Bulgarian Journal of Veterinary Medicine, 2014, **17**, No 4, 241–266
ISSN 1311-1477; online at http://tru.uni-sz.bg/bjvm/bjvm.htm

Review

BOTULISM IN MAN AND ANIMALS

S. M. NUM[1] & N. M. USEH[2,3]

[1]Department of Veterinary Pathology and Microbiology, University of Agriculture, Makurdi, Nigeria; [2]Department of Veterinary Pathology, Ahmadu Bello University, Zaria, Nigeria; [3]Laboratory of Molecular Biology of Infectious Diseases, Department of Population Medicine & Diagnostic Sciences, Cornell University, Ithaca, New York, United States of America

Summary

Num, S. M. & N. M. Useh, 2014. Botulism in man and animals. *Bulg. J. Vet. Med.,* **17**, No 4, 241–266.

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). Botulism 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 baratii* 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. baratii* 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 toxaemia 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; Umeda *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, Bohnel *et al*., 2003; Elad *et al*., 2004; Lindstrom *et al*., 2004; Böhnel *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 underdiagnosed or undetected in many countries (Bianco *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 salivation, 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, normal cranial reflexes, except for reduced ear, eye and lip reflexes, indicating impaired function of the facial (VII) nerve, response to deep pain stimuli by vocalizing, without moving the legs or head (dog), mild depression, anorexia, recumbency, flaccid paralysis, dyspnoea, quadriplegia (cat), flaccid paralysis affecting several individual animals simultaneously or within a few days (cattle) (van der Lugt *et al*., 1995; Elad *et al*., 2004; Bruchim *et al*., 2006; Johnson *et al*., 2010; Payne *et al*., 2011; Sharpe *et al*., 2011). The epidemiology of animal botulism has been discussed exhaustively by Anza *et al*. (2014a,b).

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 pCO_2 (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, AST 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

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 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 (Couesnon *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).

PATHOLOGY

Very few reports are available in the literature about autopsy of food borne botulism in human beings. The autopsy findings on a man who died after a prolonged illness caused by botulinum toxin exposure likely attributable to a commercially prepared food source included pulmonary embolus adherent to the left lower lobe pulmonary artery. The lungs displayed diffuse congestion with chronic inflammation. An adherent thrombus was also present in the left posterior tibial vein. The heart was mildly enlarged (440 g) with mild atherosclerotic and hypertensive cardiac disease. There was a maximum of 75% focal narrowing of the right coronary artery and a maximum of 50% focal narrowing of the left anterior descending coronary artery. Other findings included a patent foramen ovale (0.2 cm in diameter) and mild hepatosplenomegaly. Apparent diffuse muscular atrophy of the upper and lower extremities was also observed. Microscopically, sections of quadriceps and gastrocnemius muscle showed scattered degenerating muscle fibres, with basophilic change along with scattered angular atrophic fibres. Sural and peroneal nerves displayed no significant histopathology (Devers & Nine, 2010). Inflammatory demyelination of cranial nerve tissue has been reported as well (Filozov *et al*., 2012). The ultrastructural pathology of Japanese patients that developed botulism following the consumption of arashi-renkon revealed neurogenic change in the skeletal muscle. There was denudation of the nerve terminal area (Tsujihata *et al*., 1987). Another autopsy diagnosis of the first outbreak of botulism in Japan revealed bronchopneumonia, congestion, haemorrhage in vagal nerve, myocardium and endometrium, gastric erosion, cloudy swelling of kidneys, enterocolitis, focal necrosis of liver and adrenals, demyelina-

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 toxicoses 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 BoTest 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 immunosorbent 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

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 3.0×10^3 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* neutoxin type A. Several other immune-detection methods have been reported (Cadieux *et al*., 2005; Gessler *et al*., 2005; Dixit *et al*., 2006; Stanker *et al*., 2008). Denaturation high-performance liquid chromatography has been used as a tool to detect neurotoxigenic *Clostridium botulinum* (Franciosa *et al*., 2004).

Amplified fragment length polymorphism has been used to identify strain types (Myllykoski *et al*., 2009). Lindström & Korkeala (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) (Akbulut *et al*., 2005a; Fenicia *et al*., 2007b; Fach *et al*., 2009; Kirchner *et al*., 2010; Satterfield *et al*., 2010; Fenicia *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-

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 Ca^{2+} influx, driving signal transduction and ACh release at the synapse. The mechanism by which aminopyridines inactivate the K_v 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 presynaptic 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.

ACKNOWLEDGEMENTS

Research funding by the Academy of sciences for the developing world (TWAS), Trieste, Italy (grant no. UNESCO FR: 3240262728) to N. M. Useh is gratefully acknowledged. The authors thank Professor Dr. Manfred Reinacher, Director, Institute of Veterinary Pathology, Justus Liebig University, Giessen, Germany for providing the facility to prepare this manuscript via a Deutscher Akademischer Austauch Dienst (DAAD) grant no. A/ 11/ 06980 to N. M. Useh.

REFERENCES

Abe, Y., T. Negasawa, C. Monma & A. Oka, 2008. Infantile botulism caused by *Clostridium butyricum* type E toxin. *Paediatric Neurology*, **38**, 55–57.

Adler, M., B. Capacio & S. S. Deshpande, 2000. Antagonism of botulinum toxin A-mediated muscle paralysis by 3, 4-diaminopyridine delivered via osmotic minipumps. *Toxicon*, **38**, 1381–1388.

Akbulut, D., K. A. Grant & J. McLauchlin 2005a. Improvement in laboratory diagnosis of wound botulism and tetanus among injecting illicit-drug users by use of Real-Time PCR assays for neurotoxin gene fragments. *Journal of Clinical Microbiology*, **43**, 4342–4348.

Akbulut, D., J. Dennis, M. Gent, K. A. Grant, V. Hope, C. Ohai, J. McLauchlin, V. Mithani, O. Mpamugo, F. Ncube & L. de Souza-Thomas L, 2005b. Wound botulism in injectors of drugs: upsurge in cases in England during 2004. *Euro Surveillance*, **10**, 172–174.

Allwright, D. M., M. Wilson, & W. J. J. van Rensburg, 1994. Botulism in ostriches (*Struthio camelus)*. *Avian Pathology*, **23**, 183–186.

Alpers, K., U. van Treeck & C. Frank, 2005. Outbreak of wound botulism in injecting

drug users in Germany, October-December 2005. *Euro Surveillance*, **10**, 2859. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=2859 (12 June 2014 date last accessed).

Angulo, F. J., J. Getz, J. P. Taylor , K. A. Hendricks, C. L. Hatheway, S. Z. Barth, H. M. Solomon, A. E. Larson, E. A. Johnson, L. N. Nickey & A. A. Ries, 1998. A large outbreak of botulism: The hazardous baked potato. *The Journal of Infectious Diseases*, **178**, 172–177.

Anza, I., H. Skarin, D. Vidal, A. Lindberg, V. Båverud & R. Mateo, 2014a. The same clade of *Clostridium botulinum* strains is causing avian botulism in southern and northern Europe. *Anaerobe*, **26**, 20–23.

Anza, I., D. Vidal, C. Laguna, S. Diaz-Sánchez, S. Sánchez, A. Chicote, M. Florin & R. Mateo, 2014b. Risk factors for avian botulism outbreaks in wetlands receiving effluents from urban wastewater treatment plants: Eutrophication and bacterial pathogens. *Applied and Environmental Microbiology*. Doi.10.1128/ AEM.00949-14.

Arnon, S. S., R. Schechter, S. E. Maslanka, N. P. Jewell & C. L. Hatheway, 2006. Human botulism immune globulin for the treatment of infant botulism. *New England Journal of Medicine*, **354**, 462–471.

Arnon, S. S., 1995. Botulism as an intestinal toxaemia. In: *Infection of the Gastrointestinal Tract,* eds Blaser, M. J., P. D. Smith, J. I. Ravdin, H. B. Greenberg & R. L. Guerrant, Raven, New York, pp. 257–271.

Arnon, S. S., 2004. Infant botulism. In: *Textbook of Pediatric Infectious Disease*, 5th edn, eds R. D. Feigen & J. D. Cherry, W. B. Saunders, Philadelphia, pp. 1758–1766.

Arnon, S. S., K. Damus & J. Chin, 1981. Infant botulism: Epidemiology and relation to sudden infant death syndrome. *Epidemiology Reviews*, **3**, 45–66.

Arnon, S. S., R. Schechter, T. V. Inglesby, D. A. Henderson, J. G. Bartlett, M. S. Ascher, E. Eitzen, A. D. Fine, J. Hauer, M. Layton, S. Lillibridge, M. T. Osterholm, T. O'Toole, G. Parker, T. M. Perl, P. K. Russell, D. L. Swerdlow, K. Tonat & Working Group on Civilian Biodefense, 2001. Botulinum toxin as a biological weapon: Medical and public health management. *Journal of the American Medical Association*, **285**, 1059–1070.

Artin, I., P. Bjorkman, J. Cronqvist, P. Radstrom & E. Holst, 2007. First case of type E wound botulism diagnosed using real-time PCR. *Journal of Clinical Microbiology*, **45**, 3589–3594.

Asbury, A., 2000. New concepts of Guillian-Barre syndrome. *Journal of Child Neurology*, **15**, 183–191.

Aureli, P., G. Franciosa & M. Pourshaban, 1996. Food borne botulism in Italy. *Lancet*, **348**, 1594.

Aureli, P., L. Fenicia, B. Pasolini, M. Gianfranceschi, L. M. McCroskey & C. L. Hatheway, 1986. Two cases of type E infant botulism caused by neurotoxigenic *Clostridium butyricum* in Italy. *Journal of Infectious Diseases*, **154**, 207–211.

Austin, J. W. & D. Leclair, 2011. Botulism in the North: A disease without borders. *Clinical Infectious Diseases*, **52**, 593–594.

Bakheit, A. M., C. D. Ward & D. L. McLellan, 1997. Generalized botulism-like syndrome after intramuscular injections of botulinum toxin type A: A report of two cases. *Journal of Neurology and Neurosurgical Psychiatry*, **62**, 198.

Baldwin, M. R., W. H. Tepp, A. P. Przedpelski, C. L. Pier, M. Bradshaw, E. A. Johnson & J. T. Barbieri, 2008. Subunit vaccine against the seven serotypes of botulism. *Infection and Immunity*, **76**, 1314–1318.

Barash, J. R., T. W. Tang & S. S. Arnon, 2005. First case of infant botulism caused by *Clostridium baratii* type F in California. *Journal of Clinical Microbiology*, **43**, 4280–4282.

Barsanti, J. A., M. Walser, C. L. Hatheway, J. M. Bowen & W. Crowell, 1978. Type C botulism in American Foxhounds. *Journal*

of the American Veterinary Medical Association, **17**, 809–813.

Bernard, W., T. J. Divers, R. H. Whitlock, J. Messick & E. Tulleners, 1987. Botulism as a sequel to open castration in a horse. *Journal of the American Veterinary Medical Association*, **191**, 73–74.

Bianco, M. I., C. Lúquez, L. I. T. de Jong & R. A. Fernández, 2008. Presence of *Clostridium botulinum* spores in *Matricaria chamomilla* (chamomile) and its relationship with infant botulism. *International Journal of Food Microbiology*, **121**, 357–360.

Böhnel, H., U. Wernery & F. Gessler, 2003. Two cases of equine grass sickness with evidence for soil-borne origin involving botulinum neurotoxin. *Journal of Veterinary Medicine B. Infectious Diseases and Veterinary Public Health*, **50**, 178–182.

Böhnel, H., B. Neufeld & F. Gessler, 2005. Botulinum neurotoxin type B in milk from a cow affected by visceral botulism. *The Veterinary Journal*, **169**, 124–125.

Bonventre, P. F., 1979. Absorption of botulinal toxin from the gastrointestinal tract. *Reviews on Infectious Diseases*, **1**, 663–667.

Braun, U., K. Feige, G. Schweizer & A. Pospischil, 2005. Clinical findings and treatment of 30 cattle with botulism. *The Veterinary Record*, **156**, 438–441.

Brett, M. M., 1998. Evaluation of the use of the bioMerieux rapid ID32 A kit for the identification of *Clostridium botulinum*. *Letters in Microbiology*, **26**, 81–84.

Broda, D. M., J. A. Boerema & R. G. Bell, 1998. A PCR survey of psychrotrophic *Clostridium botulinum*-like isolates for the presence of BoNT genes. *Letters in Applied Microbiology*, **27**, 219–223.

Bruchim, Y., A. Steinman, M. Markovitz, G. Baneth, D. Elad & N. Y. Shpigel, 2006. Toxicological, bacteriological and serological diagnosis of botulism in a dog. *The Veterinary Record*, **158**, 768–769.

Burnens, A., 2000. Cases of wound botulism in Switzerland. *Euro Surveillance*, **4**, 1666. Available from: http://www.eurosurveillance.org/ ViewArticle.aspx?ArticleId=1666 (12 June 2014 date last accessed).

Caballero, N. A., F. J. Meléndez, A. Niño & C. Muñoz-Caro, 2007. Molecular docking study of the binding of aminopyridines within the K^+ channel. *Journal of Molecular Modeling*, **13**, 579–586.

Cadieux, B., B. Blanchfield, J. P. Smith & J. W. Austin, 2005. A rapid chemiluminescent slot blot immunoassay for the detection and quantification of *Clostridium botulinum* neurotoxin type E in cultures. *International Journal of Food Microbiology*, **101**, 9–16.

Cameron, C. M., 2009. A brief history of botulism in South Africa. *Onderstepoort Journal of Veterinary Research*, **76**, 11–12.

Capková, K., N. T. Salzameda & K. D. Janda, 2009. Investigations into small molecule non-peptidic inhibitors of the botulinum neurotoxins. *Toxicon*, **54**, 575–582.

Carlin, C., V. Broussolle, S. Perelle, S. Litman & P. Fach, 2004. Prevalence of *Clostridium botulinum* in food raw materials used in refrigerated processed foods of extended durability (REPFEDs) manufactured in France. *International Journal of Food Microbiology*, **91**, 141–145.

Center for Disease Control (CDC), 1998. Botulism in the United States, 1899–1998. Handbook for Epidemiologists, Clinicians, and Laboratory Workers. Atlanta, Georgia, US Department of Health and Human Services, Public Health Service. http://www.cdc.gov/ncidod/dbmd/diseaseinfo/files/botulism.PDF (12 June 2014 date last accessed).

Centers for Disease Control and Prevention (CDC), 2006. Morbility and Mortality Weekly Report (MMWR). *Summary of Notifiable Diseases*, **53**, 1–79, http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5353a1.htm (12 June 2014 date last accessed).

Chaffer, M., M. Baum, K. Grinberg, T. Molad & D. Elad, 2006. Application of PCR for detection of *Clostridium botulinum* type D in bovine samples. *Journal of Veterinary Medicine*, series B, **53**, 45–47.

Chang, G. Y. & G. Ganguly, 2003. Early antitoxin treatment in wound botulism results in better outcome. *European Neurology*, **49**, 151–153.

Cherington, M., 2004. Botulism: Update and review. *Seminars in Neurology*, **24**, 155–163.

Chou, S.-J., Y.-C. Shieh & C.-Y.Yu, 2008. Haematologic and biochemistry values for black-faced spoonbills (*Platalea minor*) with and recovering from botulism. *Journal of Wildlife Diseases*, **44**, 781–784.

Coban, A., Z. Matur, H. A. Hanagasi & Y. Parman, 2010. Iatrogenic botulism after botulinum toxin type A injections. *Clinical Neuropharmacology*, **33**, 158–160.

Cobb, S. P., R. A. Hogg, D. J. Challoner, M. M. Brett, C. T. Livesey, R. T. Sharpe & T. O. Jones, 2002. Suspected botulism in dairy cows and its implications for the safety of human food. *The Veterinary Record*, **150**, 5–8.

Coleman, E. S., 1998. Clostridial neurotoxins: Tetanus and botulism. *Compendium on Continuing Education for the Practising Veterinarian*, **20**, 1089–1097.

Collins, M. D. & A. K. East, 1998. Phylogeny and taxonomy of the food borne pathogen *Clostridium botulinum* and its neurotoxins. *Journal of Applied Microbiology*, **84**, 5–17.

Couesnon, A., Y. Pereira & M. R. Popoff, 2008. Receptor-mediated transcytosis of botulinum neurotoxin A through intestinal cell monolayers. *Cellular Microbiology*, **10**, 375–387.

Cowden, J., 2011. Food borne *Clostridium botulinum* intoxication from mass produced foodstuffs in Europe. *Euro Surveillance*, **16**, 20033, http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId =20033 (12 June 2014 date last accessed).

Cox, N. & R. Hinkle, 2002. Infant botulism. *American Family Physician*, **65**, 1388–1392.

Crauste, F., M. L. Hbid & A. Kacha, 2008. A delay reaction-diffusion model of the dynamics of botulinum in fish. *Mathematical Biosciences*, **216**, 17–29.

Cunha, C. E., G. M. Moreira, F. M. Salvarani, M. S. Neves, F. C. Lobato, O. A. Dellagostin & F. R. Conceiä, 2014. Vaccination of cattle with a recombinant bivalent toxoid against botulism serotypes C and D. *Vaccine*, **32**, 214–216.

Elad, D., E. Y.-N. Aroch, M. H. Shamir, S. Kleinbart, D. Hadash, M. Chaffer, K. Greenberg & A. Shlosberg, 2004. Natural *Clostridium botulinum* type C toxicosis in a group of cats. *Journal of Clinical Microbiology*, **42**, 5406–5408.

Dahlenborg, M., E. Borch & P. Rådström, 2001. Development of a combined selection and enrichment PCR procedure for *Clostridium botulinum* types B, E, and F and its use to determine prevalence in faecal samples from slaughtered pigs. *Applied and Environmental Microbiology*, **67**, 4781–4788.

Dawar, M., L. Moody, J. D. Martin, C. Fung, J. Isaac-Renton & D. M. Patrick, 2002. Two outbreaks of botulism associated with fermented salmon roe – British Columbia, August 2001. *Canadian Communicable Diseases Report*, **28**, 45–49.

de Medici, D., F. Anniballi, G. M. Wyatt, M. Lindström, U. Messelhaüßer, C. F. Aldus, E. Delibato, H. Korkeala, M. W. Peck & L. Fenicia, 2009. Multiplex PCR for detection of botulinum neurotoxin-producing clostridia in clinical, food, and environmental samples. *Applied and Environmental Microbiology*, **75**, 6457–6461.

Delgado, M. R., 2003. Botulinum neurotoxin type A. *Journal of American Academy of Orthopaedic Surgeons*, **11**, 291–294.

Devers, K. G. & J. S. Nine, 2010. Autopsy findings in botulinum toxin poisoning. *Journal of Forensic Science*, **55**, 1649–1651.

Dixit, A., S. I. Alam, R. K. Dhaked & L. Singh, 2006. Development of an immunodetection test for a botulinum-like neurotoxin produced by *Clostridium* sp. RKD. *Indian Journal of Medical Research*, **124**, 355–362.

Dohms, J. E., P. H. Allen & J. K. Rosenberger, 1982. Cases of type C botulism in broiler chickens. *Avian Diseases*, **26**, 204–210.

Dong, M., F. Yeh, W. H. Tepp, C. Dean, E. A. Johnson, R. Janz & E. R. Chapman, 2006. SV2 is the protein receptor for botulinum neurotoxin A. *Science*, **312**, 592–596.

Dong, M., D. A. Richards, M. C. Goodnough, W. H. Tepp, E. A. Johnson & E. R. Chapman, 2003. Synaptotagmin I and II mediate entry of botulinum neurotoxin B into cells. *Journal of Cell Biology*, **162**, 1293–1303.

Doutre, M. P., 1967a. Type C botulism in a dove (*Streptopelia roseogrisea bornuensis*) from Ferlo (Senegal). *Revue de'Élevage et de Médecine Vétérinaire des Pays Tropicaux*, **20**, 601–604.

Doutre, M. P., 1967b. First case of beta C botulism in swine in Senegal. *Revue de'Élevage et de Médecine Vétérinaire des Pays Tropicaux*, **20**, 351–353.

Doutre, M. P., 1983. Second case of botulism type D in the dog in Senegal. *Revue de'Élevage et de Médecine Vétérinaire des Pays Tropicaux*, **36**, 131–132.

Doyle, M. P., 1989. Food Borne Bacterial Pathogens, Marcel Dekker Inc., New York, N.Y. p. 147.

Elad, D., E. Y.-N. Aroch, M. H. Shami, S. Kleinbart, D. Hadash, M. Chaffer, K. Greenberg & A. Shlosberg, 2004. Natural *Clostridium botulinum* type C toxicosis in a group of cats. *Journal of Clinical Microbiology*, **42**, 5406–5408.

Fach, P., S. Perelle, F. Dilasser, J. Grout, C. Dargaignaratz, L. Botella, J.-M. Gourreau, F. Carlin, M. R. Popoff & V. Broussolle, 2002. Detection by PCR-enzyme-linked immunosorbent assay of *Clostridium botulinum* in fish and environmental samples from a coastal area in Northern France. *Applied and Environmental Microbiology*, **68**, 5870–5876.

Fach, P., P. Micheau, C. Mazuet, S. Perelle & M. Popoff, 2009. Development of real-time PCR tests for detecting botulinum neurotoxins A, B, E, F producing *Clostridium botulinum*, *Clostridium baratii and Clostridium butyricum*. *Journal of Applied Microbiology*, **107**, 465–473.

Fach, P., L. Fenicia, R. Knutsson, P. R. Wielinga, F. Anniballi, E. Delibato, B. Auricchio, C. Woudstra, J. Ågren, B. Segerman, D. de Medici & B. J. van Rotterdam, 2011. An innovative molecular detection tool for tracking and tracing *Clostridium botulinum* types A, B, E, F and other botulinum neurotoxin producing clostridia based on the GeneDisc cycler. *International Journal of Food Microbiology*, **145**, 145–151.

Farrow, B. R., W. G. Murrell, M. L. Revington, B. J. Stewart & R. M. Zuber, 1983. Type C botulism in young dogs. *Australian Veterinary Journal*, **60**, 374–377.

Fenicia, L. & F. Anniballi, 2009. Infant botulism. *Annali dell'Istituto Superiore di Sanità*, **45**, 134–146.

Fenicia, L., F. Anniballi & P. Aureli, 2007a. Intestinal toxemia botulism in Italy, 1984–2005. *European Journal of Clinical Microbiology and Infectious Diseases*, **26**, 385–394.

Fenicia, L, F. Anniballi, D. de Medici, E. Delibato & P. Aureli, 2007b. SYBR green real-time PCR method to detect *Clostridium botulinum* type A. *Applied and Environmental Microbiology*, **73**, 2891–2896.

Fenicia, L., P. Fach, B. J. van Rotterdam, F. Anniballi, B. Segerman, B. Auricchio, E. Delibato, R. A. Hamidjaja, P. R. Wielinga, C. Woudstra, J. Ågren, D. de Medici & R. Knutsson, 2011. Towards an international standard for detection and typing botulinum neurotoxin-producing Clostridia types A, B, E and F in food, feed and environmental samples: A European ring trial study to evaluate a real-time PCR assay. *International Journal of Food Microbiology*, **145**, 152–157.

Filozov, A., J. A. Kattan, L. Jitendranath, C. G. Smith, C. Lúquez, Q. N. Phan & R. P. Fagan, 2012. Asymmetric type F botulism with cranial nerve demyelination. *Emerging Infectious Diseases*, **18**, 102–104.

Flet, L., E. Polard, O. Guillard, E. Leray, H. Allain, L. Javaudin & G. Edan, 2010. 3,4-diaminopyridine safety in clinical practice: An observational, retrospective cohort study. *Journal of Neurology*, **257**, 937–946.

Forrester, D. J., K. C. Wenner, F. H. White, E. C. Greiner, W. R. Marion, J. E. Thul, G. A. Berkhoff, 1980. An epizootic of avian botulism in a phosphate mine settling pond in northern Florida. *Journal of Wildlife Diseases*, **16**, 323–327.

Fox, C. K., C. A. Keet & J. B. Strober, 2005. Recent advances in infant botulism. *Paediatric Neurology*, **32**, 149–154.

Franciosa, G., L. Fenicia, C. Caldiani & P. Aureli, 1996. PCR for detection of *Clostridium botulinum* type C in avian and environmental samples. *Journal of Clinical Microbiology*, **34**, 882–885.

Franciosa, G., M. Pourshaban, A. de Luca, A. Buccino, B. Dallapiccola & P. Aureli, 2004. Identification of type A, B, E, and F botulinum neurotoxin genes and of botulinum neurotoxigenic clostridia by denaturing high-performance liquid chromatography. *Applied and Environmental Microbiology*, **70**, 4170–4176.

Frean, J., L. Arntzen, J. van den Heever & O. Perovic, 2004. Fatal type A botulism in South Africa, 2002. *Transactions of Royal Society of Medicine and Hygiene*, **98**, 290–295.

Fujinaga, Y., K. Inoue, S. Watanabe, K. Yokota, Y. Hirai, E. Nagamachi & K. Oguma, 1997. The haemagglutinin of *Clostridium botulinum* type *C* progenitor toxin plays an essential role in binding of toxin to the epithelial cells of guinea pig small intestine, leading to the efficient absorption of the toxin. *Microbiology*, **143**, 3841–3847.

Fujinaga, Y., Y. Sugawara & T. Matsumura, 2013. Uptake of botulinum neurotoxin in the intestine. *Current Topics in Microbiology and Immunology*, **364**, 45–59.

Gaunt, P. S., S. R. Kalb & J. R. Barr, 2007. Detection of botulinum type E toxin in channel catfish with visceral toxicosis syndrome using catfish bioassay and endopep mass spectrometry. *Journal of Veterinary Diagnostic Investigation*, **19**, 349–354.

Gessler, F., K. Hampe & H. Böhnel, 2005. Sensitive detection of botulinum neurotoxin types C and D with an immunoaffinity chromatographic column test. *Applied and Environmental Microbiology*, **71**, 7897–7903.

Gil, L. A., C. E. da Cunha, F. M. Salvarani, R. A. Assis, F. C. Lobato, M. Mendonca, O. A. Dellagostin & F. R. Conceicäo, 2013. Production and evaluation of a recombinant chimeric vaccine against *Clostridium botulinum* neurotoxin types C and D. *Plos One*, **8**, e69692.

Hardy, S. P., B. David & M. Kaldhusdal, 2011. Risk of botulism for laying hens and broilers. *Veterinary Record*, **168**, 669. Doi: 10.1136/vr.d4605.

Hatheway, C. L., 1995. Botulism: The present status of the disease. *Current Topics in Microbiology and Immunology*, **195**, 55–77.

Hatheway, C. L., 1998. *Clostridium botulinum*. In: Infectious Diseases, 2nd edn, ed S. L. Gorbach, W. B. Saunders Co, Philadelphia, Pa, pp. 1919–1925.

Hatheway, C. L. & E. A. Johnson, 1998. *Clostridium*: the spore-bearing anaerobes. In: *Topley and Wilson's Microbiology and Microbial Infections,* Vol. 2, 9th edn, eds A. Balows & B. I. Duerden, Arnold, London, pp. 731–782.

Hay, C. M., H. N. van der Made & P. C. Knoetze, 1973. Isolation of *Clostridium botulinum* type C from an outbreak of botulism in wild geese. *Journal of South African Veterinary Medical Association*, **44**, 53–56.

Hedeland, M., H. Moura, V. Båverud, A. R. Woolfitt, U. Bondesson & J. R. Barr,

2011. Confirmation of botulism in birds and cattle by the mouse bioassay and Endopep-MS. *Journal of Medical Microbiology*, **60**, 1299–1305.

Herreros, J., G. Lalli, C. Montecucco & G. Schiavo, 1999. Pathophysiological properties of clostridial neurotoxins. In: *The Comprehensive Sourcebook of Bacterial Protein Toxins,* eds J. E. Alouf & J. H. Freer, Academic Press, London, pp. 202–228.

Hill, B. J., J. C. Skerry, T. J. Smith, S. S. Arnon & D. C. Douek, 2010. Universal and specific quantitative detection of botulinum neurotoxin genes. *BMC Microbiology*, **10**, 267. http://www.biomedcentral.com/1471-2180/10/267 (12 June 2014 date last accessed).

Hoarau, G., I. Pelloux, A. Gayot, I. Wroblewski, M. R. Popoff, C. Mazuet, M. Maurin & J. Croizé, 2012. Two cases of type A infant botulism in Grenoble, France: No honey for infants. *European Journal of Paediatrics*, **171**, 589–591.

Horowitz, B. Z., 2010. Type E botulism. *Clinical Toxicology (Philadelphia)*, **48**, 880–895.

Humeau, Y., F. Doussau, N. J. Grant & B. Poulain, 2000. How botulinum and tetanus neurotoxins block neurotransmitter. *Biochimie*, **82**, 427–446.

Hunter, J. M., B. W. Rohrbach, F. M. Andrews & R. H. Whitlock, 2002. Round bale grass hay: A risk factor for botulism in horses. *Compendium of Continuing Education for the Practicing Veterinarian*, **24**, 166–169.

Hutson, R. A., D. E. Thompson, P. A. Lawson, R. P. Schocken-Itturino, E. C. Bottger & M. D. Collins, 1993. Genetic interrelationships of proteolytic *Clostridium botulinum* types A, B, and F and other members of the *Clostridium botulinum* complex as revealed by small-subunit rRNA gene sequences. *Antonie van Leeuwenhoek*, **64**, 273–283.

Hyytiä, E., S. Hielm & H. Korkeala, 1998. Prevalence of *Clostridium botulinum* type E in Finnish fish and fishery products. *Epidemiology and Infection*, **120**, 245–250.

Inui, K., H. Ito, K. Miyata, T. Matsuo, R. Horiuchi, T. Ikeda, T. Watanabe, T. Ohyama & K. Niwa, 2010. Involvement of sialic acid in transport of serotype C1 botulinum toxins through rat intestinal epithelial cells. *Journal of Veterinary Medical Sciences*, **72**, 1251–1255.

Johnson, A. L., S. C. McAdams & R. H. Whitlock, 2010. Type A botulism in horses in the United States: A review of the past ten years (1998–2008). *Journal of Veterinary Diagnostic Investigation*, **22**, 165–173.

Johnson, E. A., W. H. Tepp, M. Bradshaw, R. J. Gilbert, P. E. Cook & E. D. G. McIntosh, 2005. Characterization of *Clostridium botulinum* strains associated with an Infant botulism case in the United Kingdom. *Journal of Clinical Microbiology*, **43**, 2602–2607.

Jones, A. N., 1980. Outbreak of botulism in Kenya after ingestion of white ants. *British Medical Journal*, **281**, 1682.

Kalb, S. R., M. C. Goodnough, C. J. Malizio, J. L. Pirkle & J. R. Barr, 2005. Detection of botulinum neurotoxin A in a spiked milk sample with subtype identification through toxin proteomics. *Analalytical Chemistry*, **77**, 6140–6146.

Kalb, S, R., W. I. Santana, I. N. Geren, C. Garcia-Rodriguez, J. Lou, T. J. Smith, J. D. Marks, L. A. Smith, J. L. Pirkle & J. R. Barr, 2011. Extraction and inhibition of enzymatic activity of botulinum neurotoxins /B1, /B2, /B3, /B4, and /B5 by a panel of monoclonal anti-BoNT/B antibodies. *BMC Biochemistry*, **12**, 58. http://www.biomedcentral.com/1471-2091/12/58 (12 June 2014 date last accessed).

Keet, C. A., C. K. Fox, M. Margeta, E. Marco, A. L. Shane, S. J. DeArmond, J. B. Strober & S. P. Miller, 2005. Infant botulism, type F, presenting at 54 hours of life. *Paediatric Neurology*, **32**, 193–196.

Kelch, W. J., L. A. Kerr, J. K. Pringle, B. W. Rohrbach & R. H. Whitlock, 2000. Fatal *Clostridium botulinum* toxicosis in eleven Holstein cattle fed round bale barley haylage. *Journal of Veterinary Diagnostic Investigation*, **12**, 453–455.

Khoo, L. H., A. E. Goodwin, D. J. Wise, W. E. Holmes, L. A, Hanson, J. M. Steadman, L. M. McIntyre & P. S. Gaunt, 2011. The pathology associated with visceral toxicosis of catfish. *Journal of Veterinary Diagnostic Investigation*, **23**, 1217–1221.

Kinde, H., R. L. Bettey, A. Ardans, F. D. Galey, B. M. Daft, R. L. Walker, M. W. Eklund & J. W. Byrd, 1991. *Clostridium botulinum* type-C intoxication associated with consumption of processed alfalfa hay cubes in horses. *Journal of the American Veterinary Medical Association*, **199**, 742–746.

King, L. A., T. Niskanen, M. Junnikkala, E. Moilanen, M. Lindström, H. Korkeala, T. Korhonen, M. Popoff, C. Mazuet, H. Callon, N. Pihier, F. Peloux, C. Ichai, H. Quintard, P. Dellamonica, E. Cua, M. Lasfargue, F. Pierre & H. de Valk, 2009. Botulism and hot-smoked whitefish: A family cluster of type E botulism in France, September 2009. *Euro Surveillance*, **14**, 19394. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19394 (12 June 2014 date last accessed).

Kirchner, S., K. M. Kraemer, M. Schulze, D. Pauly, D. Jacob, F. Gessler, A. Nitsche, B. G. Dorner & M. B. Dorner, 2010. Pentaplexed quantitative real-time PCR assay for the simultaneous detection and quantification of botulinum neurotoxin-producing clostridia in food and clinical samples. *Applied and Environmental Microbiology*, **76**, 4387–4395.

Kitamura, M., K. Takamiya, S. Aizawa & K. Furukawa, 1999. Gangliosides are the binding substances in neural cells for tetanus and botulinum toxins in mice. *Biochemistry and Biophysics Acta*, **1441**, 1–3.

Kiyatkin, N., A. B. Maksymowych & L. L. Simpson, 1997. Induction of an immune response by oral administration of recombinant botulinum toxin. *Infection and Immunity*, **65**, 4586–4591.

Koepke, R., J. Sobel & S. S. Arnon, 2008. Global occurrence of infant botulism, 1976–2006. *Paediatrics*, **122**, e73–e82.

Kozaki, S., Y. Kamata, S. Watarai, T. Nishiki & S. Mochida, 1998. Ganglioside GT1b as a complementary receptor component for *Clostridium botulinum* neurotoxins. *Microbiology and Pathology*, **25**, 91–99.

Leclair, D., F. Pagotto, J. M. Farber, B. Cadieux & J. W. Austin, 2006. Comparison of DNA fingerprinting methods for use in investigation of type E botulism outbreaks in the Canadian arctic. *Journal of Clinical Microbiology*, **44**, 1635–1644.

Lee, J. C., H. J. Hwang, Y. Sakaguchi, Y. Yamamoto, H. Arimitsu, T. Tsuji, T. Watanabe, T. Ohyama, T. Tsuchiya & K. Oguma, 2007. C terminal half fragment (50 kDa) of heavy chain components of *Clostridium botulinum* type C and D neurotoxins can be used as an effective vaccine. *Microbiology and Immunology*, **51**, 445–55.

Lévêque, C., G. Ferracci, Y. Maulet, C. Grand-Masson, M. Blanchard, M. Seagar & O. El-Far, 2013. A substrate sensor chip to assay the enzymatic activity of Botulinum neurotoxin A. *Biosensors and Bioelectronics*, **49**, 276–281.

Lindström, M., R. Keto, A. Markkula, M. Nevas, S. Hielm & H. Korkeala, 2001. Multiplex PCR assay for detection and identification of *Clostridium botulinum* types A, B, E, and in food and faecal material. *Applied and Environmental Microbiology*, **67**, 5694–5699.

Lindström, M., M. Nevas, J. Kurki, R. Sauna-Aho, A. Latvalakiesila, I. Polonen, H. Korkeala, 2004. Type C botulism due to toxic feed affecting 52,000 farmed foxes and minks in Finland. *Journal of Clinical Microbiology*, **42,** 4718–4725.

Lindström, M. & H. Korkeala, 2006. Laboratory diagnostics of botulism. *Clinical Microbiology Reviews*, **19**, 298–314.

Long, S. S., J. L. Gajewski, L. W. Brown & P. H. Gilligan, 1985. Clinical, laboratory, and environmental features of infant botulism in Southeastern Pennsylvania. *Paediatrics*, **75**, 935–941.

Lund, B. M. & M. W. Peck, 2000. *Clostridium botulinum*. In: *The Microbiological Safety and Quality of Food*, eds B. M. Lund, T. C. Baird-Parker & G. W. Gould, Aspen Publishers Inc., Gaithersburg, MD, pp. 1057–1109.

Lúquez, C., M. I. Bianco, L. I. T. de Jong, M. D. Sagua, G. N. Arenas, A. S. Ciccarelli & R. A. Ferna´ndez, 2005. Distribution of botulinum toxin-producing clostridia in soils of Argentina. *Applied and Environmental Microbiology*, **71**, 4137–4139.

Lúquez, C., M. I. Bianco, M. D. Sagua, C. P. Barzola, L. I. T. de Jong, S. M. Degarbo & R. A. Fernández, 2007. Relationship between the incidence of infant botulism and the presence of botulinum toxin-producing clostridia in the soil of Argentina, 1982–2005. *Journal of Pediatric Neurology*, **5**, 1–8.

Lúquez, C., J. K. Dykes, P. A. Yu, B. H. Raphael & S. E. Maslanka, 2010. First report worldwide of an infant botulism case due to *Clostridium botulinum* type E. *Journal of Clinical Microbiology*, **48**, 326–328.

Mahrhold, S., A. Rummel, H. Bigalke, B. Davletov & T. Binz, 2006. The synaptic vesicle protein 2C mediates the uptake of botulinum neurotoxin A into phrenic nerves. *FEBS Letters*, **580**, 2011–2014.

Maksymowych, A. B., M. Reinhard, C. J. Malizio, M. C. Goodnough, E. A. Johnson & L. L. Simpson, 1999. Pure botulinum neurotoxin is absorbed from the stomach and small intestine and produces peripheral neuromuscular blockade. *Infection and Immunity*, **67**, 4708–4712.

Martinez, R. & G. Wobeser, 1999. Immunization of ducks for type C botulism. *Journal of Wildlife Diseases,* **35**, 710–715.

Mengiste, B., T. Mesfin, B. G. Egziabher & C. L. Duarte, 1990. Cattle poisoning and mortality associated with tortoise clostridial toxicity in the Beletu district of Ethiopia. *Tropical Animal Health and Production*, **3**, 195–196.

Merivirta, L. O., M. Lindström, K. J. Björkroth & J. K. Korkeala, 2006. The prevalence of *Clostridium botulinum* in European river lamprey (*Lampetra fluviatilis*) in Finland. *International Journal of Food Microbiology*, **109**, 234–237.

Middlebrook, J. L. & D. R. Franz, 1997. Botulinum toxins. In: *Medical Aspects of Chemical and Biological Warfare*. eds F. R. Sidell, T. E. Takafuji & D. R. Franz, Borden Institute, Walter Reed Army Medical Center, Washington, DC, pp. 643–654.

Midura, T. F. & S. S. Arnon, 1976. Infant botulism. Identification of *Clostridium botulinum* and its toxins in faeces. *Lancet*, **2**, 934–936.

Montecucco, C., O. Rossetto & G. Schiavo, 2004. Presynaptic receptor arrays for clostridial neurotoxins. *Trends in Microbiology*, **12**, 442–446.

Myllykoski, J., M. Lindström, R. Keto-timonen, H. Söderholm, J. Jakala, H. Kallio, A. Sukura & H. Korkeala, 2009. Type C bovine botulism outbreak due to carcass contaminated non-acidified silage. *Epidemiology and Infection*, **137**, 284–293.

Myllykoski, J., M. Lindström, E. Bekema, I. Pölönen & H. Korkeala, 2011. Fur animal botulism hazard due to feed. *Research in Veterinary Science*, **90**, 412–418.

National Institute of Occupational Safety and Health, 1996. Registry of toxic effects of chemical substances (R-TECS). Cincinnati, Ohio: National Institute of Occupational Safety and Health. http://www.cdc.gov/niosh/docs/97-119/pdfs/97-119.pdf (12 June 2014 date last accessed).

Neimanis, A., D. Gavier-Widen, F. Leighton, T. Bollinger, T. Rocke & T. Morner, 2007. An outbreak of type C botulism in herring gulls (*Larus argentatus*) in southeastern Sweden. *Journal of Wildlife Diseases*, **43**, 327–336.

Nevas, M., M. Lindström, A. Virtanen, S. Hielm, M. Kuusi, S. S. Arnon, E. Vuori & H. Korkeala, 2005. Infant botulism acquired from household dust presenting as sudden infant death syndrome. *Journal of Clinical Microbiology*, **43**, 511–513.

Nishida, H., M. Shiota, K. Nakagawa, T. Minamigata, M. Takuwa, T. Morishima, H. Kumakura, T. Yoshioka, A. Uematsu, N. Haneda, D. Hata, K. Umeda, J. Ogasawara & M. Takahashi, 2007. An infant botulism case due to *Clostridium botulinum* type B toxin, October 2005 – Osaka City. *Infectious Agents Surveillance Report*, **28**, 168–169.

Nishiki, T., Y. Kamata, Y. Nemoto, A. Omori, T. Ito, M. Takahashi & S. Kozaki, 1994. Identification of protein receptor for *Clostridium botulinum* type B neurotoxin in rat brain synaptosomes. *Journal of Biological Chemistry*, **269**, 10498–10503.

Niwa, K., T. Yoneyama, H. Ito, M. Taira, T. Chikai, H. Kouguchi, T. Suzuki, K. Hasegawa, K. Miyata, K. Inui, T. Ikeda, T. Watanabe & T. Ohyama, 2010. Sialic acid-dependent binding and transcytosis of serotype D botulinum neurotoxin and toxin complex in rat intestinal epithelial cells. *Veterinary Microbiology*, **141**, 312–320.

Nol, P., J. L. Williamson, T. E. Rocke & T. M. Yuill, 2004. Detection of *Clostridium botulinum* type C cells in the gastrointestinal tracts of Mozambique tilapia (*Oreochromis mossambicus*) by polymerase chain reaction. *Journal of Wildlife Diseases*, **40**, 749–753.

Okoye, J. O., 1988. An outbreak of type-C botulism in broiler chickens in Nigeria. *Revue de'Elevage et de Médecine Vétérinaire des Pays Tropicaux*, **41**, 51–52.

Ostrowski, S. V. K., J. Palmero, C. M. Reilly, J. K. Higgins, S. Cook-Cronin, S. N. Tawde, B. M. Crossley, P. Yant, R. Cazarez & F. A. Uzal, 2012. An outbreak of equine botulism type A associated with feeding grass clippings Stephanie R. *Journal of Veterinary Diagnostic Investigation*, **24**, 601. Doi: 10.1177/1040638712440987.

Park, J. B. & L. L. Simpson, 2003. Inhalational poisoning by botulinum toxin and inhalation vaccination with its heavy-chain component. *Infection and Immunity*, **71**, 1147–1154.

Pascuzzi, R. M. & J. D. Fleck, 1997. Acute peripheral neuropathy in adults: Guillain-Barre syndrome and related disorders. *Neurology Clinics*, **15**, 529–547.

Payne, J. H., R. A. Hogg, A. Otter, H. I. J. Roest & C. T. Livesey, 2011. Emergence of suspected type D botulism in ruminants in England and Wales (2001–2009), associated with exposure to broiler litter. *Veterinary Record*, **168**, 640–643.

Peck, M. W., S. C. Stringer & A. T. Carter, 2011. *Clostridium botulinum* in the post-genomic era. *Food Microbiology*, **28**, 183–191.

Peck, M. W., 2006. *Clostridium botulinum* and the safety of minimally heated, chilled foods: an emerging issue? *Journal of Applied Microbiology*, **101**, 556–570.

Peck, M. W. & S. C. Stringer, 2005. The safety of pasteurized in-pack chilled meat products with respect to the food borne botulism hazard. *Meat Science*, **70**, 461–475.

Pflug, I. J., 2010. Science, practice, and human errors in controlling *Clostridium botulinum* in heat-preserved food in hermetic containers. *Journal of Food Protection*, **73**, 993–1002.

Piazza, T. M., D. S. Blehert, F. M. Dunning, B. M. Berlowski-Zier, F. N. Zeytin, M. D. Samuel & W. C. Tucker, 2011. *In vitro* detection and quantification of botulinum neurotoxin type E activity in avian blood. *Applied Environmental Microbiology*, **77**, 7815–7822.

Pingeon, J. M., C. Vanbockstael, M. R. Popoff, L. A. King, B. Deschamps, G. Pradel, H. Dupont, A. Spanjaard, A. Houdard, C. Mazuet, B. Belaizi, S. Bourgeois, S. Lemgueres, K. Debbat, P. Courant, R. Quirin & P. Malfait, 2011. Two outbreaks of botulism associated with consumption of green olive paste, France, September

2011. *Euro Surveillance*, **16**, 20035. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20035 (12 June 2014 date last accessed).

Poli, M. A., V. R. Rivera & D. Neal, 2002. Development of sensitive colorimetric capture ELISAs for *Clostridium botulinum* neurotoxin serotypes E and F. *Toxicon*, **40**, 797–802.

Poulain, B., B. G. Stiles, M. R. Popoff & J. Molgo, 2006. Attack of the nervous system by clostridial toxins: physical findings, cellular and molecular actions. In: *The Sourcebook of Bacterial Protein Toxins,* eds J. E. Alouf & M. R. Popoff, Elsevier, Academic Press, Amsterdam, pp. 348–389.

Prévot, V., F. Tweepenninckx, E. van Nerom, A. Linden, J. Content & A. Kimpe, 2007. Optimization of polymerase chain reaction for detection of *Clostridium botulinum* type C and D in bovine samples. *Zoonoses and Public Health*, **54**, 320–327.

Rasooly, R. & P. M. Do, 2008. Development of an *in vitro* activity assay as an alternative to the mouse bioassay for *Clostridium botulinum* neurotoxin type A. *Applied and Environmental Microbiology*, **74**, 4309–4313.

Raymundo, D. L., R. von Hohendorf, F. M. Boabaid, M. C. Both, L. Sonne, R. A. Assis, R. P. Caldas & D. Driemeier, 2012. Outbreak of type C botulism in captive wild birds. *Journal of Zoology and Wildlife Medicine*, **2,** 388– 390.

Rebagliati, V., R. Philippi, M. Tornese, A. Paiva, L. Rossi & A. Troncoso, 2009. Food-borne botulism in Argentina. *Journal of Infections in Developing countries*, **3**, 250–254.

Reller, M. E., R. W. Douce, S. E. Maslanka, D. S. Torres, S. R. Manock & J. Sobel, 2006. Wound botulism acquired in the amazonian rain forest of Ecuador. *American Journal of Tropical Medicine and Hygiene*, **74**, 628–631.

Rowlands, R. E. G., C. A. Ristori, G. E. S. L. Lopes, A. M. R. de Paula, H. Sakuma, R. Grigaliunas, R. L. Filho, D. S. Gelli, M. B. de Paula Eduardo & M. Jakabi, 2010. Botulism in Brazil, 2000–2008: Epidemiology, clinical findings and laboratorial diagnosis. *Revista do Instituto de Medicina Tropical de São Paulo*, **52**, 183–186.

Rummel, A., S. Mahrhold, H. Bigalke & T. Binz, 2004a. The Hcc-domain of botulinum neurotoxins A and B exhibits a singular gangliosside binding site displaying serotype specific carbohydrate interaction. *Molecular Microbiology*, **51**, 631–643.

Rummel, A., T. Karnath, T. Henke, H. Bigalke & T. Binz, 2004b. Synaptotagmins I and II act as nerve cell receptors for botulinum neurotoxin G. *Journal of Biological Chemistry*, **279**, 30865–30870

Sanford, D. C., R. E. Barnewall, M. L. Vassar, N. Niemuth, K. Metcalfe, R. V. House, I. Henderson & J. D. Shearer, 2010. Inhalational botulism in rhesus macaques exposed to botulinum neurotoxin complex serotypes A1 and B1. *Clinical and Vaccine Immunology*, **17**, 1293–1304.

Satterfield, B. A., A. F. Stewart, C. S. Lew, D. O. Pickett, M. N. Cohen, E. A. Moore, P. F. Luedtke, K. L. O'Neill & R. A. Robison, 2010. A quadruplex real-time PCR assay for rapid detection and differentiation of the *Clostridium botulinum* toxin genes A, B, E and F. *Journal of Medical Microbiology*, **59**, 55–64.

Schiavo, G., M. Matteoli & C. Montecucco, 2000. Neurotoxins affecting neuroexocytosis. *Physiology Reviews*, **80**, 717–766.

Schroeter, M., K. Alpers, U. V. Treeck, C. Frank, N. Rosenkoetter & R. Schaumann, 2009. Outbreak of wound botulism in injecting drug users. *Epidemiology and Infection*, **137**, 1602–1608.

Schwarz, B., R. Brunthaler, C. Hahn & R. van den Hoven, 2012. Outbreaks of equine grass sickness in Hungary. *Veterinary Record*, **170**, 75. Doi:10.1136/vr.100141.

Setlow, P. & E. A. Johnson, 1997. Spores and their significance. In: *Food Microbiology, Fundamentals and Frontiers*, eds M. P. Doyle, L. R. Beuchat & T. J. Montville, ASM Press, Washington, pp. 30–65.

Sevenier, V., S. Delannoy, S. André, P. Fach & F. Remize, 2012. Prevalence of *Clostridium botulinum* and thermophilic heat-resistant spores in raw carrots and green beans used in French canning industry. *International Journal of Food Microbiology*, **155**, 263–268.

Shapiro, R. L., C. Hatheway & D. L. Swerdlow, 1998. Botulism in the United States: A clinical and epidemiologic review. *Annals of Internal Medicine*, **129**, 221–228.

Sharpe, A. E., E. J. Sharpe, E. D. Ryan, H. J. Clarke & S. A. McGettrick, 2011. Outbreak of type C botulism in laying hens. *Veterinary Record*, **168**, 669a. Doi: 10.1136/vr.d1090.

Shayegani M, W. B. Stone & G. E. Hannett, 1984. An outbreak of botulism in waterfowl and fly larvae in New York State. *Journal of Wildlife Diseases*, **20**, 86–89.

Sheppard, Y. D., D. Middleton, Y. Whitfield, F. Tyndel, S. Haider, J. Spiegelman, R. H. Swartz, M. P. Nelder, S. L. Baker, L. Landry, R. MacEachern, S. Deamond, L. Ross, G. Peters, M. Baird, D. Rose, G. Sanders & J. W. Austin, 2012. Intestinal toxaemia botulism in 3 adults, Ontario, Canada, 2006–2008. *Emerging Infectious Diseases*, **18**, 1–6.

Simpson, L. L., 2004. Identification of the major steps in botulinum toxin action. *Annual Reviews in Pharmacology and Toxicology*, **44**, 167–193.

Smart, J. L., P. W. Laing & C. E. Winkler, 1983. Type C botulism in intensively farmed turkeys. *Veterinary Record*, **113**, 198–200.

Smith, L. A., 2009. Botulism and vaccines for its prevention. *Vaccine*, **27**, 3–39.

Smith, L. D. S. & H. Sugiyama, 1988. Cultural and serological characteristics. In: *Botulism: the Organism, its Toxins, the Disease,* eds L. D. S. Smith & H. Sugiyama, Charles C. Thomas, Springfield, USA, pp. 23–37.

Sobel, J. 2005. Botulism. *Clinical Infectious Diseases*, **15**, 1167–1173.

Sobel, J., N. Tucker, A. Sulka, J. McLaughlin & S. Maslanka, 2004. Foodborne botulism in the United States, 1990-2000. *Emerging Infectious Diseases*, **10**, 1606–1611.

Sobel, J., M. Malavet & S. John, 2007. Outbreak of clinically mild botulism type E illness from home-salted fish in patients presenting with predominantly gastrointestinal symptoms. *Clinical Infectious Diseases*, **45**, e14–e16.

Sobel, J. & S. Maslanka, 2012. Botulism. In: *Harrison's Principles of Internal Medicine.* 18th edn, eds D. L. Longo, K. L. Kasper, J. L. Jameson, A. S. Fauci, S. L. Hauser & J. Loscalzo, McGraw-Hill, New York, pp. 1200–1203.

Stanker, L. H., P. Merrill, M. C. Scotcher & L. W. Cheng, 2008. Development and partial characterization of high-affinity monoclonal antibodies for botulinum toxin type A and their use in analysis of milk by sandwich ELISA. *Journal of Immunological Methods*, **336**, 1–8.

Sugii, S., I. Ohishi & G. Sakaguchi, 1977. Intestinal absorption of botulinum toxins of different molecular sizes in rats. *Infection and Immunity*, **17**, 491–496.

Swaan, C. M., I. M. van Ouwerkerk & H. J. Roest, 2010. Cluster of botulism among Dutch tourists in Turkey, June 2008. *Euro Surveillance*, **15**, 19532. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19532 (12 June 2014 date last accessed).

Tacket, C. O., W. X. Shandera, J. M. Mann, N. T. Hargrett & P. A. Blake, 1984. Equine antitoxin use and other factors that predict outcome in type A food borne botulism. *American Journal of Medicine*, **76**, 794–798.

Takeda, M., H. Kasai, Y. Torii, M. Mukamoto, T. Kohda, K. Tsukamoto & S. Kozaki, 2006. Protective effect of botulinum C/ D mosaic toxoid against avian botulism. *Journal of Veterinary Medical Science*, **68**, 325–330.

Thiongane, Y., Y. Leforban & M. P. Doutre, 1984. Botulism type D in Senegal. A new

outbreak of water origin responsible for a high mortality. *Revue de'Elevage et de Médecine Vétérinaire des Pays Tropicaux*, **37**, 152–154.

Townes, J. M., P. R. Cieslak, C. L. Hatheway, H. M. Solomon, J. T. Holloway, M. P. Baker, C. F. Keller, L. M. McCroskey & P. M. Griffin, 1996. An outbreak of type A botulism associated with a commercial cheese sauce. *Annals of Internal Medicine*, **125**, 558–563.

Toyoda, H., K. Omata, K. Fukai & K. Akai, 1980. A report on the pathology of type A botulism. *Acta Pathology* (Japan), **30**, 445–450.

Tsujihata, M., I. Kinoshita, M. Mori, K. Mori, S. Shirabe, A. Satoh & S. Nagataki, 1987. Ultrastructural study of the motor endplate in botulism and Lambert-Eaton myasthenic syndrome. *Journal of Neurological Sciences*, **81**, 197–213.

Tsukamoto, K., T. Kohda, M. Mukamoto, K. Takeuchi, H. Ihara, M. Saito & S. Kozaki, 2005. Binding of *Clostridium botulinum* types C and D neurotoxins to ganglioside and phospholipid. *Journal of Biological Chemistry*, **280**, 35164–35171.

Turton, K., J. A. Chaddock & K. R. Acharya, 2002. Botulism and tetanus neurotoxins: Structure, function and therapeutic utility. *Trends in Biochemical Sciences*, **27**, 552–558.

Umeda, K., Y. Seto, T. Kohda, M. Mukamoto & S. Kozaki, 2009. Genetic characterization of *Clostridium botulinum* associated with type B infant botulism in Japan. *Journal of Clinical Microbiology*, **47**, 2720–2728.

US Food and Drug Administration, 2012. 21 CFR 113. Chapter I Food and Drug Administration. Subchapter B. Food for human consumption. Part 113. Thermally processed low-acid foods packaged in hermetically sealed containers. Revised 1 April 2012. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=113.3 (12 June 2014 date last accessed).

Van der Lugt, J. J., S. C. De Wet, S. S. Bastianello, T. S. Kellerman & L. P. Van Jaarsveld, 1995. Two outbreaks of type C and type D botulism in sheep and goats in South Africa. *Journal of South Africa Veterinary Association*, **66**, 77–82.

Vossen, M. G., K.-B. Gattringer, J. Wenisch, N. Khalifeh, M. Koreny, V. Spertini, F. Allerberger, W. Graninger, C. Kornschober, H. Lagler, A. Reitner, T. Sycha, F. Thalhammer, 2012. The first case(s) of botulism in Vienna in 21 years: A case report. *Case Reports in Infectious Diseases*. Doi:10.1155/2012/438989.

Walker, N., L. Redmond, J. Payne, S. Kennedy & S. Wyllie, 2009. Change to FSA advice on botulism in sheep and goats. *Veterinary Record*, **164**, 666–667.

Wang, D., J. Baudys, S. R. Kalb & J. R. Barr, 2011. Improved detection of botulinum neurotoxin type A in stool by mass spectrometry. *Analytical Biochemistry*, **412**, 67–73.

Webb, R. P., T. J. Smith, P. M. Wright, V. A. Montgomerya, M. M. Meagher & L. A. Smith, 2007. Protection with recombinant *Clostridium botulinum* C1 and D binding domain subunit (Hc) vaccines against C and D neurotoxins. *Vaccine*, **25**, 4273–4282.

Werner, S. B., D. Passaro, D. McGee, R. Schechter & D. J. Vugia, 2000. Wound botulism in California, 1951–1998: recent epidemic in heroin injectors. *Clinical Infectious Diseases*, **31**, 1018–1024.

Whitlock, R. H. & S. McAdams, 2006. Equine botulism. *Clinical Techniques in Equine Practice*, **5**, 37–42.

Williams, J. H., L. Bester, L. Venter, D. Pretorius & F. Greyling, 2011. Barbiturate ingestion in three adult captive tigers (*Panthera tigris*) and concomitant fatal botulism of one. *Journal of South African Veterinary Association*, **82**, 244–249.

Willison, H. J. & G. M. O'Hanlon, 1999. The immunopathogenesis of Miller Fisher syndrome. *Journal of Neuroimmunology*, **100**, 3–12.

Wobeser, D. J. R., T. B. Smith-Windsor & G. Bogdan, 1983. Avian botulism during late autumn and early spring in Saskatchewan. *Journal of Wildlife Diseases*, **19**, 90–94.

Wobeser, K. B., R. G. Clark & A. W. Deyo, 1997. Type C botulism in cattle in association with a botulism die-off in waterfowl in Saskatchewan. *Canadian Veterinary Journal*, **38**, 782.

Woo, G.-H., H.-Y. Kim, Y.-C. Bae, Y. H. Jean, S.S. Yoon, E.-J. Bak, E. K. Hwang & Y.-S. Joo, 2010. Outbreak of Botulism (*Clostridium botulinum* type C) in wild waterfowl, Seoul, Korea. *Journal of Wildlife Diseases*, **46**, 951–955.

Wu, H. C., Y. L. Huang, S. C. Lai, Y. Y. Huang & M. F. Shaio, 2001. Detection of *Clostridium botulinum* neurotoxin type A using immuno-PCR. *Letters in Applied Microbiology*, **32**, 321–325.

Yamakawa, K., S. Kamiya, K. Yoshimura & S. Nakamura, 1992. *Clostridium botulinum* type C in healthy swine in Japan. *Microbiology and Immunology*, **36**, 29–34.

Yoneyama, T., K. Miyata, T. Chikai, A. Mikami, T. Suzuki, K. Hasegawa, T. Ikeda, T. Watanabe, T. Ohyama & K. Niwa, 2008. *Clostridium botulinum* serotype D neurotoxin and toxin complex bind to bovine aortic endothelial cells via sialic acid. *FEMS Immunology and Medical Microbiology*, **54**, 290–298.

Yule, A. M., J. W. Austin, I. K. Barker, B. Cadieux & R. D. Moccia, 2006a. Persistence of *Clostridium botulinum* neurotoxin type E in tissues from selected freshwater fish species: Implications to public health. *Journal of Food Protection*, **69**, 1164–1167.

Yule, A. M., I. K. Barker, J. W. Austin & R. D. Moccia, 2006b. Toxicity of *Clostridium botulinum* type E neurotoxin to Great Lakes fish: implications for avian botulism. *Journal of Wildlife Diseases*, **42**, 479-493.

Zakhari, J. S., I. Kinoyama, M. S. Hixon, A. Di Mola, D. Globisch & K. D. Janda, 2011. Formulating a new basis for the treatment against botulinum neurotoxin intoxication: 3,4-Diaminopyridine prodrug design and characterization. *Bioorganic and Medicinal Chemistry*, **19**, 6203–629.

Zhou, Y. & B. R. Singh, 2004. Cloning, high-level expression, single-step purification, and binding activity of His-6-tagged recombinant type B botulinum neurotoxin heavy chain transmembrane and binding domain. *Protein Expression and Purification*, **34**, 8–16.

Zichel, R., A. Mimran, A. Keren, A. Barnea, I. Steinberger-Levy, D. Marcus, A. Turgeman & S. Reuveny, 2010. Efficacy of a potential trivalent vaccine based on Hc fragments of botulinum toxins A, B, and E produced in a cell-free expression system. *Clinical and Vaccine Immunology*, **17**, 784–792.

Paper received 02.12.2014; accepted for publication 25.04.2014

Correspondence:

Nicodemus M. Useh, DVM, PhD (ABU),
FSB (London)
Laboratory of Molecular Biology of Infectious Diseases, Department of Population Medicine & Diagnostic Sciences,
College of Veterinary Medicine,
Cornell University,
Upper Tower Road,
14853 Ithaca, New York
e-mail: nicodemus.useh@fulbrightmail.org