Review

BOTULISM IN MAN AND ANIMALS

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Summary


Botulism is a rare, acute and highly fatal neuroparalytic disease that affects both man and animals. It is a worldwide problem that has been reported in several countries, with limited management options that are still being researched. Confirmation of diagnosis of the disease is a problem, especially in localities where facilities for conducting the traditional mouse bioassay are not readily available. In this review paper, the authors highlighted the epidemiology of the disease in man and animals and summarised the new research trends and reports on novel diagnostic methods that could save time and enhance patient survival. It was concluded that apart from ensuring a sustained global research on the molecular dynamics of the disease, with the aim to evolving the most effective management protocols, with the highest probability of patient survival, human and veterinary public health officials in the endemic areas should routinely educate the rural and urban communities on the public health significance of botulism and the need to keep safe through strict adherence to standard preventive and control measures.

Key words: animals, botulism, man

INTRODUCTION

Botulism is an acute, severe neuroparalytic disease affecting human beings and animals that occurs as a result of blockage of acetylcholine (neurotransmitter) release from the synaptic vesicles at the neuromuscular junctions due to the specific action of botulinum neurotoxins (BoNT) (Hatheway, 1995; Hatheway & Johnson, 1998). The disease is rare, but could cause severe illness that is potentially lethal if not treated rapidly (Arnon et al., 2001; Coban et al., 2010). In vivo BoNT cleaves proteins necessary for nerve signal transmission. The enzymatic cleavage results in the inhibition of nerve impulses, leading to flaccid muscular paralysis, which
can affect the lungs and may require ventilator support (Kalb et al., 2011). A clinical syndrome of cranial neuropathy and descending asymmetrical flaccid paralysis has been linked to botulism by some authors (Shapiro et al., 1998). Cranial palsies also characterise the disease (Sobel et al., 2007). Generalised muscular weakness, which is classical, may extend gradually to all skeletal muscles leading to death due to respiratory dysfunction (Takeda et al., 2006). In waterfowl, drowning is a common cause of death, because the neck muscles of the affected bird become paralysed and the bird is no longer able to hold its head above water (Sharpe et al., 2011). Botulinum toxin is the most potent toxic substance known to man, with seven (A to G) antigenically distinct neurotoxins (Peck & Stringer, 2005). Since the first reported cases of food borne botulism in the late 18th century, botulism has gained attention not only as a threat to food producers and consumers, but also as a potential cause of crib death of small babies, as a deadly trap for intravenous drug abusers, and as a potent weapon for bioterrorism (Lindström & Korkeala, 2006).

TYPES OF BOTULISM AND AETIOLOGY

Four clinical forms of botulism have been described and they include food borne, adult intestinal, infant and wound, although rare, iatrogenic and inhalation forms have been described as well (Arnon et al., 2001; Coban et al., 2010). Other authors writing exclusively on botulism in human beings categorised it into three main types namely food borne, infant and adult intestinal colonisation, and wound botulism (Turton et al., 2002). Neurotoxins produced by Clostridium botulinum, Clostridium baratti and Clostridium butyricum are known to cause the disease (Schiavo et al., 2000). C. botulinum produces all seven known serotypes (A to G), whereas C. baratti and C. butyricum produce only one serotype each (F and E, respectively) (Hutson et al., 1993; Simpson, 2004). In human beings, botulism is caused mainly by serotypes A, B and E and rarely by serotype F (Sobel et al., 2004), while botulism in animals is mainly caused by serotypes B, C and D (Coleman, 1998). The anaerobic spore forming C. botulinum serotypes/strains above have been further classified into groups I–IV based on metabolism and pathogenesis. Groups I and II include human pathogenic strains. Group I consists of proteolytic strains producing types A, B and F toxins, and group II of non-proteolytic strains producing types B, E and F toxins (Smith & Sugiyama, 1988). Group III strains produce either serotype C or D and are associated with botulism in animals, and group IV contains only strains producing BoNT serotype G (Collins & East, 1998; Lund & Peck, 2000). In the Baltic Sea area, where non-proteolytic group II has predominated, a particularly high prevalence of type E has been reported (Hyytiä et al., 1998).

Food borne botulism is a paralytic illness that is usually caused by ingestion of the toxins of Clostridium botulinum and closely related species (Sobel et al., 2007). Consumption of contaminated food in which neurotoxin has been produced can result in food borne botulism, a severe disease with a high fatality rate. As little as 30 ng of neurotoxin can be fatal (Peck, 2006). Infant botulism is an intestinal toxæmia that affects children <12 months of age; a similar disease also very rarely affects adults, and occurs when competing bacteria in the normal intestinal microbiota have been suppressed (e. g., by antibi-
otic treatment). Infant botulism has been reported in many countries, and in the United States, it is the commonest manifestation of the disease. Some reports suggest a link to sudden infant death syndrome (Arnon, 2004; Fox et al., 2005). Wound botulism is an infection in which growth and neurotoxin formation occur in wound in the body. For adult and infant botulism to occur there has to be intestinal colonisation by C. botulinum, while wound botulism occurs following wound contamination by C. botulinum (Werner et al., 2000).

SUSCEPTIBLE HOSTS

Typical C. botulinum infection causes disease and mortality (Forrester et al., 1980; Wobeser et al., 1997) and has been reported worldwide in man (Midura & Arnon, 1976; Arnon et al., 1981; Aureli et al., 1986; Cox & Hinkle, 2002; Carlin et al., 2004; Frean et al., 2004; Barash et al., 2005; Fox et al., 2005; Johnson et al., 2005; Keet et al., 2005; Arnon et al., 2006; Reller et al., 2006; Artin et al., 2007; Fenicia et al., 2007a; Nishida et al., 2007; Abe et al., 2008; Cameron, 2009; Fenicia & Anniballi, 2009; Uméda et al., 2009; Lúquez et al., 2010; Rowlands et al., 2010; Sevenier et al., 2012; Fujinaga et al., 2013), and other mammals (Doutre, 1967b; Barsanti et al., 1978; Doutre, 1983; Farrow et al., 1983; Thiongane et al., 1984; Bernard et al., 1987; Yamakawa et al., 1992; van der Lugt et al., 1995; Fujinaga et al., 1997; Wobeser et al., 1997, Bohnél et al., 2003; Elad et al., 2004; Lindstrom et al., 2004; Böhnél et al., 2005; Walker et al., 2009; Johnson et al., 2010; Williams et al., 2011; Schwarz et al., 2012). The disease is known to also affect birds (Doutre, 1967a; Hay et al., 1973; Forrester et al., 1980; Dohms et al., 1982; Smart et al., 1983; Wobeser et al., 1983; Shayegani et al., 1984; Okoye, 1988; Allwright et al., 1994; Woo et al., 2010; Hardy et al., 2011; Sharpe et al., 2011; Raymundo et al., 2012). There are several reports of botulism in cold blooded animals (Mengiste et al., 1990; Nol et al., 2004; Merivirta et al., 2006; Yule et al., 2006a,b; Gaunt et al., 2007; Neimanis et al., 2007; Crauste et al., 2008; King et al., 2009; Horowitz, 2010; Khoo et al., 2011). Wound botulism has been reported by several famous authors (Werner et al., 2000; Akbulut et al., 2005a,b; Schroeter et al., 2009).

EPIDEMIOLOGY

Botulism in humans is a disease that has been reported worldwide. In the coastal areas of the North including Alaska, the Pacific coast of British Columbia, northern Canada and Greenland it occurs following consumption of traditionally prepared foods (Dawar et al., 2002; Austin & Leclair, 2011). Majority of the outbreaks are caused by consumption of the flesh of marine mammals and fish. Infant botulism seems to be on the increase in the United States. Since 2004, the Centers for Disease Control and Prevention (CDC) have documented more than 2000 cases of infant botulism in the United States, principally produced by types A and B (NIOSH, 1996; CDC, 2006). The average annual incidence of infant botulism in Argentina (2.2 per 100,000 live births) (Lúquez et al., 2005; 2007) is similar to that in the United States (1.9 per 100,000 live births) (Centers for Disease Control and Prevention, 1998). Only a small number of cases have been reported in Italy, Germany, the United Kingdom, Spain, Denmark, Japan, Australia (Fox et al., 2005), and Finland (Nevas et al., 2005). Although 26 coun-
tries in 5 continents have reported infant botulism, with United States, Argentina, Australia, Canada, Italy and Japan reporting the largest number of cases in that order (Koepke et al., 2008), it is believed that infant botulism is most likely under-diagnosed or undetected in many countries (Myllykoski et al., 2008; Koepke et al., 2008; Rebagliati et al., 2009). Risk factors for infant botulism are multifactorial and include breast feeding, and the introduction of first formula feeding, consumption of honey and residence in a region of high spore density and soil disruption (Long et al., 1985). Constipation appears to be a risk factor but also is an early manifestation of intoxication (Sobel, 2005).

Both food-borne and wound botulism are extremely rare in Ireland, unlike many European countries which routinely report food-borne cases each year. Wound botulism is much rarer, but both sporadic cases and outbreaks have been reported in European countries in the past several years (Burnens, 2000; Alpers et al., 2005). Most cases of food borne botulism in the United States are due to improperly handled (primarily home-preserved) foods (Sobel & Maslanka, 2012). Botulism attributed to commercially canned foods is rare. Proper commercial canning, owing to controlled temperature and processing time, renders food commercially sterile (free of viable microorganisms, including those of public health significance such as spores of C. botulinum, capable of reproducing under normal non-refrigerated conditions during storage and transport) (US Food and Drug Administration, 2012). In 2008, Dutch tourists on a mini-cruise in Turkey reportedly developed food-borne botulism (Swaan et al., 2010). In animals, food-borne botulism is usually associated with feed contamination (Myllykoski et al., 2011; Ostrowski et al., 2012) and mortality, especially in sheep and cattle, range between 5–80% (Payne et al., 2011). There are several reports of food-borne botulism in European countries (Aureli et al., 1996; Cowden, 2011; Pingeon et al., 2011). The fatality rate is approximately 5-10% of cases. The economic and medical costs associated with food borne botulism are extremely high (Setlow & Johnson, 1997; Peck et al., 2011).

Intestinal toxaemia botulism is an infectious form of botulism in which illness results from ingesting spores, which is followed by spore germination and intraluminal production of botulinum neurotoxins over an extended period (Arnon, 1995; Fenicia et al., 2007a). Intestinal toxaemia botulism is rarely reported in adults. Inhalational botulism is not a naturally occurring disease. The syndrome was described once among German laboratory workers in 1962, with symptoms resembling those of food borne botulism (Middlebrook & Franz, 1997). Deliberate dissemination of botulinum toxin by aerosol could produce an outbreak of inhalational botulism (Arnon et al., 2001). Iatrogenic botulism is caused by injection of botulinum toxin for cosmetic or therapeutic purposes. Doses recommended for cosmetic treatment are too low to cause systemic disease. Higher doses injected for treatment of muscle movement disorders have caused anecdotal cases of systemic botulism-like symptoms (Bakheit et al., 1997).

Clinically, human botulism presents as descending flaccid paralysis, beginning in the bulbar muscles and involving at least one cranial nerve. The patients may complain about difficulty swallowing, a dry mouth, double vision, dysarthria, constipation and general fatigue. The onset of the symptoms usually takes place after
two hours up to eight days after toxin ingestion (Vossen et al., 2012). Some case reports of intestinal toxaemia botulism showed abdominal pain, blurred vision, diarrhoea, dysarthria, dysphagia, horizontal binocular diplopia, imbalance, and weakness in the arms and hands, minimal Orbicularis oculi contractions and quadriparesis with normal reflexes. After admission to the hospital, respiratory arrest occurred requiring intubation and ventilation support (Sheppard et al., 2012).

In animals clinical signs usually observed depend on the species involved and these include sudden death, without clinical signs, standing with head lowered, drooling saliva, terminal paresis and/or paralysis (sheep), progressive muscle weakness, recumbency, decreased tail and/or tongue tone, dysphagia, respiratory distress, and death (horse), sternal recumbency, reluctance to move, flaccid paralysis of the neck with the head and beak resting on the ground in front of them, wings drooping to the sides, closed eyes and diarrhoea-stained vent feathers (chicken), acute onset, hind limb paralysis, quadriplegia within hours, diffuse lower motor neuron dysfunction with impaired spinal reflexes in all limbs, lateral recumbency, reluctance to move, flaccid paralysis of the head, and flaccid weakness in the arms and hands, minimal Orbicularis oculi contractions and quadriparesis with normal reflexes. After a mission to the hospital, respiratory arrest occurred requiring intubation and ventilation support (Sheppard et al., 2012).

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HAEMATOLOGIC AND BIOCHEMICAL CHANGES

Laboratory investigation results were normal in a case of fatal human botulism in Kenya (Jones, 1980). In another report, no significant biological abnormalities, except hypoglycaemia, were observed in a 2-month old breast fed child with botulism (Hoarau et al., 2012). Braun et al. (2005) documented the haematologic and plasma biochemical changes in cattle with botulism. The report showed that packed cell volume (haematocrit) was either normal or increased in some animals and the same pattern was observed for total leukocytes. Total protein, fibrinogen, aspartate amino transferase (AST), alanine amino transferase (ALT), and bilirubin were also normal or increased in some animals. Serum electrolytes (calcium, inorganic phosphorus, magnesium, sodium, potassium, chloride, bicarbonate) were either normal or decreased. Venous gases and pCO₂ (mmHg) were also normal or decreased. Neutrophilia and hyperglycaemia with no other consistent haematologic or biochemical abnormalities have also been reported (Cobb et al., 2002).

In an outbreak of botulism in black-faced spoonbills, Chou et al. (2008) compared the haematologic and biochemical changes in apparently healthy and sick birds before treatment and after recovery (about 17–30 days after treatment). No botulism-related differences were found for RBC count, Hb, PCV, MCV, MCH and MCHC (P>0.05). Values of creatinine, uric acid, ALP, ALT, and triglycerides all decreased (P<0.025) in recovered birds. Median BUN, UA, ALP, ALT, and triglycerides were more than double the levels observed in recovered
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Birds; mean creatinine was eightfold higher before recovery. The authors attributed the elevation in creatinine, uric acid, ALP, ALT, AST, and triglycerides in affected birds to anorexia, dehydration, and liver, kidney, and muscle damage. Although not statistically significantly different ($P=0.1$), CK and mean phosphorus levels were also elevated by more than twofold in birds with botulism. The authors attributed the elevation in creatinine, uric acid, ALP, ALT, AST, and triglycerides in affected birds to anorexia, dehydration, and liver, kidney, and muscle damage. Although not statistically significantly different ($P=0.1$), CK and mean phosphorus levels were also elevated by more than twofold in birds with botulism. The authors believed that the higher levels of uric acid and BUN in birds with botulism may have occurred as a result of anorexia, dehydration, and kidney damage and all these factors may have contributed to these elevated levels. Sanford et al. (2010) found no biologically relevant changes in haematologic and clinical chemistry parameters of monkeys (Rhesus macaques) exposed to either BoNT/A1 or BoNT/B1 in the low-, middle-, and high challenge-dosage groups.

PATHOGENESIS

Botulinum neurotoxins (BoNTs) are synthesised by Clostridium botulinum as single chain proteins (approximately 150 kDa). The toxins are exported outside the bacteria by a yet to be known mechanism and are proteolytically cleaved into a heavy chain (H; approximately 100 kDa) and a light chain (L; approximately 50 kDa), which remain linked by a disulfide bridge. The di-chain molecule constitutes the active neurotoxin. In culture medium or in food, BoNTs are non-covalently associated to non-toxic proteins (ANTPs) including haemagglutinin (HA) subunits and a single non-toxin non-haemagglutinin (NTNH) component, to form botulinum complexes of various sizes (Couesson et al., 2008). The induction of neuromuscular paralysis by BoNTs requires three biochemical steps. First, BoNT protein binds to gangliosides on the presynaptic cholinergic nerve terminal through interactions with the heavy chain. These interactions allow subsequent endocytosis into the neuron through several possible mechanisms involving synaptotagmins I and II (BoNT/B and BoNT/G) and SV2 (BoNT/A) (Dong et al., 2006). The toxin is then translocated into the cytosol where its light chain (LC), a metalloprotease, binds to, and cleaves soluble N-ethylmaleimide-sensitive factor attachment protein receptor proteins (SNAREs) (Simpson, 2004). This action halts the release of acetylcholine (ACh) at the neuromuscular junction, leading to the cessation of neurotransmission.

Many authors described the pathogenesis of botulism and botulism neurotoxin (BoNT) in such a way as to portray the toxin as one whose lethality is second to none. Botulinum toxin blocks the release of acetylcholine at neuromuscular junctions resulting in flaccid paralysis (Hill et al., 2010). BoNTs target and penetrate cholinergic nerve endings by receptor-mediated endocytosis. Upon acidification of endosome-containing toxin molecules, the L chain translocates into the cytosol and catalyses a zinc-dependent proteolysis of one of three proteins of the SNARE complex, which play an essential role in evoked neurotransmitter exocytosis. The BoNT/A L chain cleaves the synaptosomal-associated protein SNAP25 at neuromuscular junction (Herreros et al., 1999; Humeau et al., 2000; Schiavo et al., 2000; Poulain et al., 2006). The highly specific binding of BoNTs to the target nerve endings involves protein and ganglioside receptors that localise at the neuronal plasma membrane (Montecucco et al., 2004). Gangliosides of GD1b and GT1b series are involved in binding and functional entry into cells of BoNT/A and
BoNT/B (Kozaki et al., 1998; Kitamura et al., 1999; Rummel et al., 2004a). The protein receptor on neuronal cells has been identified as synaptotagmin I and II for both BoNT/B and BoNT/G, and as synaptic vesicle protein SV2 (isoforms A, B and C) for BoNT/A (Nishiki et al., 1994; Dong et al., 2003; 2006; Rummel et al., 2004b; Mahrhold et al., 2006). In contrast, BoNT/C and BoNT/D seem to interact only with gangliosides (GD1b and GT1b) or phosphatidylethanolamine respectively (Tsukamoto et al., 2005).

BoNT escapes the gastrointestinal tract to reach the target cholinergic nerve endings, probably through the blood and lymph circulation (Maksymowych et al., 1999). The upper small intestine is known to be the primary site of toxin absorption (Sugii et al., 1977; Bonventre, 1979), but BoNT can also be absorbed from the stomach (Maksymowych et al., 1999). In addition, BoNT is able to cross other epithelial cell barriers, such as the epithelium of the respiratory system, explaining why botulism can also be acquired by toxin inhalation (Park & Simpson, 2003). Penetration of BoNT through an epithelial cell barrier and its subsequent migration to cholinergic nerve endings are the essential first steps of botulinum intoxication. In another study, it was demonstrated that BoNTs and large toxin complex (L-TC) bound to bovine aortic endothelial cells via sialic acid, suggesting a possible trafficking pathway for BoNT in food borne botulism (Yoneyama et al., 2008). The binding of serotype C1 and D BoNT and L-TC to sialic acid on rat intestinal epithelial cells promoted their transport through the cell layers (Inui et al., 2010; Niwa et al., 2010) to corroborate the report of Yoneyama et al. (2008).

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tion of cranial nerves, and focal hyaline degeneration of striated, smooth muscles and myocardium (Toyoda et al., 1980).

The necropsy findings in suspected C. botulinum neurotoxin type E intoxication in catfish (Ictalurus punctatus) included intestinal intussusceptions, ascites, pale proximal intestines with engorged serosal blood vessels, splenic congestion, and a reticular pattern to the liver. Significant histopathologic findings were limited to cerebral, splenic, and hepatic congestion, splenic lymphoid depletion and perivascular oedema, vascular dilation and oedema of the gastrointestinal tract, and perivascular oedema in the anterior and posterior kidneys (Khoo et al., 2011). In an outbreak of botulism type C in herring gulls, affected gulls had dry, tacky subcutaneous tissues at post mortem. The proventriculus and ventriculus were bile-stained and empty, the gall bladder was full, and the cloaca was distended with urates and faeces. All affected and healthy birds had moderate to abundant fat stores (Neimanis et al., 2007). Histopathologically, amyloid (Congo red stain) was seen in vessel walls of the spleen and occasionally in other organs. Aﬀected gulls often had abundant haemosiderin (Perl’s stain) in Kupffer cells, and two affected birds had moderate, multifocal distension of renal tubules with urates and the tubular epithelium was attenuated. Mild, acute skeletal myocyte degeneration in limb and pectoral muscles and mild to moderate, subacute muscle necrosis in limbs were observed in aﬀected gulls.

Typical or pathognomonic necropsy lesions are not considered a feature of equine botulism (Ostrowski et al., 2012). However, it is believed that oedema of the head and neck may be prominent, although inconsistently observed, in necropsy finding associated with cases due to types A and C (Whitlock & McAdams, 2006). Oedema of the cervical fascia along the nuchal and supraspinous ligaments, extending caudally as far as the lumbar region (Kinde et al., 1991) and muscle fibres of the inguinal area have also been reported in equine botulism (Ostrowski et al., 2012). Aspiration pneumonia and pulmonary oedema have been reported at euthanasia in cows (Braun et al., 2005). Cobb et al. (2002) found no conclusive gross or histopathological lesions in cows.

DIFFERENTIAL DIAGNOSES

In human beings the differential diagnoses of botulism includes Guillain-Barré syndrome (GBS), myasthenia gravis, stroke syndromes, Eaton-Lambert syndrome, and tick paralysis. Less likely conditions include tetrodotoxin and shellfish poisoning, antimicrobial-associated paralysis, and a host of conditions due to even rarer poisons. A thorough history and meticulous physical examination can effectively eliminate most competing diagnoses. GBS, a rare autoimmune, demyelinating polyneuropathy that follows an acute infection (with Campylobacter jejuni in one-third of cases), presents in 95% of cases as an ascending paralysis and never occurs in outbreaks (Pascuzzi & Fleck, 1997). Five percent of GBS cases present with the Miller Fisher variant that is characterised by the triad of ophthalmoplegia, ataxia, and areflexia, which are easily mistaken for descending paralysis (Willison & O’Hanlon, 1999; Asbury, 2000; Sobel, 2005). Other authors have also documented the differential diagnoses of infant botulism and categorised them as infectious (sepsis, meningitis, encephalitis), metabolic (electrolyte abnormalities – hyponatremia), Reye’s syndrome, hepatic...
encephalopathy, hypothyroidism, organic acidurias, subacute necrotising encephalomyelitis, toxins (heavy metals, alcohols, organophosphates, anticholinergics, narcotics) and neuromuscular (poliomyelitis, infantile spinal muscular atrophy, acute polyneuropathy: Guillain-Barré syndrome), congenital myasthenia gravis, muscular dystrophy and congenital myopathy, tick paralysis) respectively (Cox & Hinkle, 2002). Diphtheritic neuropathy is also listed elsewhere as a differential diagnosis for botulism (Cherington, 2004). In cattle, differential diagnoses of botulism include hypocalcaemia, hypomagnesaemia, carbohydrate overload, and several toxicoxes including mycotoxin, lead, nitrate, organophosphate, atropine or atropine-like alkaloid (Kelch et al., 2000).

**DIAGNOSIS**

Mouse bioassay of serum, gastric contents/vomitus, stool/enema material, suspect foodstuff, wound tissue, environmental swab (in case of bioterrorism); stool microbiologic culture positive for *Clostridium botulinum* organisms, wound microbiologic culture positive for *C. botulinum*, electromyography and rapid repetitive electromyography (20–50 Hz) findings compatible with botulism, miscellaneous (essentially exclusionary) studies of possible help in diagnosis: edrophonium (tensilon) challenge, serum toxicology screens, porphyria evaluation, lumbar puncture with appropriate ancillary studies, brain studies, imaging studies (computed tomography, magnetic resonance imaging) are necessary in confirming clinical botulism in the hospital (Doyle, 1989; Angulo et al., 1998; CDC, 1998; Hatheway, 1998). The limitation of mouse lethality bioassay is that it is labour-intensive, low throughput and can take up to 7 days to complete. Rasooly & Do (2008) developed an *in vitro* cleavage assay for SNAP-25 (synaptosome-associated proteins of 25 kDa) for measuring the toxin activity with the same sensitivity as that of the mouse bioassay. The assay was reported to be far more rapid and could be automated and adapted to many laboratory settings, and has the potential to be used for toxin typing. The authors were of the view that the method was a better alternative to mouse bioassay, since it was an *in vitro* experiment that required no animal use that could be associated with complications. Piazza et al. (2011) developed a rapid and sensitive *in vitro* assay, the Bo-Test Matrix E assay that combines immunoprecipitation with high-affinity endopeptidase activity detection by Förster resonance energy transfer (FRET) to rapidly quantify BoNT/E activity in avian blood with detection limits comparable to those of the mouse lethality assay. BoTest Matrix E detected picomolar quantities of BoNT/E following a 2-h incubation and femtomolar quantities of BoNT/E following extended incubation (24 h) with 100% diagnostic specificity and 91% diagnostic sensitivity. Sensitivities close to mouse bioassay, without the use of animals, in a simpler format were achieved by Poli et al. (2002) who developed sensitive and specific colorimetric capture enzyme linked immunoassortent assays (ELISAs) to detect *Clostridium botulinum* neurotoxin serotypes E (BoNT E) and F (BoNT F) in assay buffer and human serum. The use of the rapid ID32 kit A produced by bioMérieux identified *Clostridium botulinum* within 4 hours, with a setback, as some of the strains could not be correctly identified (Brett, 1998).

Several polymerase chain reaction methodologies have been developed and improved over time for the laboratory
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diagnosis of botulism (Franciosa et al., 1996; Chaffer et al., 2006; Prévot et al., 2007; Hill et al., 2010). A specific and sensitive combined selection and enrichment PCR procedure was developed for the detection of Clostridium botulinum types B, E, and F in faecal samples from slaughtered pigs. Two enrichment PCR assays, using the DNA polymerase rTth, were constructed. One assay was specific for the type B neurotoxin gene, and the other assay was specific for the type E and F neurotoxin genes. Based on examination of 29 strains of C. botulinum, 16 strains of other Clostridium spp., and 48 non-Clostridium strains, it was concluded that the two PCR assays detect C. botulinum types B, E, and F specifically. Sample preparation prior to the PCR was based on heat treatment of faecal homogenate at 70 °C for 10 min, enrichment in tryptone-peptone-glucose-yeast extract broth at 30 °C for 18 h, and DNA extraction. Detection limits after sample preparation were established as 10 spores per g of faecal sample for non-proteolytic type B, and 3.0×10^2 spores per g of faecal sample for type E and non-proteolytic type F with a detection probability of 95% (Dahlenborg et al., 2001). The use of degenerate primers to amplify A, B, E, F BoNT for botulism diagnoses had been reported several years earlier (Broda et al., 1998). Multiplex PCR have been developed and improved diagnosis of botulism (Lindström et al., 2001; de Medici et al., 2009). A PCR-enzyme linked immunosorbent assay (PCR-ELISA) is used to detect Clostridium botulinum infections (Fach et al., 2002; Carlin et al., 2004). Wu et al. (2001) used immuno-polymerase chain reaction (Immuno-PCR) to detect Clostridium botulinum neurotoxin type A. Several other immune-detection methods have been reported (Cadieux et al., 2005; Gesler et al., 2005; Dixit et al., 2006; Stanger et al., 2008). Denaturation high-performance liquid chromatography has been used as a tool to detect neurotoxic Clostridium botulinum (Franciosa et al., 2004).

Amplified fragment length polymorphism has been used to identify strain types (Myllykoski et al., 2009). Lindström & Korkeaala (2006) exhaustively reviewed the application of DNA fingerprinting methods such as random amplified fragment length polymorphism, repetitive element-based PCR, and pulse-field gel electrophoresis in the diagnosis of botulism. In a study that compared the various DNA fingerprinting methods for use to investigate type E botulism outbreaks, it was reported that strain differentiation was unsuccessful with the automated ribotyping system, producing a single characteristic EcoRI fingerprint common to all group II strains. Random amplified polymorphic DNA (RPAD) analysis of C. botulinum group II strains was not consistently reproducible with primer OPJ-6 or OPJ-13, apparently discriminating between epidemiologically related strains. A modified PFGE protocol was judged to be the most useful method for typing epidemiologically related C. botulinum type E strains, based on its ability to type all strains reproducibly and with an adequate level of discrimination (Leclair et al., 2006).

SYBR green and other quantitative real-time PCR methods showed very high specificity for the detection of C. botulinum (inclusivity and exclusivity, 100% depending on the method used) (Akbult et al., 2005a; Fencia et al., 2007b; Fach et al., 2009; Kirchner et al., 2010; Satterfield et al., 2010; Fencia et al., 2011). Fach et al. (2011) developed a robust macro-array method based on the
GeneDisc Cycler designed for simultaneously testing the bont/A, bont/B, bont/E and bont/F genes encoding the botulinum neurotoxins types A, B, E and F. BoNT producing clostridia and non-BoNT-producing bacteria isolated from clinical, food and environmental samples were tested using this macro-array and results were compared to the reference lethality test on mice. The bont genes were correctly detected in all C. botulinum type A, B, E and F strains available, as well as in toxigenic C. baratii type F and toxigenic C. butyricum type E. No cross reactivity was observed with non human-toxigenic bacteria, C. botulinum types C, D and G.

The identification of the bont genotype using the macro-array was correlated to toxino-typing of the BoNTs as determined by the mouse bioassay. An “evaluation trial” of the GeneDisc array performed blind in four European laboratories with 77 BoNT-producing Clostridia as well as 10 food and clinical samples showed that the developed macro-array is specific and reliable for identifying BoNT/A-, BoNT/B-, BoNT/E- and BoNT/F-producing clostridial strains and for screening naturally contaminated food and faecal samples. The test has a low detection limit (c.a. 5 to 50 genome copies in the PCR reaction microwell) and is promising for monitoring BoNT-producing clostridia in different kinds of samples including food and clinical samples.

The advent of toxin proteomics in the molecular diagnosis of botulism marked the beginning of an end to the limitations associated with the identification of botulism neurotoxins. Endopep-MS, a mass spectrometry-based endopeptidase method for detecting and differentiating BoNT in buffer has been developed. This method rapidly determines the presence of BoNT in a sample and differentiates the toxin type of BoNT present. Subtype identification has also been achieved through mass spectrometric analysis of the protein toxin itself and does not require the presence of DNA from the toxin-producing bacteria. Tryptic digests of A1 and A2 subtypes of BoNT were analysed by mass spectrometry, and peptides unique to either the A1 or A2 subtypes were subjected to tandem mass spectrometry analysis to confirm their identities. With this method, BoNT typing is accomplished in a few hours and subtype identification within 24 h (Kalb et al., 2005). Endopep-MS is also used for diagnosis of botulism in animals (Hedeland et al., 2011). Wang et al. (2011) developed an improved detection method for BoNT type A in stool by mass spectrometry. Very recently, also, the detection of Botulinum neurotoxin Type A via BoNT/A endopeptidase activity was reported (Lévêque et al., 2013). This method was confirmed to be 100 times more sensitive than the traditional mouse assay, potentially providing rapid read-out of small amounts of toxin for environmental surveillance and quality control of pharmaceutical preparations.

MANAGEMENT, PREVENTION AND CONTROL

Currently, the only approved therapies against BoNT intoxication are pre-exposure prophylaxis with a vaccine and post-exposure administration of sera containing anti-BoNT antibodies (Arnon et al., 2001). Upon cellular intoxication, however, it is imperative to provide fast acting neuro-modulatory drugs to recover neurotransmission through acetylcholine (Ach) release, to at least restore partial muscle function. Thus, a potential small molecule pharmacological treatment could provide many benefits over these anti-
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body-based approaches. Most small molecule research efforts have targeted the metallo-proteolytic properties of the BoNT light chain (LC) protease. However, no small molecule therapeutics has been approved to date (Capkova et al., 2009).

The cholinergic agonist 3, 4 diaminopyridine (3,4-DAP) is a potent reversible inhibitor of voltage-gated potassium channels, which has been shown to facilitate recovery of neuromuscular action potential post botulinum intoxication by blocking K⁺ channels (Flet et al., 2010). Aminopyridines 3,4-DAP and 4-aminopyridine (4-AP) facilitate recovery of neuromuscular action potential post botulism intoxication by reversibly blocking voltage-dependent K⁺ channels (Adler et al., 2000). This action promotes Ca²⁺ influx, driving signal transduction and ACh release at the synapse. The mechanism by which aminopyridines inactivate the K⁺ channel is unknown, but through molecular modelling it has been hypothesised that two putative receptor sites found within the tetrameric channel are important in this overall process (Caballero et al., 2007). Unfortunately, 3,4-DAP displays toxicity largely due to blood-brain-barrier (BBB) penetration. This motivated the design of carbamate and amide conjugates of 3,4-DAP. The carbamate prodrug was intended to be a slowly reversible inhibitor of acetylcholinesterase (AChE) along the lines of the stigmines thereby allowing increased persistence of released acetylcholine within the synaptic cleft. As a secondary activity, cleavage of the carbamate prodrug by AChE should afford the localised release of 3,4-DAP, which in turn, should enhance the pre-synaptic release of additional acetylcholine. Being a competitive inhibitor with respect to acetylcholine, the activity of the prodrug was intended to be greatest at the synaptic junctions most depleted of acetylcholine (Zakhari et al., 2011).

Vaccination with an appropriate antigen usually produces neutralizing antibodies that bind to and clear toxin from the circulation before it enters nerve cells and block neurotransmission. Immunity from botulism, however, has the disadvantage of precluding an individual from realising the potential benefits of therapeutic botulinum toxin, if such a need were to arise. Vaccination is an effective strategy of providing specific protection against exotoxins such as botulinum toxin by eliciting neutralising antibodies that would prevent the binding of the toxin to an appropriate receptor and promote clearance and degradation by phagocytes. Toxoid and recombinant vaccines have been used to treat botulism and several research works have been documented on these (Kiyatkin et al., 1997; Martinez & Wobeser, 1999; Zhou & Singh, 2004; Lee et al., 2007; Webb et al., 2007; Baldwin et al., 2008; Smith, 2009; Zichel et al., 2010). Recent reports suggest these recombinants are effective against cattle botulism (Gil et al., 2013; Cunha et al., 2014).

Supportive intensive care together with antitoxin therapy helps recovery from botulism (Sobel, 2005). Arnon et al. (2006) reported that prompt treatment of infant botulism type A or type B with human botulism immunoglobulin G intravenous (BIG-IV) was safe and effective in shortening the length and cost of the hospital stay and the severity of illness. To date, the only specific treatment for botulism is administration of botulinum antitoxin. Antitoxin arrests the progression of paralysis and decrease the duration of paralysis and dependence on mechanical ventilation. Antitoxin should be given early in the course of illness, ideally <24 h after onset of symptoms (Tacket et al.,...
1984; Chang & Ganguly, 2003), because antitoxin neutralises only toxin molecules that have yet to be bound to nerve endings. To prevent botulism, low acidity, low oxygen and high water concentration of forage which favour C. botulinum spore germination (Townes et al., 1996) should be avoided. Wet and moldy hays which are risk factors of botulism (Hunter et al., 2002) should not be fed to animals (Johnson et al., 2010). Pflug (2010) suggested that to effectively control food borne botulism, efforts should be concentrated on reducing human errors in the delivery of the specified process to containers of food. All other risk factors of botulism that are applicable strictly to human beings should be avoided as preventive and control measures.

CONCLUSION

Botulism is rare, yet very important, because of its lethality. Therefore, a sustained global research on the molecular dynamics of all the serotypes that affect man and animals in different countries is what is required to regularly evolve the best management protocols at all times. Proper prevention and control measures for man and animals in the endemic areas should be the top priority of human and veterinary public health officials, who should routinely educate the rural and urban communities on the public health significance of the disease and the need to keep safe through strict adherence to standard prevention and control measures. With the volume of research that is currently ongoing about the disease, it is predicted that in the future, better diagnostic methods for disease detection will evolve for better prevention and control.

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