine activates the epsilon prototoxin to active epsilon toxin (ETX). Sialidases helps in breaching the host mucosal barrier (mucus layer) and thus establishing bacterial attachment and colonization. CPA disrupts the intestinal epithelial cell membrane, leading to necrosis, mucosal sloughing and pseudomembrane formation. Formation of pores in epithelial cells by ETX cause leakage of sodium ions and water which results in secretory diarrhoea. ETX also cleaves the mucosal tight junctions and enters the lamina propria (paracellular pathway). In lamina propria, ETX binds to endothelial cells of mucosal capillaries and increases its permeability via endothelial injury. There is resultant systemic absorption of ETX, haemorrhage and diapedesis of leucocytes to the site of injury. *Prominent features of caprine enterotoxaemia.

by *C. perfringens* type D [54]. The interaction between *C. perfringens* and intestinal cells was studied using type D isolates and cultured enterocyte-like Caco-2 cells [55] [56] [57]. This study revealed that *C. perfringens* senses the presence of enterocytes and contact with host cell is essential for upregulation of epsilon toxin production.

And it was later found that the host cell induces *C. perfringens* to utilize Agr-like quorum sensing system for ETX production [95]. Also the sialidases were found to enhance ETX binding and cytotoxicity [52]. Production of ETX is a highly regulated process and found to be produced maximally at neutral pH [96]. In order to exert its toxic effects, the prototoxin form of epsilon toxin is needed to be converted by intestinal trypsin and lambda protease of *C. perfringens* to more active form. This activation requires removal of the 13 N-terminal and 29 C-terminal residues of prototoxin by trypsin or 10 N-terminal residues by lambda protease [54] [89]. This activation process was also studied *ex vivo* using caprine small intestinal contents which showed that it is activated in a stepwise manner into a ~27-kDa protein [97]. Once activated, it increases the permeability of the gut wall, facilitates its own absorption and then transported to several target organs, including brain, lungs and kidney [54] [98]. On the other hand, alpha toxin (CPA) is trypsin sensitive and does not require activation. In an experiment to study the role of ETX on the small intestinal permeability in mice and rats, it was evident that ETX alters the intestinal permeability by opening the mucosal tight junction and this allowed the passage of macromolecules through these tight junctions [99]. This explains the massive absorption of ETX via paracellular pathway. ETX was also found to increase the vascular permeability of rat mesentery by directly damaging the endothelium [100]. Effect of intestinal microenvironment, differential absorption and corresponding toxicity of ETX from different intestinal segments was extensively studied in mice model. It was evident that low pH or increased glucose level enhance ETX absorption and lethality of ETX was more when absorbed from colon than from small intestine [101]. The author also reported that ETX absorption occurred from small and large intestines but not from stomach. The effects of alpha toxin on intestinal barrier are not yet studied in caprine intestine but peculiar findings were observed in ovine and bovine gut. In cattle, this include exfoliation in alpha toxin treated small intestine loops while ETX treated colonic loops showed haemorrhage histologically and dilation of the intercellular spaces ultrastructurally [102]. In sheep, alpha toxin treated ileal and colonic loops retained more fluid along with the net reduction in water absorption from lumen. There was moderate neutrophilic infiltration in lamina propria and submucosa of ileum and colon [103]. Beside this, some recent studies in mice unravels the biological action of alpha toxin. This includes impairment of neutrophil differentiation [59] [104], impaired erythropoiesis via inhibition of erythroid differentiation and impaired granulopoiesis via degradation of granulocyte colony-stimulating factor receptor (G-CSFR) [105].

Reverse genetics approach had proved that epsilon toxin is responsible for all clinical manifestations and pathology of disease [106]. On the other hand, the role

of alpha toxin in enteric disease is poorly understood [107]. Upon absorption, epsilon toxin affects wide range of organs as studied in different animal models, including mice [108] [109] [110], rats [111], sheep [112], goats [113] and cattle [114] [115]. The pathogenic effects on different cell types and organs of animals and in cell cultures are discussed here under.

Kidney is one of the target organs of epsilon toxin. The so called "pulpy kidney" lesion has a diagnostic significance in ovine enterotoxaemia whereas renal lesions are not considered as diagnostic facet of caprine enterotoxaemia [5]. However, it is still not proved that epsilon toxin is associated with this condition or not. Thus, it is considered to be a postmortem change rather an antemortem lesion [54]. But association of ETX with kidney was demonstrated in mice in which immunolabelling of ETX was seen predominantly in glomeruli, capillaries and collecting ducts [116]. Cytotoxic activity of ETX has been studied using cell lines from renal origin of dogs, mice and human. In mice, ETX caused degeneration of distal tubules in nephrons and haemorrhages in the medulla [68]. These findings are further supported by an experiment on mouse kidney sections which revealed that ETX specifically binds to distal tubule [117].

In addition to kidneys, Brain is also a prime target of epsilon toxin [118]. ETX initially binds to endothelial cells of the blood-brain-barrier (BBB) which subsequently causes swelling, vacuolation and necrosis of affected vascular endothelial cells with rupture of perivascular astrocyte processes [69] [108] [119]. This results in leakage of fluid and proteins, hypoxia of the neural parenchyma [120] [121]. Microscopic lesions such as proteinaceous perivascular oedema and degeneration of white matter, astrocytes are considered diagnostic in sheep but similar findings are rare in goats [5] [113] [122]. However, proteinaceous intramural vascular oedema was recently reported in brain sections of goats died of *C. perfringens* type D enterotoxaemia [123]. Brain responds to oedema by upregulating the expression of a water channel protein, aquaporin 4 (AQP4) [124]. This overexpression was seen in astrocytes of mice, sheep and goats [78] [123]. Apart from its effect on BBB, ETX can directly damage neurons, astrocytes and oligodendrocytes resulting in neuronal damage and demyelination [70] [71] [125] [126] [127]. Recently, a myelin and lymphocyte protein (MAL) expressed on endothelial cells, oligodendrocytes, myelin, maturing T-lymphocytes and renal cell is proposed as a specific ETX receptor [128] [129] (**Figure 3**). Action of ETX on MAL expressing oligodendrocytes explains the reason behind demyelination which is supported by the decrease in immunoreactivity to myelin basic protein [130]. After binding, ETX causes demyelination by inhibiting potassium inward rectifier (Kir) channels in oligodendrocytes [131]. ETX was known to stimulate the release of glutamate, an excitatory neurotransmitter, by targeting the hippocampal glutamatergic system and cerebellar granule cells in mice [71] [132]. This might be the reason behind nervous signs in affected animals. However, another study contradicts these findings as no glutamate was released from GFP-ETX incubated nerve terminals [133]. A subsequent study revealed the indirect role of ETX induced vasogenic oedema and neuronal toxicity behind permanent neu-

ronal degeneration and resulting behavioural changes [134]. Experimental studies in sheep and goats showed the preferential action of ETX on certain regions of brain namely, cerebral cortex, basal ganglia, superior colliculi, pons, cerebellum, thalamus, cerebellar peduncles and obex [119] [135].

Other than intestine, kidneys and brain, some other cells and organs are also susceptible to epsilon toxin. One of this is T-cells as shown in an experimental study. They demonstrate that MAL protein is essential for ETX binding and cytotoxicity in T-cells [74]. This cytotoxicity might be a result of hampered MAL protein functions such as T-cell maturation [136], exosome secretion [137] or intracellular membrane traffic [138]. Not only vascular endothelial cells of intestine and brain are susceptible to ETX, blood vessels in few other organs are also seems to be susceptible. ETX was shown to damage retinal microvessels in rat which was evidenced by loss of immunoreactivity to endothelial barrier antigen [139]. The damaged retinal microvessels may subsequently affects the function of retina. This may be the reason behind blindness, as one of the clinical signs of type D enterotoxaemia in sheep and goats [140] [141].

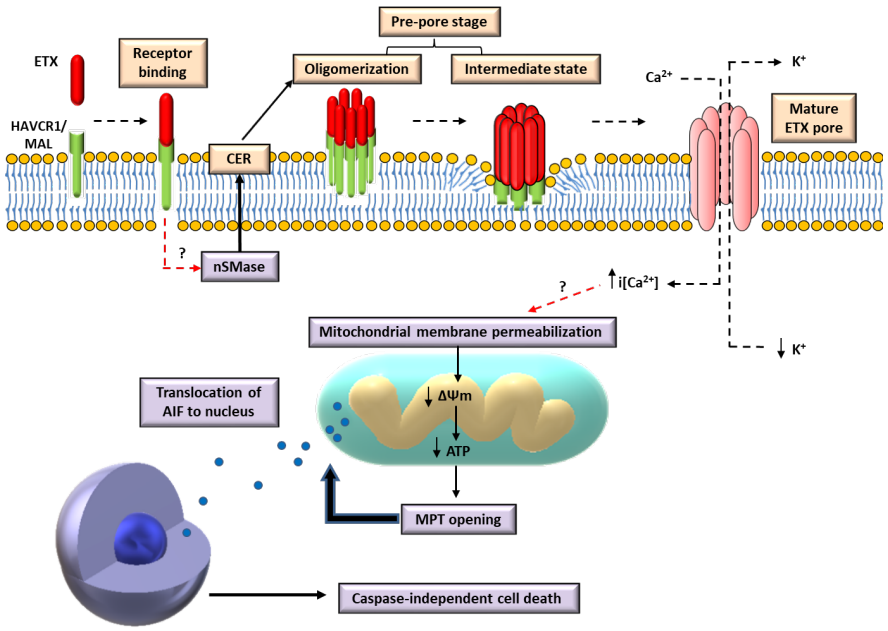

Figure 3. Cellular mechanism of action of epsilon toxin. Epsilon toxin (ETX) binds with their putative receptors, Hepatitis A virus cellular receptor 1 (HAVCR1) and myelin and lymphocyte (MAL) protein distributed on cell membrane of different cells. This is followed by oligomerization of ETX and subsequent membrane insertion. This constitutes the pre-pore stage. This oligomerization is suggested to be facilitated by ceramide (CER) which is in turn produced by ETX activated neutral sphingomyelinase (nSMase). Maturation of ETX pore results in efflux of K^+ ions and influx in Ca^{2+} ions. This increased intracellular ionised calcium ion concentration is suggested to increase mitochondrial membrane permeability. This reduces mitochondrial membrane potential ($\Delta\Psi m$), thereby causing ATP depletion and opening of membrane permeability transition (MPT). This results in translocation of Apoptosis-inducing factor (AIF) from mitochondria to nucleus which results in caspase-independent cell death. Red dashed arrows depict proposed cellular effects of ETX.

C. perfringens type D enterotoxaemia in goats is responsible for the severe enterocolitis with clinically prominent diarrhoea which is considered as the most consistent finding [141]. Besides, other important toxins produced by *C. perfringens* are enterotoxin and β2-toxin, and both have been reportedly associated with the disease [142]. Few experimental studies were successful in reproducing disease in goats [43] [135] [143] [144] [145]. Whole culture of *C. perfringens* type D, its culture supernatant and washed cells were administered intra-duodenally in three different animal groups. A starch solution was also injected into the abomasum of each animal so as to mimic the natural condition. Most of the animals experienced diarrhoea, necrotizing pseudomembranous colitis and cerebral oedema are salient necropsy findings. The reason for this difference in clinical manifestation of diarrhoea was studied in a comparative intestinal loop assay using epsilon toxin in sheep and goats. This study showed that there is a rapid and greater accumulation of fluid and sodium in ileal and colonic loops of goats than sheep. This suggested that the fluid quickly flushes away toxins from gut of goats, thereby reducing transit time for toxin absorption. The delayed response in sheep allows greater absorption of toxin from gut resulting in systemic disease [103].

Experimental type D enterotoxaemia in sheep was studied by many authors [119] [146] [147] [148]. In a study, prominent nervous signs were observed such as paddling, convulsions, ophisthotonus, blindness and bleating but diarrhoea was absent in experimental animal [119]. Similarly, intestinal lesion was absent in lambs but severe haemorrhagic enterocolitis was found in kids at necropsy [147]. Systemic findings such as lung oedema, hydropericardium and cerebral oedema are more prominent in sheep with minor and inconsistent changes in intestine [119] [149]. These prominent systemic findings may be the effect of epsilon toxin which was found in different body fluids like pericardial fluid, aqueous humour including intestinal contents from duodenum, colon and ileum of experimentally infected sheep [148].

Cellular mechanism of action of epsilon toxin

At molecular level, ETX act on cells in a step-wise manner. Attachment of ETX with cell receptor occur prior to or after activation by intestinal proteases (**Figure 3**). Few authors had suggested that some receptors may involve in facilitating cytotoxicity such as hepatitis A virus cellular receptor (HAVCR1) in MDCK cells [150] and myelin and lymphocyte (MAL) protein in different cells [128] [129]. This results in oligomerization to form a pre-pore complex [118]. The oligomerization is observed in rat synaptosomes [151] [152] and MDCK cells [152] [153]. ETX forms a large membrane complex (155 kDa) on the cell membranes of MDCK. The structure of ETX pore and dynamics of ETX pore formation was recently unveiled [154]. Action of ETX on plasma membrane is most commonly studied on Madin-Darby canine kidney (MDCK) cell line due to its high degree of specificity [155]. Recently, Fischer rat (FRT) thyroid cell line was identified as a novel model to study ETX action [156]. The importance of ETX oligomerization in mediating cytotoxicity was recently came in light when a

novel set of rabbit monoclonal antibodies blocked this step which subsequently abolishes ETX endocytosis and cellular vacuoaltion [157].

This oligomerization is reported to be facilitated by ETX activated neutral sphingomyelinase which in turn produce ceramide in the plasma membrane [158]. The binding affinity of ETX and pro-ETX to liposomes was shown to increase by the presence of sulphatide in liposomes and calcium enhances this effect for ETX but not pro-ETX [159] [160]. Action of ETX as a pore-forming toxin is supported by the early efflux of K^+ ions and influx of Na^+ and Cl^- ions followed by rise in intracellular Ca^{2+} levels in MDCK cells [153] [161]. This leads to membrane permeabilisation, decrease in mitochondrial membrane potential and a subsequent ATP depletion. There is translocation of apoptosis-inducing factor (AIF) from mitochondria to nucleus which results in caspase-independent cell death [162]. Apart from its effect on cell membranes, it was demonstrated that ETX blocks mitosis at early stage of cell cycle with a resultant increase in duration of S phase in MDCK cells [163]. However, cellular dynamics can be affected even without pore-formation in oligodendrocytes suggesting some undefined mechanism behind demyelination [70] [130].

7. Clinical History

In sheep and goats, most cases of enterotoxaemia occur soon (a few hours to a few days) after overeating or sudden changes in diet, usually diets rich in highly fermentable carbohydrates [164] [165]. In addition to dietary factors, other unknown prerequisites seem to be necessary for the development of enterotoxaemia in sheep and goats. An outbreak of enterotoxaemia was reported in a herd of goats that had consistently been fed a diet of hay and concentrates for many months before the outbreak [166]. Also, some outbreaks of enterotoxaemia type D have been reported to occur in goats under extensive grazing systems without known diet change [33]. Other factors that disturb the intestinal environment include heavy tapeworm infestation and dosing with phenothiazine in sheep. In goats, a triad of an accidental overdose of netobimin, cold weather stress and a concomitant coccidial infestation were suggested as possible predisposing factors in an outbreak of caprine enterotoxaemia [33]. Heavy worm burden could also be a predisposing factor in goats. A history of sudden change in diet is therefore a useful indicator of a possibility of enterotoxaemia, but the absence of this precedent should not be used to preclude a possible diagnosis of this disease. Vaccination history is frequently used by animal owners and veterinarians to rule out infections by *C. perfringens*. However, the quality of the *C. perfringens* vaccines varies greatly between countries and manufacturers, and vaccines are not always correctly transported, stored and/or administered. In addition, individual variation in antibody responses between animals occurs frequently in both sheep and goats [6]. The history of vaccination as an indicator of possible disease is even less valuable in goats than in sheep. This is due to the fact that vaccination against *C. perfringens* in the former produces antibody titers of

lower level and lower duration than in sheep. Also, the occurrence of enterotoxaemia in goats that have been vaccinated and that have serum levels of epsilon antitoxin which would be protective for sheep [6] suggests that the enteric form of the disease is partially independent of circulating epsilon toxin. Vaccination history alone should therefore not be used to rule out a diagnosis of enterotoxaemia in sheep or goats.

8. Clinical Signs

Clinical signs are suggestive at the most of *C. perfringens* infections, and no final diagnosis can be based on clinical grounds only. The clinical signs vary according to the type of *C. perfringens* involved. In sheep, *C. perfringens* type A produces a rare form of acute enterotoxaemia in lambs, also known as yellow lamb disease, characterized clinically by depression, anemia, icterus and haemoglobinuria. *C. perfringens* types B and C produce similar clinical diseases in sheep that are characterized by sudden death or acute neurological signs with presence or absence of hemorrhagic diarrhoea [165]. Lamb dysentery and hemorrhagic enteritis (*C. perfringens* type B) occur in lambs under 3 weeks of age, whilst struck (*C. perfringens* type C) is a condition of adult sheep [165]. *C. perfringens* type D produces an acute to chronic neurological condition in sheep. The condition is characterized clinically by sudden death or acute to chronic neurological signs including blindness, opisthotonous, convulsions, bleating and recumbency. Enterotoxaemia in goats occurs in four forms, *i.e.*, peracute, acute, subacute and chronic form. In this, peracute form is frequent in young goats and characterized by sudden loss of appetite, marked abdominal pain, kicking at belly, fever up to 40.5˚C and death within twenty-four hours and acute form frequently occurs in adult goats and is clinically characterized by diarrhoea, abdominal pain, severe shock, opisthotonus and convulsions [32] [123] and ultimately causes recovery or death within 2 - 4 days after the onset of clinical signs [147] [167]. Subacute form is characterized by initial softening of faeces which subsequently become diarrhoeic with occasional presence of shreds of intestinal mucosa but abdominal pain is absent [15]. Chronic form of disease lasting days or weeks may also occur in adult animals and shows signs of profuse watery diarrhoea (sometimes mixed with blood and mucus) weakness, abdominal discomfort, weight loss, anaemia and may terminate in death or recovery [141].

9. Postmortem Lesions

In sheep, *C. perfringens* type A enterotoxaemia is characterized by generalized icterus and enlarged, pale and friable livers. Red tinged urine is found in the urinary bladder. *C. perfringens* types B and C in sheep produce similar intestinal lesions consisting of diffuse or multifocal haemorrhagic enteritis, predominantly in the ileum, with excess of sero-sanguineous fluid in the abdominal cavity. *C. perfringens* type D in sheep may produce brain lesions that are pathognomonic for this form of enterotoxaemia. This includes herniation of cerebellum in fora-

men magnum (cerebellar coning) in acute or subacute cases and focal symmetrical encephalomalacia (FSE) in chronic cases, characterised by symmetrical haemorrhagic foci in corpus striatum, thalamus, midbrain, cerebellar peduncles and white matter [5]. Other lesions are pulmonary oedema and pulpy kidney condition.

In case of goats, the disease caused by *C. perfringens* type A, B and C are not well documented and the lesions produced are not specific for these conditions. In enterotoxaemia by *C. perfringens* type D in goats, there may be changes that are suggestive of the disease, but none of these alterations is pathognomonic of enterotoxaemia. An excess of straw colored pericardial fluid rich in protein that clots on exposure to air, interstitial lung edema and sub-endocardial hemorrhages are frequently seen in the per-acute form of the disease [145]. In chronic form of the disease, fibrino-haemorrhagic enterocolitis and pulmonary oedema seem to be the most consistent lesions [141]. Mucosal ulceration has been reported in goats with chronic enterotoxaemia [149]. Kidney lesions are inconsistently seen and any such change could be considered to be suggestive of caprine enterotoxaemia. The lesion in kidneys includes petechial and ecchymotic subcapsular haemorrhages but pulpy kidney condition in case of goats is inconsistent or absent [33] [141]. Recently, cerebellar coning was reported in a goat with *C. perfringens* type D enterotoxaemia for the first time [123]. This lesion is considered pathognomonic in sheep.

10. Histopathology

In sheep and goats, the most of the histopathological changes due to enterotoxaemia are not very specific but if present, can be highly suggestive of the disease. Histopathological findings in *C. perfringens* type B and C enterotoxemia in sheep and goats is characterised by coagulative necrosis in intestinal mucosa, thrombosis of mucosal and submucosal blood vessels and a variable inflammatory response [5]. In sheep, the histopathological changes produced by *C. perfringens* type D epsilon toxin in the brain are unique and pathognomonic for this type of enterotoxaemia. The most consistent change is perivascular proteinaceous oedema, consistent of acidophilic lakes of protein surrounding small and medium sized arteries and veins [168]. This change can be seen in animals after a few hours of the onset of clinical signs. If the animals survive for several hours, degeneration of white matter, hemorrhage, astrocyte and axonal swelling can also be observed [168]. In the chronic form of the disease the lesion is characterized by necrosis of white matter, grossly known as focal symmetric encephalomalacia (FSE). Both the perivascular edema and the degeneration and necrosis of brain parenchyma are always bilateral and symmetrical and they have been described most frequently in corpus striatum, internal capsule, thalamus, mid brain, cerebellar peduncles and cerebellar white matter [112] [168].

In a study, severe congestion, haemorrhage and necrosis were found at the tip of the villi in duodenum, ileum and of colonic epithelium of goats [33]. Fibrino-necrotic or pseudo-membranous colitis was also observed in sub-acute and

chronic cases of enterotoxaemia caused by *C. perfringens* type D [166]. Histological lesions in the kidney are not a characteristic of sheep or goat enterotoxaemia. The so-called pulpy kidney disease described in cases of this diseases is assumed to be a post-mortem change due to rapid autolysis of the tissues [149] and it has very little, if any, diagnostic significance. Changes in the brain are not considered to be a common feature of caprine enterotoxaemia, although meningeal congestion with perineuronal oedema in the cerebral cortex and cerebellum may be observed in per-acute and acute cases [42] [43] [113] [145] [149]. However, few reports describe about the neuropathology involved in caprine enterotoxaemia. Bilaterally symmetrical homogenous eosinophilic, proteinaceous perivascular oedema in the internal capsule and superior colliculi was observed in goat kids died of *C. perfringens* type D enterotoxaemia [135]. Recently, intramural vascular oedema in brain of *C. perfringens* type D enterotoxaemic goats was described in which the highly proteinaceous oedema was present in between the muscularis and adventitia of the vascular wall [123].

11. Clinical Pathology

There is limited literature on haematologic and clinical biochemistry studies on enterotoxaemia. The presence of urinary glucose in any amount is strongly indicative of enterotoxaemia in both sheep and goats [5]. In contrast to ovine enterotoxaemia, hyperglycaemia and glycosuria are inconsistent [33] [147] and thus not considered pathognomonic in goats. In context to urine protein and glucose, no significant difference was found in treated and control groups of goats in an experimental study [135]. The effects of *C. perfringens* type D infection on haematological and biochemical parameters in sheep and goats are studied. In goats, mean erythrocytes count (RBC) and haemoglobin level decreased significantly while the white blood cells (WBC) increased significantly in diseased animals compared to the healthy animals [145]. Non-significant changes were observed in packed cell volume and platelet count. Similarly biochemical analysis showed a significant increase in liver enzymes, serum creatinine, total bilirubin, blood urea and glucose in diseased goats [169]. In sheep, mean leukocyte count and packed cell volume was significantly higher in infected than control animals. Serum biochemistry revealed a significant rise in blood glucose, urea and serum creatinine levels in infected animals as compared to control groups [140].

12. Diagnosis

A tentative diagnosis can be given on the basis of clinical history and signs, predisposing factors, postmortem lesions. The definitive diagnosis of enterotoxaemia can be given on the basis of detection of epsilon toxin in intestinal fluid, tissue, culture supernatants and other body fluids with the help of serological tests and molecular techniques such as ELISA, PCR, real time PCR, immunohistochemistry etc. Various techniques used for diagnosis of enterotoxaemia are outlined in **Figure 4**.

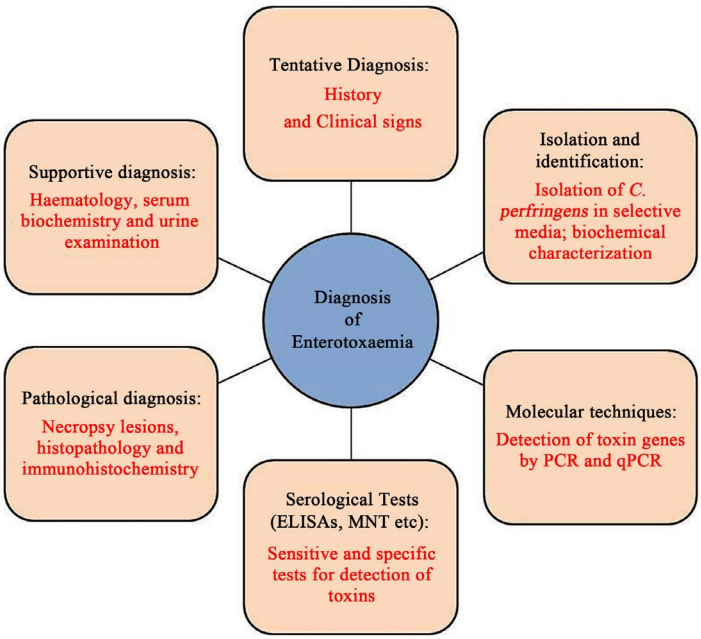

Figure 4. Methods for diagnosis of enterotoxaemia.

1) Field diagnosis—It is based on a combination of characteristic clinical history and signs, necropsy. Sudden death without any premonitory signs and the presence of acute haemorrhagic enterocolitis with haemorrhagic intestinal contents at necropsy examination are highly specific. Information must be taken into account with particular attention to any stress observed 12 to 36 hours before death. The ideal sample obtained at post-mortem examination for bacterial analysis is a ligated intestinal loop in the location of the entero-haemorrhagic lesions. For bacteriological and culture examination, samples should be obtained from freshly dead animals. Therefore, in practice, post-mortem examination must be performed on-site (or on-farm) as quickly as possible and the ligated intestinal loop should be kept at 4˚C in appropriate transport conditions. To further maintain the stability of toxins, one drop of chloroform can be added in each 10 ml of intestinal content [170].

2) Laboratory diagnosis—It is based on isolation of *C. perfringens* from the faeces or gut lumen [164] and identification of epsilon toxin or the gene expressing epsilon toxin in *C. perfringens* cultures from faeces or gut lumen content [5].

Bacteriological and Biochemical Tests

Isolation and cultural examination of *C. perfringens* from gut contents of animals is of merely importance in diagnosis for enterotoxaemia because this organism is a normal inhabitant of intestine in most of the small ruminants. A bacterial count of 10^4 - 10^7 CFU/g of faecal sample is considered as normal in the sheep and goats [119]. However, impression smears from the small and large intestine of affected animals' revealed almost pure population of short, thick usually non-sporulated, Gram positive rods [32]. This can be suggestive of enterotoxaemia and thus helpful in further diagnostic interventions to be followed.

The common culture media utilized for isolation are Robertson's cooked meat media (RCMM), thioglycolate media, reinforced clostridial media, clostridial supplemented brucella blood agar (CLS-BBA), tryptose sulphite cycloserine (TSC) agar, TSC-egg yolk agar (TSC-EYA) [43] [171] [172] etc. Gas production in RCMM, double zone of hemolysis in CLS-BBA [43] [145] and pitch black colonies with opaque halos in TSC-EYA are presumptive of *C. perfringens* [171]. Various biochemical tests for *C. perfringens* type D were depicted in **Table 3**.

Detection of Toxin Genes

PCR

In the beginning, in 1990s, polymerase chain reaction test were developed to detect the presence of bacterial genes for production of *C. perfringens* toxins based on the genes encoding the respective toxins [173]. Polymerase chain reaction (PCR) provides a useful alternative to *in vivo* toxin neutralization tests for typing of *C. perfringens* [10]. Therefore, detection of epsilon toxin encoding gene (etx) is very important and helpful in the diagnosis of enterotoxaemia [174]. Multiplex PCR based toxinotyping of *C. perfringens* strains present in the intestinal contents or faeces [20] [174] [175] and intestinal tissue of sheep and goats [23] [43] [176] are now commonly used for the diagnosis of disease. Multiplex PCR based toxinotyping revealed the high prevalence of beta2 gene in type A isolates (73%) and type D isolates (67%) in faecal samples from healthy and diseased sheep and goats [175].

Real-time PCR

Real-time PCR technique was used by many authors for detection of toxin genes of C. perfringens. It was used for detection of *C. perfringens* in horses affected by enterocolitis and the results showed that prevalence of infection by *C. perfringens* type C was 40% (12/30) in 30 faecal samples [177]. Similarly, alpha toxin gene was detected in 40% and 70% of cattle and sheep faecal samples [178]. Real-time fluorogenic PCRs were developed for the detection of *C. perfringens* α, β, $\beta 2$, ε, entero- and ι-toxin [179]. Dual-labeled fluorescence hybridization probe (TaqMan®)-based real-time multiplex PCR assay was developed for detection of toxin genes alpha (*cpa*), beta (*cpb*), iota (*ia*), epsilon (*etx*), beta2 (*cpb*2) and enterotoxin (*cpe*) of *C. perfringens* directly from cattle faeces [180].

Detection of Toxins

Immunohistochemistry

The immunohistochemistry represents possible diagnostic significance in those cases in which culture and PCR detection is not possible. The immunohistochemistry has been used surpassing to detect the presence of clostridial toxins in tissues, including *Clostridium perfringens* enterotoxin (CPE) in the colon of rabbits [181], ileal mucosa of goat [182], different parts of gastrointestinal tract of horses [183] and *Clostridium perfringens* beta toxin in the small intestine of piglets [184]. CPB2 was demonstrated in small and large intestine of goats with beta2 toxin/enterotoxin-positive *C. perfringens* type D enterotoxaemia [17]. Further studies are required for demonstration of alpha and epsilon toxin in sheep and goat tissues.

Table 3. Biochemical characteristics of *C. perfringens* type D [172].

Biochemical tests	*C. perfringens* type D
Sugar fermentation-glucose, maltose, lactose, sucrose, fructose, galactose, dulcitol	+
Indole production	–
Methyl red	–
Voges Proskauer	–
Catalase	–
litmus milk	+
Urease	–
H_2S production	+
Nitrate reduction	+
Phospholipase reaction	+

+, positive; –, negative reaction.

Serological and biological neutralization tests

Detection of toxin in the intestinal contents from enterotoxaemia affected animals is considered as best diagnostic test for enterotoxaemia. There are several tests available for detection of toxin from intestinal contents as well as other body fluids and culture supernatants. The techniques are several including ELISAs, counter-immunoelectrophoresis and mouse neutralization test [185].

1) Mouse neutralization test (MNT)

It is a commonly used biological neutralization test to detect epsilon toxin in biological and non-biological samples. Its use has been reduced as other serological tests are developed. MNT is commonly used as a comparative test for validating or comparing the diagnostic efficacy of other serological tests and also used for potency testing of clostridial vaccines. MNT has absolute sensitivity and specificity of 54.54% and 100%, respectively in detecting epsilon toxin in intestinal contents and body fluids [186].

2) Enzyme-linked immunosorbent assay (ELISA)

ELISA is considered satisfactory test for detection of epsilon toxin in gut contents [187]. Sandwich ELISA has high sensitivity and specificity rates, respectively of 97.4% and 94.6% for epsilon toxin assay in intestinal contents [188]. Recently, a highly sensitive sandwich immunoassay and immunochromatographic test was developed to detect epsilon toxins with fivefold detection limits in different matrices such as serum, intestinal contents [189]. In a comparative study using polyclonal capture-ELISA (PC-ELISA), monoclonal capture-ELISA (MC-ELISA), counter-immunoelectrophoresis and MNT for the detection of *C. perfringens* type D epsilon toxin in intestinal contents and different body fluids of sheep and goats, PC-ELISA was found to be the most sensitive technique re-

gardless of fluid type [186]. Recently, CPA and ETX are detected in the small and large intestine contents of the goats with type D enterotoxaemia using monoclonal antibody-based capture ELISA [123].

3) Latex agglutination test (LAT)

Latex agglutination test (LAT) was developed as a qualitative test to detect epsilon toxin in intestinal contents of animals suspected of dying from enterotoxaemia. It has showed a sensitivity of 96.5 per cent and a specificity of 95.2 per cent which was found slightly less than that of ELISA [190]. In another study, ELISA was found significantly more sensitive than LAT in detecting *C. perfringens* toxins in intestinal contents of sheep with suspected enterotoxaemia. ELISA and LAT detected toxins in 84.61% and 58.46% of intestinal contents, respectively [21]. However, the LAT has some additional advantages over ELISA. It is comparatively simple, cheap and quick to perform and thus can serve as a potential field test for diagnosing enterotoxaemia. Unlike ELISA, it can demonstrate the biological activity of epsilon toxin [191].

4) Indirect haemagglutination test (IHT) and Indirect haemagglutination inhibition test (IHIT)

Indirect haemagglutination test was effectively used to measure serum antibody titre against adjuvanated toxoid vaccines [192]. Likewise, these tests were developed in our laboratory to detect anti-epsilon toxin antibodies in the sera and epsilon toxin in intestinal contents of enterotoxaemia affected animals respectively (Unpublished data). In IHT, glutaraldehyde tannic acid treated sheep RBCs were sensitized separately with both crude-ETX and peptide ETX antigens, both of which gave encouraging results as mode of measuring protective antibody titre in vaccinated animals and passive protective titre in neonates.

Mass spectrometry techniques

Novel MS techniques are now utilized in some specialized laboratories to detect and quantify epsilon toxin in different biological and non-biological samples [193] [194] [195]. This includes liquid chromatography-mass spectrometry (LC-MS), ultraperformance liquid chromatography-tandem mass spectrometry (UPLC-MS) and MALDI-TOF tandem mass spectrometry. Despite of high sensitivity in detecting ETX, upto 0.2 ppb [194], MS techniques are used infrequently due to its high initial investment cost which limits its use in laboratories dealing with biological warfare agents. In addition to this, it cannot determine the biological activity of toxin.

13. Treatment

In general, the clinical course of disease is too rapid for effective treatment. The treatment should be focused on following targets—inhibiting bacterial proliferation, preventing absorption of toxins from intestine, neutralization of already absorbed toxins (serotherapy) and supplementary treatment to counteract dehydration, acidosis and shock, especially in per-acute and acute cases. Bacterial proliferation can be inhibited by oral and/or parenteral antibiotic therapy. Re-

cently, an extensive *in vitro* antibiotic sensitivity testing and *in vivo* antibiotic trials against *C. perfringens* in diarrhoeic sheep and goats revealed ciprofloxacin, penicillin and ceftriaxone as most effective antibiotics [196]. In face of bacterial proliferation, the second important step is to flush away released toxins from intestinal tract so as to inhibit its absorption. Cathartics can be helpful in this situation but condition of animal especially dehydration status must be kept in mind. As disease progresses very rapidly, administration of antitoxin (serotherapy) is considered better option over antimicrobial therapy [197]. Serotherapy with ETX antitoxin has been used in experimental *C. perfringens* type D infected sheep and goats [54]. The immediate losses in outbreaks can be prevented by administering epsilon antitoxin at a dose rate of 200 IU/Kg BW in sheep [171]. The lack of identification of the toxins involved the cost and unavailability of antitoxin and the existence of a risk of anaphylaxis (equine origin antitoxin) renders this approach very uncommon in field conditions. However, the combined treatment with antisera and antibiotics was found more effective than antibiotics or antitoxin alone in few experimental studies in goats [143] [144]. The efficacy of combined therapy with hyperimmune sera and procaine penicillin, hyperimmune sera and oxytetracycline dihydrate, procaine penicillin alone, hyperimmune serum alone and oxytetracycline dihydrate alone was 83.33%, 50%, 50%, 33% and 33% [143]. Beside these, intravenous administration of electrolyte fluid mixed with bicarbonate can be helpful in alleviating dehydration, acidosis and toxaemic state [32].

14. Prevention and Control

Immunoprophylaxis

Small ruminants are considered highly susceptible to enterotoxaemia and it is universally recommended that they should be vaccinated against the disease. There is marked variation in immune response in sheep and goats against toxins. The commonly available commercial vaccines do not offer the proper protection in goats [6] [135] [198]. The goats with titre below 0.1 IU/ml were found to be unprotected [6] while titre of 0.25 IU/ml is considered protective in goats [135]. A single administration of enterotoxaemia-cum-lamb dysentery vaccine in sheep and goats produced an antibody titre of 6.5 and 2.64 respectively and the booster dose of same vaccine produced titre of 59.7 and 12.12. This depicts that protective serum antibodies level is maintained for limited period of time in goats [199]. In another study, a booster dose after 40 days from first vaccination was found successful in generating satisfactory antibody levels in goat kids [198]. So, in order to generate adequate protection, goats should be vaccinated at intervals of three to four months or at least semi-annually with currently available commercial vaccines [32]. In case of sheep, two doses of vaccine, about 4 - 6 weeks apart followed by subsequent annual booster is sufficiently protective against enterotoxaemia [54]. A polyvalent enterotoxaemia vaccine seems to be effective in sheep as shown by a significant difference in disease occurrence

among vaccinated (3.3%) and unvaccinated groups (64%) [200]. In terms of antibody response in goats, incomplete Freund's adjuvanted vaccine was found best among aluminium hydroxide adjuvanted and liposome-adjuvanted *C. perfringens* type epsilon toxoid vaccine [7]. Recombinant *C. perfringens* epsilon toxoid vaccine confers protection against enterotoxaemia with protective antibody titers of 14.3 and 26 IU/ml in goats and sheep respectively [201]. Recently, a recombinant trivalent vaccine against alpha, beta and epsilon toxins generated a good antibody response in multiple species (sheep, goats, cattle and rabbits) than commercial vaccines [202]. Despite of exciting results, the recombinant vaccines are not commonly used.

Feeding Strategies

Smart feeding of goats can reduce the occurrence of disease in flock or herd. It is better to give hay before feeding of carbohydrate rich feed stuffs and these should be divided into many small feedings. Additionally avoid excessive feeding of carbohydrates, decrease amount of feed with intermittent feeding schedule and increase animal movement [46]. Recently it was also identified that, vegetable tannins can inhibit the *in vitro* growth of *C. perfringens* in a dose-dependent manner [203]. But practical utility needs to be explored to establish the effect of tannins and more studies are required in this direction to mitigate the disease. To summarise, timely vaccination, avoiding sudden feed changes, preventing overeating and preventing accidental access to grains and other stored feeds are keys to control enterotoxaemia in small ruminants.

15. Conclusion

Enterotoxaemia is a devastating disease of sheep and goats throughout the world. Though there are significant differences between caprine and ovine enterotoxaemia, documentations and research specifically on the condition in goats are very little. At the same time caprine enterotoxaemia continues to cause economic losses to goat farmers all over the world. The characteristics of enterotoxaemia in goats differ from sheep mainly in terms of clinical signs and lesions in different organs at necropsy and failure of vaccination strategy. Hence, basis for all these differences needs to be clarified for timely diagnosis and effective control of this disease in goats. In the field settings, enterotoxaemia incurs huge cost and loss of valuable germplasm. Further research studies are required to provide better understanding of the pathogenicity of *C. perfringens*, which in turn will help in the development of molecular biomarkers/diagnostics to provide confirmatory diagnosis, vaccination strategies, better management and feeding practices for preventing enterotoxaemia in small ruminants, particularly in goats.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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