ADVISORY COMMITTEE ON THE MICROBIOLOGICAL SAFETY OF FOOD

AD HOC GROUP ON BOTULISM IN CATTLE

REPORT ON BOTULISM IN CATTLE

Advises the Food Standards Agency on the Microbiological Safety of Food

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The ACMSF accepts full responsibility for the final content of the report.

SUMMARY

1. In this report we have considered the potential risk to human health from food chain issues linked to botulism or suspected botulism in cattle, particularly in relation to the spreading of poultry litter on agricultural land.

2. The report comes against the background of a marked increase in the reported incidence of suspected cattle botulism in England, Wales and Ireland since 2003. There is some evidence to suggest that access to litter from deep litter broiler houses containing carcases of dead birds is a factor in the occurrence of the disease in a proportion of these recent outbreaks. In some cases the litter had been spread onto land on which the cattle were grazing or on adjacent fields, in others the animals gained access to a stack of stored litter.

3. In June 2004 we decided to set up an *ad hoc* Group to examine food chain issues linked to botulism or suspected botulism in cattle. The terms of reference for the Group were to consider the potential human health risk associated with botulism or suspected botulism in cattle, particularly in relation to the spreading of poultry litter on agricultural land, and to report back with recommendations to the full Committee. The Group met on seven occasions over a period of 12 months.

4. At the beginning of our investigations we considered information on the molecular biology and structure of *Clostridium botulinum* toxins and toxin types. We concluded that in cattle affected by botulism, toxin delivered from a food source is rapidly and irreversibly bound to the motor end plates in muscles in a form that is unlikely to be able to cause re-intoxication following consumption of meat or milk from affected cattle.

5. Subsequently, we examined the epidemiology and diagnosis of botulism including the occurrence of botulism and clinical signs of the disease in cattle. We concluded that the proportion of animals affected by this disease was very variable and that classical signs of botulism related to varying degrees of paralysis of the muscles. As part of our considerations of clinical and laboratory diagnosis of botulism, the Group considered clinical and diagnostic testing protocols used by government agencies including use of a mouse bioassay to identify botulinum toxin in cattle. We concluded that diagnosis of botulism was difficult and usually made on the basis of clinical signs and the identification of a likely toxin source. There were also disadvantages associated with existing diagnostic tests for the detection of botulinum toxins. In light of these limitations we recommended that the mouse bioassay be applied to gastrointestinal samples to aid diagnosis and help assess risk by determining whether the toxin types involved were associated with botulism in humans. In addition samples collected during clinical investigations should be archived to support development of future testing methods. Consideration should also be given to further work to understand the occurrence of botulinum toxins in cattle gastrointestinal contents. To confirm diagnosis, effort should be

targeted to develop sensitive *in vitro* tests for the detection of *C. botulinum* toxins or assessment of food safety risks.

6. We reviewed definitions, types and sources of poultry waste, including poultry litter and manure, and assessed the risk sources associated with botulism in cattle. We concluded that where a source was identifiable in outbreaks of cattle botulism, carrion or putrefying material was the most common source. Litter from broiler chicken production was also commonly, if often indirectly, associated with outbreaks of cattle botulism. Whilst recognising that practical solutions would need to take account of local factors, we have recommended good practice in litter management and disposal, including expansion of biosecurity messages to broiler farmers to highlight the risks of disease transmission caused by poor carcase removal practices.

7. The Group examined risk assessment work conducted in France, which did not identify a significant risk to the public from food associated with botulism in cattle. We also considered toxin availability in meat and milk including the impact of the reduced activity of the bound toxin in affected animals, toxico-infection, and the role of heat treatment/food processing effects on both these foods and dilution factors on the availability of toxin in milk. We concluded that botulinum toxin types identified in animals (C and D) have rarely been associated with disease in man, and that the presence of these toxins was not a significant risk due to the low apparent susceptibility of humans to these toxin types. Also any disease occurring in cattle would result in affected cattle being removed from the food chain. We also concluded that the risks associated with toxin in meat and milk were similar. Therefore the likelihood of active toxin entering meat or milk was low, although the possibility of cross-contamination from environmental spores was a consideration. However, should toxico-infection be found to be a more common means of infection of animals in the UK, or should toxin types other than C and D emerge as a cause of disease in cattle, then we have recommended that the risk to foods be re-assessed.

8. Evidence for the occurrence of human cases associated with meat and milk consumption was also reviewed. The Group concluded that there was no evidence to suggest that any clinical cases of botulism were associated with consumption of meat or milk derived from animals from herds affected by botulism. No reports were identified by the Group of suckling calves acquiring botulism, or of the transmission of botulism to other animals such as dogs via fresh meat.

9. Finally, following examination of measures to prevent outbreaks of botulism in animals and for the management of outbreaks in the UK, including the availability of guidance to farmers, and in light of the other views outlined in this report, the Group concluded that statutory notification did not currently appear to be merited. Although voluntary reporting of botulism outbreaks in cattle may result in under-reporting of the disease, clinically affected animals were likely to be investigated. Voluntary restrictions on meat and milk from clinically affected animals appeared

appropriate, but restriction of unaffected animals was considered over-cautious. However this would need to be reviewed if evidence emerged that other toxin types more commonly associated with humans were causing outbreaks in animal populations.

10. Therefore, from the evidence presented to the Group we have recommended that, in the absence of other signs, there should be no requirement to restrict sales of meat or milk from clinically healthy cattle from farms where there have been clinically suspected cases of botulism in cattle. In addition, there should be no requirement to restrict the slaughter of healthy cattle from herds where cases of confirmed or suspected botulism have occurred. Finally we have recommended that UK veterinary authorities continue to encourage cattle farmers to report suspected cases of botulism in cattle.

11. The assessments made and the conclusions we have reached reflect, in large measure, the evidence, oral and written, drawn from the scientific community, government departments, and the scientific literature. Our conclusions and recommendations are presented at the end of each chapter and are also drawn together at the end of the report.

Chapter 1

Introduction

Background

1.1 In 2004 the advice of the Committee was sought following a marked increase in the reported incidence of suspected cattle botulism in England, Wales and Ireland since 2003. There was some evidence to suggest that access to litter from deep litter broiler houses containing carcases of dead birds was a factor in the occurrence of the disease in a proportion of these recent outbreaks. In some cases the litter had been spread onto land on which the cattle were grazing or on adjacent fields, in others the animals gained access to a stack of stored litter. In response to this request the ACMSF established an *ad hoc* Group of Members and co-opted experts to consider the potential risk to human health associated with botulism or suspected botulism in cattle, particularly in relation to the spreading of poultry litter on agricultural land, and to report back with recommendations to the full Committee.

1.2 Botulism in cattle is caused either by ingestion of preformed toxins produced by the growth of *Clostridium botulinum* in decaying crops, vegetation or carcase material, or by a gastrointestinal infection (toxico-infection) with *C. botulinum*. It is not clear which of these exposures is the more important or whether there is a difference in clinical presentation, e.g. in the period between exposure and development of clinical signs. Cattle clinically affected by botulism will usually present with signs of progressive flaccid paralysis, which develops over time and leads after several days to recumbence and death. A small proportion of animals may recover but many of the affected cattle are euthanased to avoid welfare problems.

1.3 Cases of botulism in cattle are usually diagnosed on the basis of the clinical signs and by elimination of other conditions. It can be very difficult to obtain confirmation that botulism exists. Tests are available that can detect the toxin in the serum, tissues and gastrointestinal contents of affected animals. However, the concentration of toxin in the blood of affected cattle is often too low to be detected. The toxin or the organism can be detected in feed but identifying the affected feed is difficult. For instance, the feed left on the farm may not be representative of the feed that caused the disease because the toxin may only have been present in one part of the feed and not throughout the batch.

1.4 From 1997-2002 an average of four botulism incidents were reported each year in England and Wales. However, in 2003 the number of incidents reported in these countries rose to 29, rising to 34 incidents in 2004. There is some evidence that exposure to litter from deep litter broiler houses was a factor in the occurrence of disease in a proportion of these recent outbreaks. In Scotland the number of reports has remained constant at no more than one per year. VLA investigations of

suspected cases of botulism in cattle in England and Wales (2003-2005) (Annex 3) attempted to identify the "suspected source" in all outbreaks. A range of such suspected sources was identified, including vegetable waste, dead pheasants, a dead fox, and bread waste, but the most commonly suspected source was poultry litter. Nevertheless most associations with poultry litter were indirect. Among the 55 outbreaks, only 9 involved direct exposures to broiler litter as bedding, spreading on the pasture the cattle grazed, or spreading on silage land. In a further 7 outbreaks cattle appear to have had direct access to stacked litter. In the remaining 39 outbreaks litter was present on adjacent fields or farms, stacked or spread. If the poultry litter was in fact involved in these outbreaks, it would most probably have required the activity of wild birds or animals to transfer the toxic material to areas where the cattle could have access to it. Cases have also occurred when cattle were fed silage from fields fertilised with poultry litter. The losses suffered have been very variable, ranging from the death of a single animal up to death or euthanasia of 80% of the herd.

1.5 The Department of Agriculture and Food in the Republic of Ireland and the Department of Agriculture and Rural Development in Northern Ireland (DARDNI) have also identified a significant increase in the numbers of suspected cases of bovine botulism since 1999. For example, only two suspect cases were submitted to DARDNI's veterinary diagnostic service in 1998, compared to 17 in 1999, 121 in 2000, 31 in 2001, 73 in 2002, and 105 in 2003. However in 2004 the number of reported incidents reduced to 46. This may have been linked to the introduction of guidance offered by DARDNI (Annex 5). Epidemiological investigations have provided strong circumstantial evidence that broiler litter is a risk factor in many of these outbreaks.

1.6 The Animal By-Products Regulation (Regulation (EC) No 1774/2002) is enforced through the Animal By-Products Regulations 2003 in England, and equivalent legislation in Scotland, Wales and Northern Ireland. The Regulation prohibits the composting of poultry carcases or the spreading of litter or manure containing carcase material. Offences under the Regulations should be reported to the Local Authority who are responsible for enforcing the Regulations. Local Authorities have the right of access to farms for enforcement purposes. In Northern Ireland, offences under the Animal By-Products Regulations (NI) 2003 should be reported to DARDNI which is responsible for enforcing this aspect of the Regulations. DARDNI officials have a right of access to farms for enforcement purposes and have the power to serve Notices requiring the correct disposal of animal by-products.

1.7 The FSA adopts a precautionary approach to protect the food chain. When an outbreak of botulism is suspected, milk and meat from an affected herd are voluntarily restricted from entering the food chain for a period of 14 days from the onset of illness of the last clinical case or 17 days from removal of the source of contamination.

1.8 No reports were identified of suckling calves acquiring the disease from their dams, or of the transmission of botulism to other carnivorous animals, such as dogs, via fresh meat. Similarly, there have been no reports of fresh meat or milk from cattle affected by botulism having caused human disease. This may be because the *C. botulinum* toxins affecting cattle have little effect on humans, or because the toxins do not enter the milk of affected animals or do not persist in milk or meat in a form that remains active after ingestion and absorption. Very few cases of botulism associated with milk products, such as cheese and yoghurt, have been reported; in the case of an outbreak associated with yoghurt the toxin was found to have been present in hazelnut purée flavouring rather than in the milk product itself.

The ACMSF's approach to its work

1.9 The *ad hoc* Group met on seven occasions over a period of 12 months and considered documentary and verbal evidence relating to the risks and uncertainties associated with botulism in cattle and also management of botulism outbreaks in these animals in the UK.

1.10 The Group also considered information on the molecular biology and structure of *C. botulinum* toxins and different toxin types, and the susceptibility of humans to toxin types C and D.

1.11 Finally, the Group heard evidence relating to the mechanism of transfer *of C. botulinum* toxins into milk, and considered information on diagnostic testing for *C. botulinum* and its toxins in animals and animal products.

Chapter 2

C. botulinum and botulism

The organism, *Clostridium botulinum*

2.1 The causative agent of botulism, *Clostridium botulinum*, is a Gram-positive, spore-forming, neurotoxin-producing, obligate anaerobe.

2.2 It is essentially a soil-living bacterium; its ability to form spores that are resistant to heat, desiccation, damaging chemicals and radiation allow the organism to survive for long periods in the environment, and it is therefore distributed very widely throughout the world.

2.3 Germination of spores occurs in moist conditions, either anaerobic or aerobic. Subsequent growth of vegetative cells, during which the botulinum neurotoxins are synthesised, is restricted to anaerobic conditions only.

2.4 The spores of *C. botulinum* are very resistant to heat and easily survive conventional pasteurisation conditions. The organism in its vegetative state, on the other hand, is no more thermotolerant than other bacterial species associated with foodborne disease, and the protein neurotoxins produced by *C. botulinum* are heat labile but not totally destroyed by pasteurisation.

2.5 There is considerable genetic diversity among strains of *C. botulinum* such that the genes encoding the toxin may be chromosomal, carried by a plasmid or located in the genome of a lysogenic phage in various isolates. Normally a strain of *C. botulinum* produces a single toxin type, but there are occasional reports of an individual organism producing more that one toxin type (Gimenez & Ciccarelli, 1970), and some strains may have unexpressed 'silent' toxin genes (Minton, 1995).

Pathogenesis of disease in man (Fig. 1)

2.6 Botulism in adults is usually the result of intoxication, following ingestion of food contaminated with preformed botulinum toxin (Roberts, 2000). Botulism is not normally due to infection; *C. botulinum* appears to colonise the host poorly because it cannot compete efficiently with resident bacteria in the intestine, and so any ingested bacteria are excreted in the faeces.

2.7 There are some specific exceptions to this; infant botulism (in which *C. botulinum* does colonise the intestine because the resident flora of infants is not well established) (Münsterer, 2000; Brook, 2000), and wound botulism (in which infected deep wounds provide the anaerobic conditions necessary for growth of any contaminating bacteria). Another infectious form of botulism, similar to infant botulism, has been

reported in adults, associated with gastrointestinal operations, stagnant loops of bowel, and other pathological conditions. This form is referred to as infant-like botulism in adults, or intestinal toxaemia botulism (Cherington, 1998).

2.8 Botulinum toxins belong to a family of extremely potent neurotoxins that also includes tetanus toxin. They act on nerve cells (neurones); specificity is due to the fact that both the toxin-binding receptors (sialic acid-containing glycoproteins or glycolipids, Kozaki et al, 1998; Marxen et al, 1989; Montecucco, 1986) and the substrates for the enzymic activity of the toxin are found only in neurones.

2.9 The outcome of toxin activity is inhibition of the release of a neurotransmitter, acetylcholine, whose role is to send nerve impulses to the muscles; when acetylcholine release is blocked muscles cannot contract, resulting in flaccid paralysis.

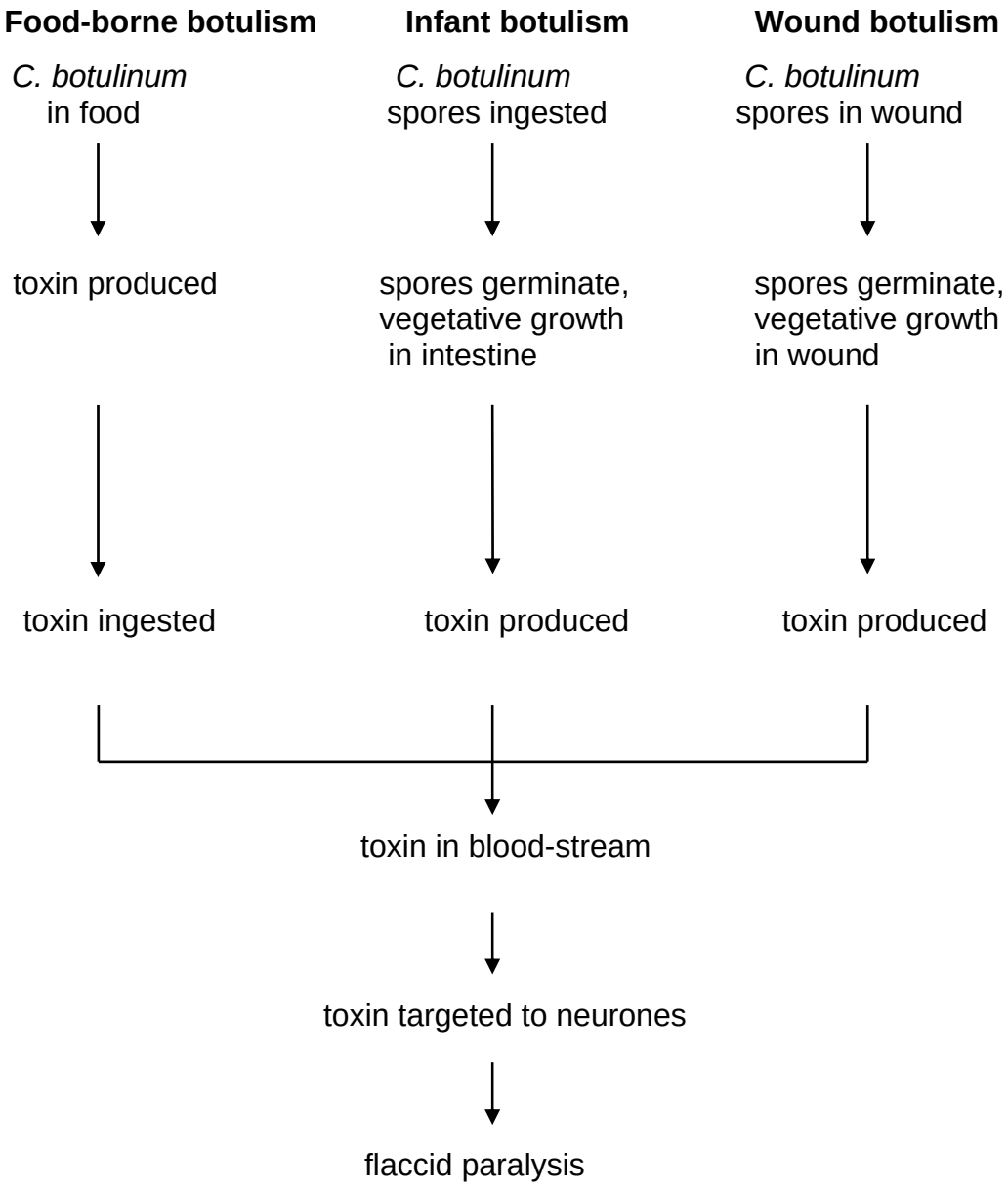

Fig. 1. Comparative pathogenesis of human food-borne botulism, infant botulism and wound botulism

Botulinum toxin structure and processing

2.10 Seven serotypes (A-G) of botulinum toxin are recognised, of which types A, B and E are most commonly associated with human disease (Cherington, 1998). Each type has a characteristic distribution; type A, for example, is found in soils in China, South America and the western USA, while type B predominates in soils in Europe and eastern USA; types C and D are primarily associated with disease in animals and birds, and type E is most often found in lake sediments and in fish (Cato et al, 1986; Henderson et al. 1997; Sugiyama, 1980). Note that some toxin serotypes are also produced by clostridial species other than *C. botulinum*, for example type F toxin by *C. baratii,* type G by *C. argentinense* and type E by *C. butyricum*.

2.11 For the purposes of this report it is important to note that toxin types C and D most often associated with recent outbreaks in cattle have only very rarely been reported to be associated with human disease. Indeed a recent report by the French Food Safety Agency (Anon, 2002) uses this observation to support its conclusion that the risks to consumers of botulism in cattle are low (see Chapter 6 for a more detailed consideration of the French report). It should be pointed out, however, that it is not clear whether the low incidence of human cases of types C and D botulism is due to inherently lower activity of these toxin types in man compared with cattle, or to the fact that the level of exposure of human populations is usually very low.

2.12 Although there is clearly sufficient amino acid sequence diversity to give rise to serological differences between toxin types, there is also significant sequence similarity (Popoff & Marvaud, 1999), especially at the functional domains described below. However, toxins of the same serotype produced by different strains may show some variation.

2.13 Botulinum toxin is synthesised as a protein of 150 kilodaltons (kDa) and is released into the environment when the producing bacteria die.

2.14 The toxin released from dying bacteria is in the form of a so-called progenitor toxin in which the 150 kDa protein forms a non-covalent complex with a variable number of associated non-toxic proteins (ANTPs) (Fig 2); these appear to protect the toxin from stomach acid and gastric proteases (Chen et al, 1998; Sakaguchi, 1983), although there is some evidence that disease signs may be induced in experimental animals fed with the 150-kDa component alone (Maksymowych et al, 1999).

2.15 In the more alkaline, less harsh conditions of the small bowel, the ANTPs dissociate from the 150-kDa protein. This non-complexed protein, known as the “derivative toxin” does not, however, have enzymic (toxic) activity; activation requires proteolytic cleavage by either bacterial or intestinal proteases to generate a 50-kDa light chain, which has toxic activity, and a 100-kDa heavy chain, which has

receptor-binding activity. A disulphide bridge links the two subunits of the active toxin.

Uptake and internalisation of botulinum toxin

2.16 The activated toxin is subsequently absorbed from the upper small intestine (by a mechanism that is still unclear) (May & Whaler, 1958). Following absorption into the blood-stream toxin molecules target neurones of the peripheral nervous system (i.e. those that make contact with muscles).

2.17 The toxin binds to receptors on the neuronal cell surface *via* a domain in the C-terminal part of the heavy chain (Kozaki et al, 1989; Lacy et al, 1998; Shone et al, 1985; Swaminathan & Eswaramoorthy, 2000; Tsukamoto et al, 2005). Local pH changes at the cell surface enhance the hydrophobicity of the toxin, enabling it to penetrate the cell membrane.

2.18 Once inside the neuronal cell the N-terminal portion of the heavy chain inserts into the membrane of the synaptic vesicles to form a pore (Oblatt-Montal et al, 1995). Changes in pH disrupt the disulphide bond between the two subunits of the toxin, and the 50-kDa light chain passes through the pore into the synaptic vesicle.

2.19 The toxin remains stably active in neuronal cells for long periods (Keller et al., 1999). It is highly unlikely, however, that ingestion of the 50-kDa form of the toxin in meat or milk products would be able to cause disease signs because it requires the 100-kDa heavy chain for internalisation in neurones (Swaminathan & Eswaramoorthy, 2000).

Mode of action of botulinum toxin

2.20 The 50-kDa component of botulinum toxin is a zinc endopeptidase (Kurazono et al, 1992: Li & Singh, 2000: Montecucco & Schiavo, 1995), which cleaves the proteins that control the release of acetylcholine at the junction between neurones and muscle cells.

2.21 Different botulinum toxin serotypes have slightly different specificities; toxins A, C and E, for example, all target a protein called SNAP-25 (the 25 kDa synaptosome-associated protein), but the cleavage site is different in each case; similarly toxins B, D, F and G all target a protein called synaptobrevin (or vesicle-associated membrane protein, VAMP), but have different cleavage sites (Montecucco & Schiavo, 1995).

2.22 Whatever the target or site of cleavage, the overall result is failure of the neurone to release acetylcholine at the neuromuscular junction and the consequent failure of transmission of nerve impulses to the muscles.

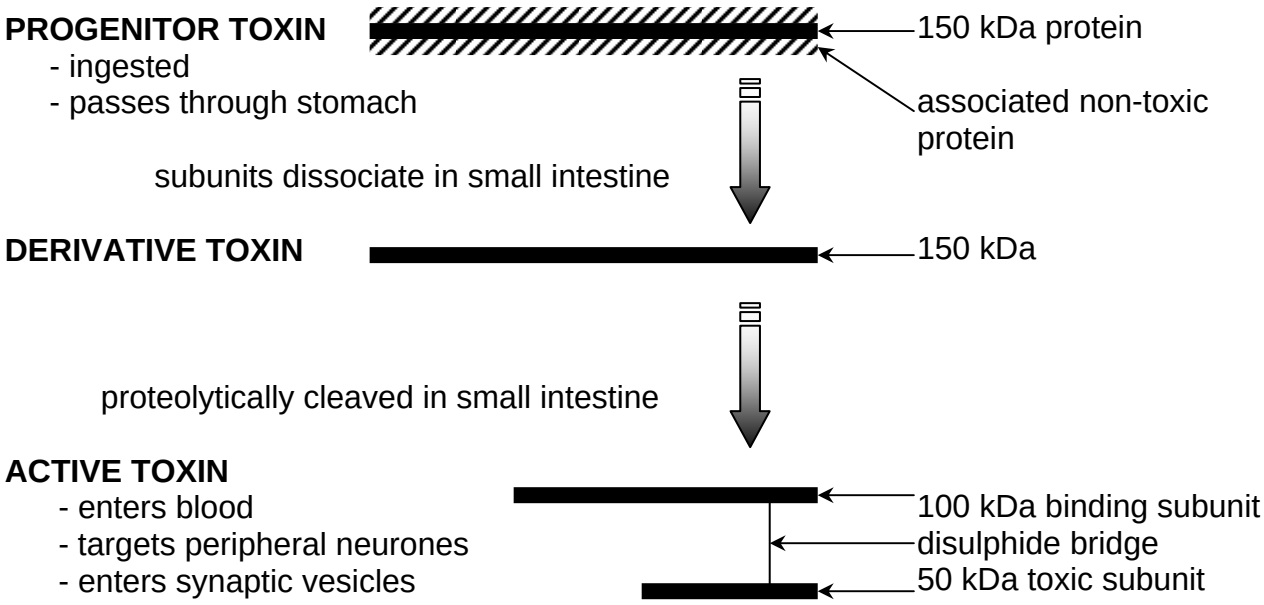

Fig. 2. Structure and processing of botulinum toxin in food-borne botulism

Conclusions

2.23 Botulinum toxins are a serologically diverse group of potent neurotoxins that inhibit the transmission of nerve impulses at neuromuscular junctions, resulting in flaccid paralysis. The process of delivery of the toxin from a food source to the neuromuscular junction involves (a) associated non-toxic proteins that are believed to protect it during passage through the stomach, and (b) a binding subunit that is required for internalisation of the active toxic subunit into the synaptic vesicles of peripheral neurones. Although the active toxin is stable at its target site, it is highly unlikely that in this form it could be responsible for another round of intoxication since by this time it lacks both the protective proteins and the binding subunit.

Chapter 3

Epidemiology and diagnosis of botulism in cattle

Occurrence

3.1 Cattle are believed to be more sensitive to the effects of botulinum toxin than birds. Outbreaks are not restricted to cattle at pasture. Current and recent information on the occurrence of botulism in animals may be found using the OIE (Office International des Epizooties) Handistatus programme. However, the information contained in this database is quite limited given that cattle botulism is not a notifiable disease. We reviewed the information for 2003. Twenty eight of 167 member countries provided information on botulism in cattle and only 8 reported no disease. Of the countries which reported botulism, only 6 provided details on the number of outbreaks, cases and deaths. Movement controls were cited as control measures by only 3 countries. No countries prohibited vaccination and 13 countries cited vaccination as a control measure.

3.2 It is normally assumed that most cases of botulism are a result of the ingestion of preformed toxin in food. It is likely that animals exposed to toxin will also be simultaneously exposed to spores of *C. botulinum*, so it is possible that the ingested toxin is supplemented with toxin arising from intestinal infection. Toxico-infection has been documented in the young of some species but not in cattle in the UK. Occasionally botulism can arise as a result of colonisation of a wound with the organism, in a similar manner to tetanus.

3.3 *C. botulinum* multiplies in putrefying organic material in the absence of oxygen and at pHs above 4.5. It may produce toxin in vegetable material such as poorly conserved silage, grass clippings, and material at the base of tussocks of grass. However the main source of toxin for cattle is believed to be carrion (putrefying carcases of mammals or birds). If the carrion is from animals or birds which have died from botulism the risk of disease may be greater but the organism may readily proliferate and produce toxin in carcases which have died from other causes.

Clinical Signs

3.4 Clinical signs are variable and not specific to botulism and may begin as soon as 24 hours after the first exposure (Radostits et al, 2000). Delayed appearance of clinical signs, sometimes described as a ‘latent period’, may relate to the delay in release of toxin from the ingested material by ruminal digestion. In none of the incidents investigated by VLA has there been a latent period of more than 17 days presumably while contaminated material has remained in the lumen of the digestive

tract. ‘Sudden deaths’ may occur but the main clinical sign is flaccid paralysis, beginning with the hind limbs and progressing forward. The clinical findings may variably include drooling, tongue paralysis, dysphagia, inability to urinate, rumen stasis, diarrhoea, constipation and sweating. Skin sensation is usually normal and withdrawal reflexes of the limbs are weak. Initially, clinical signs may resemble milk fever but affected cattle do not respond to calcium therapy and progress to sternal recumbency. Death usually occurs 6-72 hr after the onset of recumbency and recovery is rare. Böhnel et al (2001) have recently described a chronic form of the disease in cattle characterised by chronic laminitis, retracted abdomen, emaciation, apathy, reduced milk yield, and, in younger animals, delayed growth and wasting.

Clinical diagnosis

3.5 Clinical diagnosis is made through a combination of the identification of typical clinical signs and elimination of other causes of motor paralysis (such as hypocalcaemia). This may be carried out by evaluation of blood biochemistry in combination with response to treatment. In the individual animal diagnosis is not easy, particularly if it becomes a ‘downer cow’ (goes down and is unable to rise). Outbreaks are more likely to be investigated in detail than are individual cases and as a result there is likely to be under-reporting of disease in cattle. As the condition is not notifiable there is no legal obligation on the clinician or animal owner to formally report suspected cases. Outbreaks are more likely to come to notice through requests for laboratory investigation.

Laboratory diagnosis

3.6 Laboratory confirmation can be difficult since pathology is not specific and detection of toxin is not always successful.

- *Clinical Pathology*: Elevated levels of indican, protein and glucose have been reported from the urine of affected cattle although the changes are not pathognomonic.

- *Pathology*: There are no characteristic gross or microscopic lesions although haemorrhage in the small intestine has sometimes been described.

- *Toxicology*: Detection of toxin in serum or tissue in the presence of appropriate clinical signs is currently the only way to diagnose the condition definitively. The presence of toxin in gastrointestinal contents can be an aid to diagnosis.

3.7 The most sensitive test for botulinum toxin is to inoculate suspect material intraperitoneally into mice. Any samples which cause typical clinical signs in inoculated mice are confirmed by being neutralised with monovalent specific antisera. This enables identification of specific toxin types.

3.8 An ELISA for toxin detection has been described but is believed currently to be much less sensitive than the mouse test.

3.9 DARDNI, having previously tested several hundred serum samples now confines use of the mouse bioassay to samples of gastrointestinal tract (GIT) contents because of the very low rate of positivity obtained in serum samples (CDC, 1998). During 2003 and 2004, DARDNI detected botulinum toxin in 28% of GIT contents tested from 210 suspected cattle botulism cases. Type D toxin was identified in 18% of these animals, toxins that showed serological cross-reactivity between types C and D in 4%, and a mixture of type D and C/D in a further 6%. DARDNI has also identified type D toxin in a small number of samples of chicken carcases and poultry litter tested.

3.10 DARDNI's Agric-Food and Biosciences Institute is currently developing immunoassay detection systems for types C and D toxin with a view to replacement of the bioassay by more sensitive methods. Immunoaffinity chromatographic methods have been developed for toxin types C and D with much improved sensitivity (Gessler et al, 2005; Klewitz et al, 2006).

Immunology

3.11 An ELISA test has been developed to measure the antibody response to toxin. It has been shown that unvaccinated animals which are not known to have suffered clinical botulism can have positive antibody titres (Gregory et al, 1996). Clinical cases may die before they produce antibodies to the ingested toxin. A rising titre in the herd coinciding with clinical cases would be supportive of a diagnosis of botulism. However the delay inherent in using this approach would make it unlikely to be of practical benefit in managing any food-chain implications of the condition. It would be further complicated in herds in which vaccination is used.

Conclusions

3.12 Botulism can cause serious disease in cattle used for the production of beef and of milk. The proportion of animals affected is very variable. The classical clinical signs relate to varying degrees of paralysis of muscles. Diagnosis is difficult and usually made on the basis of the clinical signs and the identification of a likely source of toxin. There are no characteristic lesions at post-mortem examination. Each of the existing tests for the detection of botulin toxins has disadvantages.

3.13 While clinical diagnosis supported by exclusion of other likely causes is satisfactory for identification and clinical management of single cases and large outbreaks, it is not an adequate basis for the implementation of food safety precautions such as the exclusion of animal products from the food chain.

Recommendations

3.14 In outbreaks of clinically suspected cases of botulism in cattle we recommend that the mouse bioassay be applied to gastrointestinal samples in order to provide an aid to diagnoses and to help assess risk by determining whether the toxin types involved are those that have been associated with botulism in humans (types A, B & E).

3.15 Work should be undertaken to understand the diagnostic and clinical significance of finding botulinum toxins in gastrointestinal contents of cattle.

3.16 Because of concerns over the use of live mice for the bioassay, work should be undertaken to develop new highly sensitive and specific diagnostic tests that do not use animals for the detection of *C. botulinum* toxins and organisms in biological matrices.

3.17 Samples collected during clinical investigations should be archived to assist with the development of further assay systems.

Chapter 4

Poultry waste

Definitions

4.1 Poultry litter is a mixture of bedding material (wood shavings, chopped straw, shredded paper) and excreta of commercially reared poultry. Poultry manure is composed only of poultry excreta and is produced in systems in which birds are housed in cages or separated from a manure pit by slats. Poultry carcases are another risk waste. Birds do not normally have direct access to manure so carcases are unlikely to be present in it, but carcases may be present in litter (see paragraph 4.3 below).

Types of litter and manure

4.2 Most broilers are reared on the same litter for 5-8 weeks. In the UK, after the birds are slaughtered, the litter is removed prior to cleaning and disinfection of the housing. In some countries 3-6 successive flocks of broilers may be reared on the same litter. Commercial layers and breeding chickens are usually reared on the same litter to age 16–20 weeks and then transferred to fresh litter on the laying farms. Laying farms often have an area of conventional litter and a pit-area covered by slats under which manure will accumulate. The litter and manure from laying farms are disposed of after a single cycle of 40-60 weeks.

Sources of risk

4.3 An association between use of poultry litter as feed or bedding and the occurrence of botulism in cattle was suggested in the 1970s. An outbreak which resulted in 4 cases in yearling cattle grazing pasture on which broiler litter had been spread was documented in 1985 (Clegg et al, 1985; Smart et al, 1987). Muscle from broiler carcases retrieved from the pasture was shown to have a high concentration of botulinum toxin. The association with broiler litter has since been reported in many different countries (Hogg et al, 1990; McLoughlin et al, 1988; Ortolani et al, 1997). Both DARDNI and the VLA have reported a descriptive association between clinical outbreaks of suspected or confirmed botulism in cattle and the stacking or spreading of poultry litter on affected or neighbouring farms (Annex 3). Poultry litter has previously been associated with a number of outbreaks of the condition when it has been used for cattle feeding, mixed in silage or used for bedding cattle. There has been a temporal association between the reduction in the use of antimicrobial growth promoters in broiler production in Europe and the increase cattle botulism associated with broiler litter, although there have been no reports of an increase in botulism in poultry in the UK.

4.4 There are several possible mechanisms whereby poultry litter could be associated with botulism in other farm animals.

- the presence of putrefied poultry carcases mixed in the litter. This risk is very low for birds over 200 grams in weight under good management – at least daily collection and removal of dead birds is normal practice. There is some unavoidable risk of loss of young chick carcases through their becoming buried in the litter between one collection and the next (particularly overnight). In contrast, where adult breeding birds or commercial layers are moved on to fresh litter at 16-22 weeks of age, carcases are very unlikely to be lost because of their relatively large body size;
- the presence of other carrion in the litter. Rodents are sometimes associated with poultry farms and are routinely controlled by appropriate baits to which the stock do not have access. Stacked poultry litter could also be attractive to rodents;
- spilled feed in the litter could undergo putrefaction in the absence of carcases;
- cattle could consume any associated carrion directly, particularly if they are phosphorus deficient or have access to the stacked material;
- wildlife might transport carrion material from the stacked material onto pasture; and
- fragments of carrion may be consumed along with grass or incorporated into conserved forage.

4.5 There is not sufficient information currently available to assess the relative importance of these and other risk mechanisms. In areas where much of the broiler litter is burned for power generation or where there is a significant area of arable land the risks are clearly going to be reduced. DARDNI and VLA have produced guidelines which aim to control these risks (Annexes 4 and 5).

4.6 Poultry Carcases. It is usual practice for all poultry flocks to be checked daily for the occurrence of mortality and any carcases are removed. Birds showing signs of suffering from injury or disease are culled on humane grounds. Carcases are disposed of in accordance with the Animal By-Products Regulations (2003) (paragraph 1.6 also refers). They may be disposed of in a licensed incinerator on the poultry site or removed for disposal by a third party. Depending on the numbers of carcases and system of disposal, carcases may be held for a period at ambient temperature or in a freezer. Carcases can also result by direct predation in free-range systems. Some cases of botulism appear to have been associated with the scavenging of poultry carcases by foxes and their removal to areas of pasture.

Conclusions

4.7 Where a source is identifiable in outbreaks of cattle botulism the most common one is carrion or putrefying material of some sort.

4.8 Litter from broiler chicken production (but not manure or other sorts of poultry litter) has commonly been associated with outbreaks of cattle botulism.

Recommendations

4.9 We recognise a need to reinforce DARDNI and VLA/DEFRA messages on the use and disposal of poultry litter (Annexes 4 and 5) and recommend that the FSA works closely with the poultry industry to ensure good practice in litter management and disposal, while recognising that practical solutions will need to take into account local factors such as availability of arable land or other means of disposal of litter. This advice should also be extended to cattle farmers.

4.10 FSA messages to broiler farmers with respect to biosecurity should be expanded to highlight the risks of disease transmission through deficient practices of carcase removal. Education of cattle farmers with respect to these risks is also recommended.

Chapter 5

Management of botulism outbreaks in cattle in the UK

Management of outbreaks

5.1 There is no statutory requirement to report an incident or outbreak of botulism in cattle in the UK, which is similar to other countries in the EU. The current advice given by the FSA in suspected outbreaks of botulism is to request a voluntary restriction on the movement of livestock and of milk from affected animals. In general, if only a few animals are affected then this restriction applies to the milk of affected animals only. In larger outbreaks the restriction is applied to milk from the herd. Likewise, the farmer is requested to cease selling animals off the farm or sending them to slaughter (FSA, 2004). This restricts use of milk and meat from animals for 14 days from the date of onset of the last clinical cases or 17 days from the removal of the source. No compensation is paid to the farmer.

5.2 When reported, suspected outbreaks of botulism are investigated by the Veterinary Laboratories Agency in England and Wales and the equivalent in Scotland and Northern Ireland at no charge to the farmer to ensure that reporting of suspect cases is not discouraged. In England and Wales toxin testing of animal tissues and serum is rarely carried out due to the perceived low sensitivity of detection.

Measures to prevent outbreaks

5.3 Various measures have been recommended to avoid outbreaks of botulism in bovine herds and most focus on the prevention of access to carcases of small animals including poultry (DARDNI, 2004, Anon, 2003a, Anon, 2002). Key recommended interventions include;

- Removal of broiler carcases from the poultry house
- Storage of carcase material in enclosed and sealed containers to prevent access by scavengers
- Preventing access of cattle to broiler litter
- Precluding grazing of cattle on pasture treated with broiler litter in the same year
- Avoiding the use of poultry litter as a bedding for cattle
- Preventing capture of carcase material in grass for silage (by raising the cutting blades or not using poultry litter on land for silage)
- Avoiding the use of common vehicles for transportation of animal fodder and poultry litter or animal carcases
- Collecting water from poultry houses in tanks rather than allowing flow into adjacent fields

5.4 At present no vaccine against botulism in cattle has a full product licence in the UK. As an exceptional intervention measure, subject to a Special Treatment Authorisation (STA), a vaccine can be imported to

protect cattle against toxin types C and D. This, in conjunction with other control measures, has been successful in Northern Ireland (DARDNI, 2004). Vaccination of cattle against types C and D botulism is reported to be common practice in drought stricken regions of Australia and South Africa where bovine botulism is more common as a result of increased access to carrion.

5.5 In the event of outbreaks, advice has been issued by the Irish veterinary service to farmers to aid investigation and reduce the impact (Anon 2003b);

- Remove animals from the location where exposure to toxin is believed to have occurred to a clean area
- Identify possible sources of the toxin (such as poultry litter, rotting carcases, water from poultry houses, maggots, dead wildlife, pastures, hoppers, troughs, waterways) for evidence of contamination.

Conclusions

5.6 Reporting of botulism outbreaks in cattle is conducted on a voluntary basis. This may result in under-reporting of the disease. However, clinically affected animals are likely to be investigated and based on the low apparent risk to humans presented by toxin types C and D, which predominate in recent UK cattle outbreaks, statutory notification does not currently appear to be merited.

5.7 Advice is available to farmers to prevent and manage outbreaks of bovine botulism (Annexes 4 and 5).

Recommendations

5.8 We recommend that UK veterinary authorities continue to encourage cattle farmers to report suspected cases of botulism in cattle.

5.9 If evidence emerges of other toxin types such as A, B and E causing outbreaks in UK cattle populations the question of making botulism in cattle notifiable should be reviewed.

Chapter 6

Risk to public health

Risk assessments conducted in France by AFSSA

6.1 Following an increase in cases and outbreaks of bovine botulism and, unlike the UK, the emergence of type E botulism in chickens in France, the French Food Safety Agency (AFSSA) set up a working party to evaluate the risk of transmission of *Clostridium botulinum* to humans from infected animals, in particular birds and cattle (Anon, 2002). Outbreaks of bovine botulism in four districts of France, where the disease was more frequently recognised, had increased significantly (circa 8-fold) in the mid 1990s, reaching a peak of 42 outbreaks in 1995 before declining to 12 outbreaks at the end of the reported period (2000).

6.2 The French report considered bovine botulism to be predominantly a toxico-infection and not intoxication due to pre-formed toxin. Evidence of haemorrhagic and necrotic enteritis lesions observed in the jejunum was cited in the French report as being indicative of toxico-infection. The routes of infection/contamination were considered to be from; carcases of small birds or animals in the environment of a poultry farm and being deposited in water sources as carrion; direct access to carcases of small birds and animals in poultry manure[1]; spreading of poultry manure on, or in the vicinity of pasture intended for cattle (due to spread of spores by wind or scattering of carcases by predators). A slight seasonal variation in outbreaks of bovine botulism was noted with peaks in the summer and autumn.

6.3 The qualitative risk assessment categorised the risk to public health from products of different animal populations into 5 groups; zero risk, negligible risk, low risk, moderate risk and high risk. The risk was assessed to an exposed individual and also to the population at large. In relation to bovine products, none of the sources was considered to present either a high or a moderate risk to the health of the population at large. The risk to an exposed individual was generally higher. Key findings were as follows;

- The AFSSA report appeared not to consider the presence of types C and D toxin as a significant risk because of the low apparent susceptibility of humans to these two toxins.

- Vegetative cells or spores on the carcases of cattle from a herd in which there were cases of botulism and where the meat was used in processed foods not subject to canning or bottling (taken to mean the application of a commercial sterilisation process) were considered a low risk at the population level and a moderate risk for

[1] As used in the context of the AFSSA report

exposed individuals. The risk for both target groups was considered negligible if the meat was for fresh consumption and zero if bottled or canned properly.

- Toxin presence did not appear to be considered a risk in fresh meat from cattle affected with botulism as clinically affected animals would not enter the food chain.

- Vegetative cells or spores in the milk of dairy cows from a herd showing signs of botulism and where the milk is used in processed foods not subject to canning or bottling were considered a low risk for the population and a moderate risk for exposed individuals. The risk for both groups was considered negligible if the milk underwent little or no processing or if it was dried, and zero if bottled.

- Toxin in milk from cows was not considered to be a risk because clinically affected cows do not normally produce milk or the milk could not be collected for practical or statutory reasons.

6.4 Notwithstanding the low population risk assigned to the categories above, the report recommended additional food safety measures. This involves rapid investigation of suspected outbreaks in animals to determine toxin type; milk from dairy herds to be suspended once a case of botulism is confirmed for a period of two weeks after the last confirmed case; suspend slaughter of cattle from herds once a case is confirmed of type A or B botulism for a period of two weeks after the last confirmed case. The report also comments on the need to conduct research into better diagnostic tools.

Transfer of botulinum toxins and/or vegetative cells/spores of *C. botulinum* to meat and milk

6.5 Cattle botulism, whatever the source, is only a risk to human health if toxins are present in an active form in foods at a concentration capable of causing ill effects, and the processing of such foods prior to consumption is inadequate to inactivate the toxins. If toxico-infection is considered a potentially significant occurrence in cattle, increased levels of vegetative cells or spores may be shed resulting in increased contamination on carcases or in milk due to cross contamination during slaughter and milking, respectively.

Meat

6.6 The progenitor toxin produced by the organism, whether produced in the environment, in a food, or in the intestine, is relatively stable and protected from degradation and digestion, though it is susceptible to heat treatment. In affected animals toxin is rapidly and irreversibly bound to the motor end plate receptors in muscle. There is a theoretical risk that free toxin from the gastrointestinal tract may have contaminated meat but no human cases have been attributed to this source.

6.7 Toxico-infection leading to increased spore loading of fresh meat would only present a potential risk in foods if the spores were not subject to a process that would destroy them, i.e. an $F_0$3 (121°C for 3 minutes) process, or where the organism has an opportunity to grow. The principal foods at risk would be low acid, chilled, ready to eat foods such as cooked meats. A number of *C. botulinum* types can grow under refrigeration, i.e. non-proteolytic types B, E and F. Increasing the initial loading of *C. botulinum* in foods capable of supporting their growth could reduce safety as unsafe levels would be reached earlier in the shelf life of the product. However, given that the types most commonly associated with bovine botulism are toxin types C and D, neither of which organisms are known to grow at refrigeration temperatures, the risk of elevated initial spore loadings would not be considered significant. Likewise, given the improvements made in recent years to improving slaughter hygiene, this is not considered a significant risk.

Milk

6.8 Milk and dairy products could potentially be exposed to contamination. There has been an outbreak of human botulism associated with yoghurt but this was due to contaminated hazelnut purée, not to disease in the milk-producing cows. There have been a few large-scale outbreaks of botulism in dairy cows in California which resulted in research on the toxicity for cattle and transmission of the toxin (type C) to milk. This work validated the mouse bioassay for the detection of active toxin in milk (by 'spiking' milk samples with toxin) and demonstrated that toxin was undetectable in milk from cows given doses around the lethal dose (the mean toxic dose was determined to be 0.4 ng/kg, making cattle 13 times more sensitive than mice on a weight for weight basis) (Moeller et al 2003). A single cow was given a very much higher dose (175 ng/kg) and no toxin was found in milk up to 12 hours post toxin administration (Moeller, 2000).

6.9 Lack of transmission of active toxin to milk may also be inferred from the lack of clinical cases in suckler calves in herds in which the cows are affected by botulism among the cases investigated by the State Veterinary Service in the UK. Böhnel et al (2005) also cited a number of papers reporting cases where toxin was not found in the milk of cows clinically affected by botulism. Böhnel et al (2005) did describe a single cow in which the milk of a single mastitic quarter had a high concentration of botulinum toxin. However, they attributed this to an infection of the udder by the organism, analogous to wound botulism.

6.10 The potential for toxico-infection leading to increased levels of vegetative cells or spores contaminating milk through poor milking parlour hygiene could lead to similar risks described already for meat. However, for similar reasons (i.e. types C and D being predominant and with improvements in dairy hygiene), the risk to milk products is considered low.

6.11 Milk collection usually results in the mixing and dilution of milk from many cows, so even in small dairies, the overall risk from toxin present from mastitic cows is considered low, although this will be higher in milk not subject to mixing (e.g. for on farm consumption).

6.12 Mastitis caused by *C. botulinum* is likely to be a rare occurrence (the Group identified only one report of a single suspected case in the literature, Bohnel et al, 2005) but it could potentially result in high levels of the organism being shed into milk. High initial loading could present an increased risk of the organism growing to unsafe levels in milk if stored for long periods or in pasteurised dairy products made from the milk, e.g. cheese, yoghurt or dairy dessert, if suitable conditions prevail. The risk presented by types C and D toxins is considered low, but if the toxin types were of significance to human infection this may present an increased risk.

Conclusions

6.13 Risk assessments have not identified a significant risk to the public from food associated with botulism in cattle. This is principally because the toxin types have rarely been associated with disease in man, and the disease in animals, cattle in particular, should be noticed quickly so that affected animals would be removed from the food supply chain.

6.14 The risk assessment conducted in France is of the opinion that toxico-infection is a much more common means by which animals become infected than is considered the case in the UK. If this is correct, and if toxin types that cause disease in man begin to emerge as significant causes of cattle botulism, especially toxin type A, B and E, then the risk to foods may need reassessing.

Recommendations

6.15 Laboratory evidence suggests that recent outbreaks in cattle in the UK are associated with toxin types C and D. We recommend that the risk should be re-assessed if other toxin types emerge.

6.16 A report from Germany (Bohnel et al 2005) of the identification of botulinum toxin in the milk of a single cow with mastitis, suspected to have been caused by *Clostridium botulinum*, is noted. Although the public health significance of this finding is unknown, consideration should be given to carrying out a small study on the presence of toxin in milk from cows with botulism, especially those with concurrent mastitis.

6.17 Clostridial spore numbers are known to increase in milk when cows are fed silage. Spores may be expected to increase if botulism results from toxico-infection (caused by spores) rather than intoxication (caused by preformed toxin). Therefore investigation into the presence of spores in milk from botulinum-affected cows should be considered (Driehuis et al, 2000).

Chapter 7
Public health advice

Introduction

7.1 Cattle botulism is only a risk to human health if toxins are present in an active form in milk or meat at a concentration capable of causing ill effects, and where the processing of foods intended for human consumption is not sufficient to inactivate the toxins.

7.2 In order to assess the potential risk to human health associated with botulism or suspected botulism in cattle, and to identify appropriate recommendations to support public health advice, the following issues, amongst others, have been considered elsewhere in this Report:

- Biological activity of toxins in humans and cattle;
- Toxin availability in meat and milk;
- Evidence for human cases associated with meat and milk consumption;
- Current public health advice

Biological activity of toxins in humans and cattle

7.3 As discussed in Chapter 2, and as reported by DARDNI (2004; see Chapter 3), botulinum toxin types C and D are most commonly associated with bovine botulism while toxin types A, B and E are most commonly associated with human disease.

7.4 Risk assessment work conducted in France (reported in Chapter 6) has not identified a significant risk to the public from food associated with botulism in cattle (Anon, 2002). This is because the toxin types identified in animals have rarely been associated with disease in man, and any disease occurring in animals including cattle would be noticed quickly and affected animals removed from the supply chain.

7.5 In view of the low apparent risk to humans presented by botulinum toxin types C and D (which predominate in animal outbreaks), statutory notification does not currently appear to be merited. However this would need to be reviewed if evidence emerges of toxin types A, B and E causing outbreaks in UK animal populations.

Toxin availability in meat and milk

Binding

7.6 Chapter 2 describes the pathogenesis of botulism in man. The progenitor toxin produced by *C. botulinum* (in the environment or in a food) is relatively stable and protected from degradation and digestion

by a protein coat. In affected animals, after absorption in its protected state, the toxin is cleaved into light and heavy chains. The heavy chain forms the basis of attachment at the receptor. The light chain forms the active component of the toxin and is rapidly irreversibly localised to motor end plate in muscle. Once bound, the toxin no longer possesses its protective coat or binding sub-unit, both of which are needed for the delivery of the toxin from a food source to the neuromuscular junction. Therefore it is unlikely that the toxin would be responsible for causing re-intoxication following consumption of meat from affected cattle.

Stability to heat treatment

7.7 The effect of heat on the destruction of botulinum toxin has been studied by a number of researchers and the data reviewed by Siegal (1993). No data are available for the destruction of toxin types C and D; most work has been conducted with types A, B, E and F. Destruction of the toxin is not linear and varies depending on the food in which it is present. Generally, temperatures in foods of 75°C or above will inactivate the toxin but this may take many minutes to achieve. Woodburn et al (1979) demonstrated that the time taken to reduce initial levels of type A toxin from 10^5 LD_{50} per g or ml to undetectable levels (by the mouse bioassay) in tomato soup at pH 4.2 was 15 minutes at 79°C and 5 minutes at 85°C. The toxin appeared most resistant to heat at pH values close to pH 5. Type E toxin was less heat resistance than types A and B.

7.8 It is likely that pasteurisation of milk (71.7°C for 15 seconds) would reduce toxin levels, but not eliminate the toxins; therefore pasteurisation should not be relied upon as a control for the toxin. Similarly, cooking of meat (70°C for 2 minutes) would reduce, but not eliminate, levels of toxin. Higher heat processes such as those applied to long-life chill products (90°C for 10 minutes), and ambient low acid products (121°C for 3 minutes) would destroy the toxin.

Dilution factors

7.9 Due to dilution of milk from affected animals with other milk, probably from unaffected herds, the risk of illness following consumption of milk and dairy products would be regarded as extremely low.

Evidence for human cases associated with meat and milk consumption

7.10 Chapter 6 reviews the risk to human health associated with the transfer of botulinum toxins and/or vegetative cells or spores of *C. botulinum* to meat and milk. There is little evidence to suggest that any clinical cases of botulism from consumption of milk have occurred.

7.11 From the evidence considered by the Group, a lack of transmission of active toxin to milk may also be inferred from the lack of clinical cases of botulism occurring in suckler calves in herds in which there were

cows affected by botulism. Similarly there have been no reports of fresh meat or milk from cattle affected by botulism having caused human disease.

7.12 Risk assessment work conducted in France (Anon, 2002) concluded that vegetative cells or spores on the carcases of cattle from a herd in which there were cases of botulism, and where the meat or milk were used in processed foods not subjected to canning or bottling, were considered low risk at the population level, and moderate risk for exposed individuals. The risk was considered negligible if the meat was for fresh consumption, or if the milk underwent little or no processing or if it was dried. If meat or milk were bottled or canned properly, the risk was considered to be zero for both the population and exposed individuals.

7.13 Furthermore, use of the risk assessment work conducted in France showed that toxin presence was not considered to be a risk in fresh meat derived from cattle affected with botulism as clinically affected animals would not enter the food chain. Similarly, toxin presence in milk from cows was not considered to be a risk because clinically affected cows did not normally produce milk. In addition collection and supply of the milk from sick animals was prohibited.

7.14 The Group considered that the presence of botulinum spores in meat and milk was more likely than the presence of active toxin in these food groups. If toxico-infection was considered a significant occurrence in cattle, then increased contamination via shedding of spores could arise, resulting in increased cross-contamination of meat during slaughter or milk during milking.

7.15 The Group concluded that the risks associated with toxin in meat and toxin in milk were low, for the reasons outlined in paragraphs 7.6–7.9. Therefore the likelihood of active toxin entering meat or milk was low, although the possibility of cross-contamination from environmental spores was a consideration.

Current public health advice

7.16 As outlined in Chapter 5, there is no statutory requirement to report an incident or outbreak of botulism in cattle in the UK, which is similar to the situation in other EU Member States. The FSA currently requests the voluntary restriction of movement of livestock and milk from affected animals once a case of botulism in cattle is suspected. This restricts use of milk and meat from animals for 14 days from the last clinical case or 17 days from removal of the source of the infection. Where only one or two animals are affected, milk from affected cows is prohibited from entering the food chain. No recall of milk or milk products derived from animals before the onset of clinical disease is sought. No compensation is paid to the farmer. Advice is available to farmers to prevent and manage outbreaks of bovine botulism.

7.17 Botulinum toxin is usually undetectable in serum from clinically affected cows (Chapter 3 refers). Therefore the highest risk of toxin being transferred into meat or milk may be soon after the animal's exposure, before the activated toxin has become irreversibly bound to the motor end plates in muscles, before any clinical signs have been observed, and before the toxins have had time to degrade.

Conclusions

7.18 In view of the low apparent risk to humans presented by botulinum toxin types C and D (which predominate in animal outbreaks) statutory notification does not currently appear to be merited. Although voluntary reporting of botulism outbreaks in cattle may result in under-reporting of the disease, clinically affected animals are likely to be investigated.

7.19 Voluntary restrictions on meat and milk from clinically affected cattle appear appropriate but restrictions applied to unaffected animals could be considered over-cautious. However this would need to be reviewed if evidence emerges that toxin types such as A, B and E (more commonly associated with humans) were causing outbreaks in UK animal populations.

Recommendations

7.20 From the evidence presented to the Group, we recommend that, in the absence of other signs, there should be no requirement to restrict sales of milk from clinically healthy cattle from farms where there have been clinically suspected cases of botulism in cattle.

7.21 Only animals that are healthy should be sent for slaughter for human consumption and therefore any clinically affected animals should not pass *ante mortem* meat inspection. We recommend that there should be no requirement to restrict the slaughter of healthy cattle from herds where cases of confirmed or suspected botulism have occurred, but that meat and milk from clinically affected animals should not enter the food chain due to concern that this may pose a risk to consumers.

7.22 It would be worthwhile to undertake a small study on the stability of toxin activity in milk, for native and proteolytically activated toxin types A-E, with and without pasteurisation.

Chapter 8

Conclusions and recommendations

C. botulinum and botulism

8.1 Botulinum toxins are a serologically diverse group of potent neurotoxins that inhibit the transmission of nerve impulses at neuromuscular junctions, resulting in flaccid paralysis. The process of delivery of the toxin from a food source to the neuromuscular junction involves (a) associated non-toxic proteins that are believed to protect it during passage through the stomach, and (b) a binding subunit that is required for internalisation of the active toxic subunit into the synaptic vesicles of peripheral neurones. Although the active toxin is stable at its target site, it is highly unlikely that in this form it could be responsible for another round of intoxication since by this time it lacks both the protective proteins and the binding subunit.

Epidemiology and diagnosis of botulism in cattle

8.2 Botulism can cause serious disease in cattle for the production of beef and milk. The proportion of animals affected is very variable. The classical clinical signs relate to varying degrees of paralysis of muscles. Diagnosis is difficult and usually made on the basis of the clinical signs and the identification of a likely source of toxin. There are no characteristic lesions at post-mortem examination. Each of the existing tests for the detection of botulinum toxins has disadvantages.

8.3 While clinical diagnosis supported by exclusion of other likely causes is satisfactory for identification and clinical management of single cases and large outbreaks, it is not an adequate basis for the implementation of food safety precautions such as the exclusion of animal products from the food chain.

8.4 **In outbreaks of clinically suspected cases of botulism in cattle we recommend that the mouse bioassay be applied to gastrointestinal samples in order to provide an aid to diagnoses and to help assess risk by determining whether the toxin types involved are those that have been associated with botulism in humans (types A, B & E).**

8.5 **Work should be undertaken to understand the diagnostic and clinical significance of finding botulinum toxins in gastrointestinal contents of cattle.**

8.6 **Because of concerns over the use of live mice for the bioassay, work should be undertaken to develop new highly sensitive and specific diagnostic tests that do not use animals for the detection of *C. botulinum* toxins and organisms in biological matrices**.

8.7 **Samples collected during clinical investigations should be archived to assist with the development of further assay systems.**

Poultry waste

8.8 Where a source is identifiable in outbreaks of cattle botulism the most common one is carrion or putrefying material of some sort.

8.9 Litter from broiler chicken production (but not manure or other sorts of poultry litter) has commonly been associated with outbreaks of cattle botulism.

8.10 **We recognise a need to reinforce DARDNI and VLA/DEFRA messages on the use and disposal of poultry litter (Annexes 4 and 5) and recommend that the FSA works closely with the poultry industry to ensure good practice in litter management and disposal, while recognising that practical solutions will need to take into account local factors such as availability of arable land or other means of disposal of litter. This advice should be extended to cattle farmers.**

8.11 **FSA messages to broiler farmers with respect to biosecurity should be expanded to highlight the risks of disease transmission through deficient practices of carcase removal. Education of cattle farmers with respect to these risks is also recommended.**

Management of botulism outbreaks in cattle in the UK

8.12 Reporting of botulism outbreaks in cattle is conducted on a voluntary basis. This may result in under-reporting of the disease. However, clinically affected animals are likely to be investigated and based on the low apparent risk to humans presented by toxin types C and D, which predominate in recent UK cattle outbreaks, statutory notification does not currently appear to be merited.

8.13 Advice is available to farmers to prevent and manage outbreaks of bovine botulism (Annexes 4 and 5).

8.14 **We recommend that UK veterinary authorities continue to encourage cattle farmers to report suspected cases of botulism in cattle.**

8.15 **If evidence emerges of other toxin types such as A, B and E causing outbreaks in UK cattle populations the question of making botulism in cattle notifiable should be reviewed.**

Risk to public health

8.16 Risk assessments have not identified a significant risk to the public from food associated with botulism in cattle. This is principally because the toxin types have rarely been associated with disease in man, and

the disease in animals, cattle in particular, should be noticed quickly so that affected animals would be removed from the food supply chain.

8.17 The risk assessment conducted in France is of the opinion that toxico-infection is a much more common means by which animals become infected than is considered the case in the UK. If this is correct, and if toxin types that cause disease in man begin to emerge as significant causes of cattle botulism, especially toxin type A, B and E, then the risk to foods may need reassessing.

8.18 **Laboratory evidence suggests that recent outbreaks in cattle in the UK are associated with toxin types C and D. We recommend that the risk should be re-assessed if other toxin types emerge.**

8.19 A report from Germany (Bohnel et al 2005) of the identification of botulinum toxin in the milk of a single cow with mastitis, suspected to have been caused by *Clostridium botulinum*, is noted. Although the public health significance of this finding is unknown, consideration should be given to carrying out a small study on the presence of toxin in milk from cows with botulism, especially those with concurrent mastitis.

8.20 **Clostridial spore numbers are known to increase in milk when cows are fed silage. Spores may be expected to increase if botulism results from toxico-infection (caused by spores) rather than intoxication (caused by preformed toxin). Therefore investigation into the presence of spores in milk from botulinum-affected cows should be considered (Driehuis et al, 2000).**

Public Health advice

8.21 In view of the low apparent risk to humans presented by botulinum toxin types C and D (which predominate in animal outbreaks) statutory notification did not currently appear to be merited. Although voluntary reporting of botulism outbreaks in cattle may result in under-reporting of the disease, clinically affected animals are likely to be investigated.

8.22 Voluntary restrictions on meat and milk from clinically affected cattle appear appropriate but restrictions applied to unaffected animals could be considered over-cautious. However this would need to be reviewed of evidence emerges that toxin types such as A, B and E (more commonly associated with humans), were causing outbreaks in UK animal populations.

8.23 **From the evidence presented to the Group, we recommend that, in the absence of other signs, there should be no requirement to restrict sales of milk from clinically healthy cattle from farms where there have been clinically suspected cases of botulism in cattle.**

8.24 **Only animals that are healthy should be sent for slaughter for human consumption and therefore any clinically affected animals should not pass *ante mortem* meat inspection. We recommend that there should be no requirement to restrict the slaughter of healthy cattle from herds where cases of confirmed or suspected botulism have occurred, but that meat and milk from clinically affected animals should not enter the food chain due to concern that this may pose a risk to consumers.**

8.25 **It would be worthwhile to undertake a small study on the stability of toxin activity in milk, for native and proteolytically activated toxin types A-E, with and without pasteurisation.**

ANNEX 1

AD HOC GROUP ON BOTULISM IN CATTLE

Terms of reference

To consider the potential human health risk associated with botulism or suspected botulism in cattle, particularly in relation to the spreading of poultry litter on agricultural land. To report back with recommendations to the ACMSF.

List of Membership

Chair
Professor W J Reilly

Members
Dr M Brett
Mr A Kyriakides
Ms E Lewis
Mr P McMullin
Mr P Mepham
Professor P Williams

Assessors
Mr P Gayford (DEFRA)
Dr S Kennedy (DARDNI)

Secretariat
Dr Lucy Foster (Administrative Secretary)
Dr Jo Aish (Scientific Secretary)
Mrs L Stretton (Secretariat)

ANNEX 2

List of organisations who assisted the *ad hoc* Group

Presentations

Robert Hogg, Veterinary Laboratories Agency, Preston
Chris Livesley, Veterinary Laboratories Agency, Weybridge
Richard Sharpe, Veterinary Laboratories Agency, Penrith
Dr Seamus Kennedy, Department of Agriculture and Rural Development in Northern Ireland
Dr Cliffe Shone, Health Protection Agency

Annex 3

Table 1: Summary of botulism in cattle outbreaks (England and Wales 2003-2005)[2]

Incident No.[1]	No. Clinical Cases[2]	Herd Size	Enterprise	Age	Related Outbreaks	Previous Outbreak on Farm	Suspected Source	Exposure	How Exposed
2003/01	7	300	Dairy	>24 months			Poultry Litter	Direct	Stacked
2003/05			Beef	13 - 24 months		Y	Poultry Litter	Direct	Broiler unit on farm
2003/16	10	50	Beef	13 - 24 months			Poultry Litter	Direct	Spread on fields
2003/23	3	15	Beef	13 - 24 months			None identified	Not Known	
2003/26	3	18	Beef	13 - 24 months			Poultry Litter	Indirect	Used on neighbouring farm
2003/37	17	49	Dairy	13 - 24 months	2003/43, 2003/44, 2003/45		Poultry Litter	Direct	Stacked/spread on fields
2003/42	2	40	Beef	13 - 24 months	2003/46		Poultry Litter	Direct	Stacked in field
2003/43			Beef	> 24 months	2003/37, 2003/44, 2003/45		Poultry Litter	Indirect	Spread on neighbouring farm
2003/44			Beef	13 - 24 months	2003/37, 2003/43, 2003/45		Poultry Litter	Indirect	Spread on neighbouring farm
2003/45			Beef	> 24 months	2003/37, 2003/43, 2003/44		Poultry Litter	Indirect	Spread on neighbouring farm
2003/46	1	8	Beef	13 - 24 months	2003/42		Poultry Litter	Direct	Stacked in field
2003/47	2		Dairy	> 24 months			Poultry Litter	Direct	Used as bedding
2003/50	7	30	Beef	13 - 24 months			Poultry Litter	Indirect	Spread on adjacent field
2003/51	5	70	Dairy	> 24 months		Y	Poultry Litter	Direct	Poultry litter spread on silage field
2003/56	2	48	Dairy	> 24 months	2003/59		Poultry Litter	Indirect	Spread on adjacent field
2003/57	16		Beef	7 - 12 months			Vegetable Waste	Direct	
2003/59	3	16	Beef	> 24 months	2003/56		Poultry Litter		
2003/60	4		Beef	> 24 months			Poultry Litter	Indirect	Adjacent to farm with botulism

[2] Data on toxin types not reported

Incident No.[1]	No. Clinical Cases[2]	Herd Size	Enterprise	Age	Related Outbreaks	Previous Outbreak on Farm	Suspected Source	Exposure	How Exposed
2003/65	16	29	Dairy	13 - 24 months			Poultry Litter	Direct	Stacked in field
2003/72	1	45	Beef	13 - 24 months			Poultry Litter	Indirect	Stacked next to housing
2004/02	1		Beef	13 - 24 months			Poultry Litter	Indirect	Stacked in adjacent field
2004/05	3	31	Beef	> 24 months			Poultry Litter	Direct	Used as bedding
2004/30	5		Dairy	> 24 months	2004/31, 2004/35		Poultry Litter	Indirect	Spread on adjacent field
2004/31	2		Beef	> 24 months	2004/30, 2004/35		Poultry Litter	Direct	Stacked in field
2004/35	3		Beef	13 - 24 months	2004/30, 2004/31		Poultry Litter	Direct	Stacked in field
2004/39	8	106	Beef	13 - 24 months			Poultry Litter	Indirect	Adjacent to Poultry Farm/Litter stack
2004/46	2	20	Beef	13 - 24 months			Poultry Litter	Indirect	Stacked & spread on adjacent field
2004/47		11	Beef	13 - 24 months	2004/53		Poultry Litter	Indirect	Spread on adjacent field
2004/49	15	34	Beef	> 24 months			Poultry Litter	Direct	Stacked in field
2004/50		35	Beef	13 - 24 months			Poultry Litter	Direct	Spread on fields
2004/53			Dairy	> 24 months	2004/47		Poultry Litter	Indirect	Adjacent to poultry farm
2004/57	1		Beef	> 24 months			Pheasant carcases	Direct	In feed store/troughs
2004/58	4	24	Dairy	7 - 12 months			Not Established	Not known	
2004/60	2		Beef	13 - 24 months			Poultry Litter	Indirect	Stacked in adjacent field
2004/62			Beef	> 24 months			Poultry Litter	Direct	Stacked in field
2004/66	11		Dairy	13 - 24 months			Poultry Litter	Direct	Spread on Pasture
2004/73	4		Dairy	13 - 24 months			Poultry Litter	Indirect	
2004/75	2	40	Beef	7 - 12 months			None identified		
2004/77	2	39	Dairy	13 - 24 months			Chicken carcases	Direct	
2004/78	9	400	Beef	> 24 months			Bread Waste		
2005/02	8	50	Dairy	13 - 24 months			Poultry Litter	Indirect	Broiler unit on farm
2005/06	4	70	Dairy	> 24 months			Fox carcase	Direct	

Incident No.[1]	No. Clinical Cases[2]	Herd Size	Enterprise	Age	Related Outbreaks	Previous Outbreak on Farm	Suspected Source	Exposure	How Exposed
2005/15	5	70	Dairy	> 24 months	2005/58, 2005/61		Poultry Litter	Indirect	Stacked on neighbouring farm
2005/24	2	15	Beef	13 - 24 months			Poultry Litter	Direct	Spread on pasture cattle were grazing
2005/26	4	14	Beef	13 - 24 months			Poultry Litter	Direct	Spread on pasture cattle were grazing
2005/32	3	13	Beef	> 24 months			Silage		
2005/49	2	28	Beef	13 - 24 months		Y	Poultry Litter	Indirect	Spread in adjacent arable field
2005/50	5	22	Dairy	7 - 12 months			Poultry Litter	Direct	Stacked in field where cattle grazing
2005/58	2	48	Dairy	> 24 months	2005/15, 2005/61		Poultry Litter	Indirect	Stacked on neighbouring Farm
2005/60	2		Dairy	> 24 months			Pheasant carcases	Direct	
2005/61	5	34	Dairy	13 - 24 months	2005/15, 2005/58		Poultry Litter	Indirect	Stacked on neighbour's field
2005/62	6		Beef	> 24 months			Poultry Litter	Indirect	Spread on arable land
2005/65	3		Dairy	> 24 months			Poultry Litter	Indirect	Spread on neighbour's field
2005/66			Dairy	7 - 12 months			Poultry Litter	Indirect	Spread on Neighbour's Field
2005/67			Dairy	13 - 24 months			Poultry Litter	Indirect	Spread on Neighbour's Farm

[1] VLA incident number. Incident numbers not referred to did not involve botulism
[2] Approximate only, as the number of cattle affected may have increased after investigation of farm.

ANNEX 4

VLA Guidance on methods for the control of botulism in cattle

RECENT ASSOCIATION OF CATTLE BOTULISM WITH POULTRY LITTER; IMPLICATIONS FOR FOOD SAFETY AND INFRINGEMENT OF THE ANIMAL BY-PRODUCTS REGULATIONS 2003.

The Veterinary Laboratories Agency (VLA) recommends that farmers:

- Ensure that poultry litter recycled into agricultural land contains no poultry carcases or carcase material.
- Dispose of poultry carcases and carcase material by incineration, in accordance with the Animal By-Products Regulations 2003.
- Cease using poultry litter as fertiliser on the surface of grazing land or land used for conserving hay or silage.
- Dispose of poultry litter by incineration, deep ploughing or burial.
- Do not use facilities or equipment used for poultry litter transport and disposal for storing, mixing or distributing feeding stuffs.
- Prevent access of vermin or livestock to stored litter.
- Observe good personal hygiene precautions when handling litter because poultry litter may contain a range of human pathogens.

Emerging disease

VLA and The Department of Agriculture for Northern Ireland have independently identified a recent increase in the incidence of cattle botulism. There is strong circumstantial evidence that litter from deep-litter broiler houses is the source of infection in the majority of recent outbreaks.

Cause of botulism.

Botulism is caused by either an intoxication with toxins produced by the bacterium *Clostridium botulinum* or by an enteric infection with *Clostridium botulinum* (toxicoinfection).

Poultry litter may be a source of *Clostridium botulinum* organisms, spores or toxins following enteric colonisation of poultry with *Clostridium botulinum*. This may present as poultry botulism or may be subclinical. The source of clostridial toxins spores or organisms in poultry litter may be carcase material, litter or faecal material.

Cattle botulism has occurred when poultry litter has been used as a feed supplement, as bedding for housed cattle, as a fertiliser on grazing land or has been stored in fields where cattle are grazing.

Symptoms of botulism.

Cattle botulism usually presents as a progressive flaccid paralysis, which begins as an unsteady gait and develops over a period of several hours up to several days to recumbency and death. Some animals are found already dead or recumbent when they had been observed apparently normal a few hours earlier. A small proportion of animals may recover.

Poultry also present symptoms of progressive flaccid paralysis.

Confirmation of diagnosis.
Tests available for confirming botulism are unsatisfactory. VLA usually diagnoses cattle botulism from the presence of typical clinical symptoms and rarely confirms cattle botulism in the laboratory. Other diseases such as milk fever, bacterial and viral encephalites may present with similar symptoms to botulism and should be eliminated by autopsy and laboratory examinations when botulism is suspected.

Food safety precautions. When *suspected* botulism is diagnosed in livestock the Food Standards Agency require that no products enter the food chain from animals in the affected group for a period of two weeks after diagnosis of the last suspected clinical case of suspected botulism. This action may have very severe financial implications for farmers.
Given these controls, the ecological and economic advantages of recycling poultry litter as fertiliser are probably outweighed by the potential economic losses.

Animal By-Products Regulations 2003; disposal of poultry carcases.
Litter contamination with carcases occurs unless dead birds are identified and removed immediately. Carcases are quickly buried in litter and disintegrate into fragments.
The disposal of poultry carcases, material from disintegrated carcases or minced carcases, in litter spread on agricultural land is illegal under the Animal By-Products Regulations 2003. If VLA detects carcase material in litter they are obliged to report the incident to the Local Authority who enforce the Animal By-Products Regulations.
Local Authorities have the right of access to farms to enforce these Regulations.
Intentional disposal of carcase material in litter spread as fertiliser would be a very serious offence

A letter containing this advice was published by VLA in the Veterinary Record:

Livesey C.T. Sharpe R.T., and Hogg R.A., 2004. Recent association of cattle botulism with poultry litter. Vet Rec 154, 23, 734-735.

ANNEX 5

DARDNI Guidance on methods for the control of botulism in cattle

Background:-

Botulism has emerged as a threat to cattle in Northern Ireland in recent years. The disease is caused by toxins produced by bacteria called *Clostridium botulinum.* These bacteria are commonly found in the environment and the toxin is produced in decaying carcases and vegetable matter.

Investigations provide circumstantial evidence that broiler litter is a risk factor for many current outbreaks of botulism in cattle. The presence of the carcases of birds that have died during production is regarded as the likely source of toxin. It is speculated that even small fragments of carcases transferred onto pasture by scavenger animals, such as foxes, dogs or crows, can pose a risk to grazing cattle.

We have no evidence to suggest that exposure to hen manure e.g. from laying birds (as opposed to deep litter) presents any risk of botulism to cattle.

Scavengers may gain access to this material after it has been stacked outside or spread on pasture. Therefore, poultry litter should not be accessible to dogs, foxes, crows or other scavengers that may carry carcasses onto adjacent pasture or into cattle housing.

If you suspect botulism in your cattle:-

- Contact your veterinary surgeon as soon as possible;

- Remove the affected group of cattle to alternative grazing/housing as soon as possible

What are the clinical signs:-

Botulism causes muscle paralysis and can affect cattle of all ages.

The clinical signs include:-

- *lack of muscle tone resulting in progressive flaccid paralysis.*
- *muscle tremors, inco-ordination, hind limb stiffness, reluctance to move.*
- *muscle weakness, first in the hindquarters, then progressing to the forequarters, head and neck.*
- *animals may lie on their chest with the head turned towards the flank (similar to cows with "milk fever").*
- *inability to chew or swallow and drooling of saliva*
- *protrusion of the tongue*
- ***sudden death**.*

Protecting your herd:-
Treatment of botulism in cattle is rarely successful. It is therefore better to prevent the disease occurring. There are two important ways in which you can reduce the chances of an outbreak of botulism in your cattle:-

- **Prevent access to contaminated feedstuff and bedding,**

- **Where there is an unavoidable chance of exposure to broiler litter, vaccinate against botulism, preferably prior to turnout.**

1. Contaminated Forage

Grazing - Spreading of poultry litter on pasture cannot be recommended. If it must be spread, animals should not be allowed onto that pasture until at least the following grazing season. This is because fragments of carcasses may persist on pasture for a considerable time. If poultry litter must be spread, it should be deep-ploughed into arable ground. It should not be spread on a windy day in order to prevent contamination of adjacent fields. Any animal or bird carcases, or portions of carcasses, visible on pasture or in cattle houses, should be promptly removed. Even small fragments of carcases may be dangerous to cattle and should be disposed of carefully by incineration or rendering, as required by EU Regulation No. 1774/2002.

We have no evidence to suggest that spreading hen manure e.g. from laying birds (as opposed to litter) presents any risk of botulism to cattle.

Silage - If litter has been spread on silage ground, it is advisable to raise the cutting blades so that the grass is cut less close to the ground. This will reduce the risk of decaying matter being included in the silage cut.

Water - Washings from poultry houses and yards should be collected in tanks rather than be allowed to flow onto adjacent land.

Contaminated bedding **-** Do not use litter, or sawdust or shavings that may have had contact with broilers, as bedding for cattle.

2. Vaccination:-

An effective vaccine against botulism in cattle can now be obtained through your veterinary surgeon. A DARD survey of veterinary practitioners indicates that this vaccine has been successful in helping to control botulism in cattle in Northern Ireland. This vaccine may only be obtained by your vet through a special treatment authorisation procedure. This process takes about a week. It is important to remember that two doses of vaccine are required at an interval of 4 to 6 weeks. To be effective, the vaccine must be given prior to exposure to the disease risk. Cattle receiving only one dose may not be fully protected.

It is important to remember that vaccination should not be used as a substitute for preventing exposure of cattle to broiler litter that may contain carcass material.

REMEMBER –
Prevention is better than cure!

Protecting Public Health:-
There are seven different botulism toxins. The toxins usually associated with botulism in cattle rarely, if ever, affect humans. However, where the disease is suspected on a farm, the Food Standards Agency (FSA) in Northern Ireland requests that all milk and meat from the affected group of animals be withheld from sale for human consumption for a period of 14 days after the last case.

Further information and advice may be obtained from DARD's Veterinary laboratories at Belfast and Omagh by telephoning (028) 9052 5701 or ***(028) 8224 3337***

List of Tables and Figures

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Glossary of Terms

Broiler	A young chicken raised for meat.
Clostridium botulinum	A Gram-positive, spore-forming, neurotoxin-producing, obligate anaerobic bacterium. Associated with infant, wound and foodborne botulism.
Motor end plate	The flattened end of a motor neuron (nerve cell) that transmits nerve impulses to a muscle.
Mouse bioassay	Method for detection of botulism in which a sample is injected into selected strains of laboratory mice from which the total toxicity of the sample is assessed.
Neurotoxin	A substance that damages nerves or nerve tissue.
Neuromuscular junction	The junction between a nerve and a muscle fibre.
Neurotransmitter	Chemical substance which relays information from one nerve cell to another or from nerve cells to muscles.
Pasteurisation	A heat process designed to destroy pathogenic microorganisms. For example, milk is pasteurised at 71.7^{o}C for 15 seconds or using different time and temperature combinations to obtain an equivalent effect. Different pasteurisation processes may be used for other foods. In the cooking of burgers recommended guidelines suggests a heat treatment of 70^{o}C for 2 minutes or equivalent
Poultry litter	A mixture of bedding material (wood shavings, chopped straw, shredded paper) and faeces and urine of commercially reared poultry.
Sudden Death	Routinely monitored animal found dead without obvious cause of death and without clinical signs of disease being noted previously

Glossary of Abbreviations

AFSSA	French Food Safety Authority
ANTPs	Associated non-toxic proteins
DARDNI	Department of Agriculture and Rural Development in Northern Ireland
DEFRA	Department for Environment, Food and Rural Affairs
ELISA	Enzyme-Linked Immunosorbent Assay
SAC	Scottish Agricultural College
VLA	Veterinary Laboratories Agency

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