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Poultry waste associated type C botulism in cattle

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SUMMARY

Botulism in UK cattle has been confirmed by demonstrating type C botulinum toxin in sera from affected animals. Evidence is presented indicating the source of intoxication to be poultry carcasses containing type C *Clostridium botulinum* and its toxin. The organism was also found in poultry litter and in alimentary tract samples from slaughtered animals. The implications of these findings are discussed.

INTRODUCTION

Botulism is a well-recognized disease condition that affects many avian and mammalian species. In the UK all confirmed outbreaks of botulism affecting intensively farmed poultry have been caused by type C *Clostridium botulinum* intoxication (Blandford & Roberts, 1970; Smart & Roberts, 1977*a*; Smart, Laing & Winkler 1983). The underlying reasons why type C *Cl. botulinum* is the sole causal agent in naturally occurring outbreaks are not known. Botulism can be induced in broiler chicken by the peroral administration of spores of not only type C but also types A and D *Cl. botulinum*, the site of growth, toxin production and absorption of toxin being the caecum (Miyazaki & Sakaguchi, 1978). Prior to these studies much circumstantial evidence supporting *in vivo* toxin production was found during investigations of major outbreaks of type C poultry botulism. While *Cl. botulinum* type C was rarely found in fresh litter or feed, high numbers were found in soiled litter and voided faecal material. Despite diligent search, no source of toxin was found that could account for the high mortalities (Roberts & Collings, 1973; Smart & Roberts, 1977*a*; Smart, Laing & Winkler 1983). The potential for abnormally high numbers of type C *Cl. botulinum* in litter and in carcasses from broiler houses and the possibility that carcasses might also contain type C toxin was thus established and the hazard such contaminated material posed for other animals recognized.

Suspected bovine botulism has been reported in the UK and confirmed in many other countries (Smart & Roberts, 1977*b*). In recent years several outbreaks of suspected botulism involving cattle grazing areas on which poultry litter and carcasses had been spread or heaped have been investigated by one of the authors (J.L.S.) in collaboration with the Veterinary Investigation Service. Attempts to demonstrate the suspected relationship between the observed paralytic condition

in cattle and poultry waste however were not successful. One such incident has been described and the causal association between the observed disease condition and poultry waste claimed without proof that the disease condition was indeed botulism or that the poultry waste contained *Cl. botulinum* and or its toxin (Appleyard & Mollinson, 1985).

In this report we describe an incident in cattle and present results of a laboratory study that confirms the observed disease condition as botulism, strongly suggests an association with poultry waste and highlights the care necessary for its safe disposal. A letter briefly describing this outbreak was published previously by Clegg *et al.* (1985). Two other confirmed outbreaks of type C botulism in cattle have since been reported in which poultry litter and carcasses were available to the affected animals (Schofield, 1985; Gibson, 1986).

DESCRIPTION OF OUTBREAK

Poultry litter consisting of poultry excreta, wood shavings and the occasional broiler carcass were purchased by a farmer in January 1985 and stacked in his farm yard until mid March. Heating of stacked material occurred. It was spread over most of 10 hectares of pasture as a manure where 24 cattle aged 1–7 years were grazing. Big bale silage was being fed to these animals and the bales were opened and spread on part of this manured area. It seems likely that contamination of silage with poultry manure occurred. No predilection for poultry manure was noted by the farmer and though the pasture was bare, pica was not evident.

The affected animals were yearlings of both sexes. Symptoms were observed in the first yearling affected 24 March and this case and two subsequent cases became recumbent. Four others showed milder signs indicative of botulism. Of the three recumbent animals, two were slaughtered and one stood again after about 3 weeks, then slowly recovered. The other four affected but non-recumbent animals recovered, although their condition remained poor for several weeks. Two dogs from a neighbouring farm had access to the broiler litter and 'went off their legs' for a period of 10 days but recovered. Broiler litter had been purchased by neighbouring farmers from the same poultry farm, and stacked in some cases, but there have been no outbreaks of disease on their farms.

The cattle were removed from the pasture early in April, and returned 38 days later when good grass growth had occurred. Fourteen days later, a heifer developed symptoms of botulism. Careful search of the pasture did not reveal poultry remains. There were a few areas where poultry litter had been spilled in fair quantities at the time of spreading in March, and samples of this old litter, and grass growing at the edge of these areas were collected for testing for the presence of *Cl. botulinum* and its toxins. The cattle were again moved, and crops of hay and big bale silage taken. The field was kept empty until sheep were moved on during October and cattle in early November. There have been no cases of botulism since the case which occurred in June 1985.

The clinical signs of botulism in cattle have been described by Blood, Radostits & Henderson (1984). In this outbreak the unusual features noted were congestion of the jugular veins in recumbent animals and 'hooding' of the eyelids; this latter sign was also described by Davies *et al.* (1974). A marked feature in animals affected

Table 1. *Poultry waste associated type C botulism in cattle. Sequence of events*

Date	
1984	
Autumn	24 Cattle, 9 months to 7 years old, introduced onto 10 hectares pasture
Winter	Supplementary feeding at pasture, of big bale silage, plus 4 lb per head of mixed barley, rolled oats, beet pulp and dried brewers' grains.
1985	
January	Broiler litter (excreta, wood shavings, and a few carcasses) purchased and stacked in farm yard.
11–14 March	Broiler litter spread over pasture.
24 March	Yearling noted abnormal by farmer.
30 March	Yearling seen by veterinarian. Total of 7 become affected.
4 April	Yearling blood sampled for botulinum toxin tests.
6 April	Four yearlings, 1, 2, 3 and 4, blood sampled. Carcasses of 2 broilers collected from pasture. Cattle turned off pasture.
10 April	Animal 3 slaughtered. Samples collected.
19 April	Animal 1 slaughtered. Samples collected.
23 April	Animal 4 stood for first time since 11.4.86.
14 May	Cattle returned to pasture.
28 May	Heifer 5 develops botulism.
3 June	Heifer 5 blood sampled. Litter and grass samples collected from the pasture.
3 June	Heifer 5 sent for slaughter.
Summer	Hay and big bale silage crop taken from pasture.
October	Sheep turned onto pasture – no botulism.
November	Cattle turned onto pasture – no botulism.

in spring was constipation, which showed little response to therapy with liquid paraffin. Recumbent animals occasionally looked at their abdomens, suggesting a source of pain, presumably related to the impaction. Hanging out of the tongue was not observed although retraction of the tongue was slow after manual interference. Reflexes in affected animals were slowed.

The sequence of events in this outbreak is shown in Table 1.

SAMPLING AND LABORATORY PROCEDURES

Blood samples were not treated uniformly after collection in that some sera were stored at 4 °C for 48 h before being deep-frozen at −20 °C, others were frozen to −20 °C immediately after being decanted from clot and one from the last animal affected was at ambient temperature when received by post at the Veterinary Investigation Centre (V.I.C). Ideally sera should be deep-frozen as soon as possible after collection of blood samples to stabilize any botulinum toxin present. The various samples from animal 3 were deep frozen about 4 h after slaughter and those from animal 1 were collected onto ice at −20 °C for transportation to the V.I.C. for immediate deep freezing. Poultry carcasses were deep frozen within 2 h of collection and broiler litter and grass samples within 15 min. All samples received at the Institute of Food Research, Bristol Laboratory from the V.I.C. arrived either frozen or chilled. It is important to store samples in which *Cl. botulinum* type C might be present at temperatures below the organism's minimum growth

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Table 2. *Detection of* Cl. botulinum *type C toxin*

Sample	Toxin – mouse lethal doses
Chicken 1 muscle extract	*c.* 250/g
Chicken 2 muscle extract	*c.* 25000/g
Yearling 1 serum, 4 April*	< 1/ml
Yearling 1 serum, 6 April	2–10/ml
Yearling 2 serum, 6 April	2–10/ml
Yearling 3 serum, 6 April	20–50/ml
Yearling 3 serum, slaughtered 10 April	<1/ml
Yearling 4 serum, 6 April	20/ml
Yearling 1 serum, slaughtered 19 April	1–2/ml
Heifer 5 serum, 28 May*	1/ml
Heifer 5 serum, slaughtered 3 June	8/ml

* Specimens posted to laboratory in non-insulated containers. Some deterioration of toxin likely.

Extracts of alimentary tract contents from slaughtered animals and grass samples were toxin negative.

temperature (*c.* 12 °C) to ensure that any toxin subsequently detected was not formed between sample collection and testing. Bovine sera, extracts and enrichments of muscle from broiler chicken carcasses, bovine alimentary tract contents, litter and grass samples were tested for botulinum toxin by mouse bioassay. Toxin neutralization tests and enrichment and extraction procedures were as described by Smart & Roberts (1977*a*).

RESULTS

The samples containing *Cl. botulinum* type C toxin are listed in Table 2. Botulinum toxin was detected in sera from affected animals and in extracts of decomposing chicken muscle from carcasses found in the area grazed by the cattle. One sample of chicken muscle contained about 25000 mouse lethal doses (MLD) of type C botulinum toxin/gram of tissue. Extracts of alimentary tract content samples from slaughtered animals were toxin negative as were extracts of litter and grass samples. The organism, *Cl. botulinum* type C, was found in every sample examined including chicken muscle, poultry litter and the contents of the alimentary tracts of two of the slaughtered animals. Faeces from heifer 5 also contained *Cl. botulinum* type C.

The detection of *Cl. botulinum* type C toxin in sera from affected animals confirms the observed disease as botulism, the source of the toxin and the organism almost certainly being poultry waste spread on pasture being grazed by these cattle.

DISCUSSION

Botulism in cattle was first described in South Africa, according to Theiler (1927), by the French naturalist Le Vaillant in the latter part of the eighteenth century, the condition being named 'Lamsiekte'. Le Vaillant apparently also described 'osteophagia' in cattle but did not connect it with 'Lamsiekte'. Almost 150 years later, Theiler (1927) demonstrated that 'Lamsiekte' was indeed botulism and related the condition to the eating of carrion, including bones, containing *Cl.*

botulinum and its toxin. Many mammalian and avian species were shown to be susceptible to botulism on ingestion of contaminated animal debris and the collection of such material from grazing areas and its safe disposal was suggested as a preventive measure. Bennetts (1933) reported botulism in sheep in 1933 in Western Australia to be responsible for greater economic losses than the combined losses from all other sheep diseases and that the condition was contracted after ingestion of toxic rabbit carrion. The rabbit population in Western Australia had increased enormously over the previous 6–7 years. Vaccination with C and D *Cl. botulinum* toxoid conferred a high level of protection on both sheep and cattle (Bennetts & Hall, 1938).

Bovine botulism outbreaks in Europe have been attributed to cat cadavers in hay or silage (Prévot, Sillioc & Proute, 1955; Fjolstad & Klund, 1969; Joubert, Chirol & Beaureau, 1969; Berg, Saxegaard & Teige, 1975) and Doutre (1967) described an outbreak in Senegal affecting cattle and horses in which water from a well containing decomposing cat carcasses was considered to be the toxin source. In the UK, toxic cadavers of various species have been associated with incidents of botulism. Examples are ferrets fed a mallard duck carcass (Blandford, Roberts & Ashton, 1969), mink fed improperly refrigerated offal (Roberts *et al.* 1972), monkeys fed chopped chicken (Smart *et al.* 1980) and hounds fed uncooked meat (Marlow & Smart, 1982). United Kingdom cattle botulism (Davies *et al.* 1974; Clegg & Evans, 1974; Smart & Roberts, 1977*b*, Appleyard & Mollinson, 1985) has been strongly suspected but never confirmed by demonstrating toxin in the bloodstream of affected animals. Several unpublished UK incidents of suspected cattle botulism are known to one author (J.L.S.) in which a common feature was access to waste material from broiler poultry units. Why botulinum toxin was detected in sera from affected animals in this outbreak is not known but it might be significant that the cattle had access to decomposing chicken carcasses containing up to 25000 MLD of botulinum toxin/g. According to Springell (1968) a 500 kg beef animal has about 27·0 l of blood of which about 18·0 l is plasma. The highest level of toxin detectable in sera from affected animals in this outbreak was 50 MLD/ml which is equivalent to *c.* 900000 MLD total circulating toxin in a 500 kg animal. This amount of toxin could be extracted from 36 g of decomposing chicken muscle and it is likely that not all of the toxin would be extracted. Though it is not known how much toxin is required to kill or severely affect a bovine it is obvious that a large toxin source readily accessible to the animals has been identified. Only the younger smaller animals were affected in this outbreak, the yearlings were about 300 kg and the heifer about 400 kg body weight. Substantial numbers of *Cl. botulinum* cells must also have been ingested by the infected animals but whether or not these cells are capable of growth and toxin production in the alimentary tract of bovines is not known and requires investigation.

The observed disease condition had not been seen previously on this farm and poultry litter had not been used before as a grassland fertilizer. Our results strongly suggest that the causal association between poultry waste and confirmed bovine botulism has been made but it is also clear that decomposing animal or bird carcasses of any species might be loci for growth and toxin production by *Cl. botulinum* type C. In the absence of information on *in vivo* growth and toxin production by *Cl. botulinum* type C in bovines the major control measure would

seem to be that proposed by Theiler (1927), the safe disposal of carcasses. The spreading on grazing areas of poultry waste containing carcasses must be considered imprudent.

Due to utilization of poultry waste as feed, bedding and grassland fertilizer it is likely that *Cl. botulinum* type C could be increasingly more widely distributed in the farming environment, particularly adjacent to intensive poultry production units. The ecology of type C *C. botulinum* in relation to poultry, grassland (including silage production) and animal husbandry should be further investigated.

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REFERENCES

APPLEYARD, W. T. & MOLLISON, A. (1985). Suspected bovine botulism associated with broiler litter waste. *The Veterinary Record* **116**, 522.

BENNETTS, H. W. (1933). Botulism of sheep in Western Australia and its association with Sarcophagia. *Journal of the Council for Scientific and Industrial Research* **6**, 137–144.

BENNETTS, H. W. & HALL, H. T. B. (1938). Botulism of sheep and cattle in Western Australia: Its cause and prevention by immunisation. *Australian Veterinary Journal* **14**, 105–118.

BERG, C. R., SAXEGAARD, F. & TEIGE, J. JR. (1975). Another outbreak of botulism in cattle. *Norsk Veterinaertidsskrift* **87**, 238–241.

BLANDFORD, T. B. & ROBERTS, T. A. (1970). An outbreak of botulism in broiler chickens. *The Veterinary Record* **87**, 258–261.

BLANDFORD, T. B., ROBERTS, T. A. & ASHTON, W. L. G. (1969). Losses from botulism in mallard duck and other waterfowl. *The Veterinary Record* **85**, 541–543.

BLOOD, D. C., RADOSTITS, O. M. & HENDERSON, J. A. (1983). *Veterinary Medicine*, 6th edition. London: Bailliere Tindall.

CLEGG, F. G. & EVANS, R. K. (1974). Suspected case of botulism in cattle. *The Veterinary Record* **95**, 540.

CLEGG, F. G., JONES, T. O., SMART, J. L. & MCMURTY, M. J. (1985). Bovine botulism associated with broiler litter waste. *The Veterinary Record* **117**, 22.

DAVIES, A. B., ROBERTS, T. A., SMART, J. L. & BRADSHAW, P. R. (1974). Probable botulism in calves. *The Veterinary Record* **94**, 412–414.

DOUTRE, M. P. (1967). Le botulisme animal au Sénégal. *Bulletin de l'Office International des Epizooties* **67**, 1497–1515.

FJOLSTAD, M. & KLUND, T. (1969). An outbreak of botulism among ruminants in connection with ensilage feeding. *Nordisk Veterinaermedicin* **21**, 609–613.

GIBSON, L. A. S. (1986). Botulism in dairy cows. *The Veterinary Record* **118**, 309.

JOUBERT, L., CHIROL, C. & BEAUREAU, P. (1969). Concerning 7 cases of bovine botulism C of feline origin: epidemiological and pathogenical hypotheses. *Bulletin de la Société des Sciences Vétérinaires et de Médicine Comparée de Lyon* **71**, 95–101.

MARLOW, G. R. & SMART, J. L. (1982). Botulism in foxhounds. *The Veterinary Record* **111**, 242.

MIYAZAKI, S. & SAKAGUCHI, G. (1978). Experimental botulism in chickens: The cecum as the site of production and absorption of botulinum toxin. *Japanese Journal of Medical Science and Biology* **31**, 1–15.

PRÉVOT, A. R., SILLIOC, R. & PROUTE, J. (1955). Etude d'un foyer de botulisme bovin du a *Clostridium botulinum* C. *Annales de l'Institut Pasteur, Microbiologie* **88**, 513–515.

ROBERTS, T. A. & COLLINGS, D. F. (1973). An outbreak of type C botulism in broiler chickens. *Avian Diseases* **17**, 650–658.

ROBERTS, T. A., KEYMER, I. F., BORLAND, EDITH D. & SMITH, G. R. (1972). Botulism in birds and mammals in Great Britain. *The Veterinary Record* **91**, 11–12.

SCHOFIELD, F. (1985). Botulism in cattle. *Meat Hygienist* **48**, 27–28.

SMART, J. L., LAING, P. W. & WINKLER, C. E. (1983). Type C botulism in intensively farmed turkeys. the *Veterinary Record* **113**, 198–200.

SMART, J. L. & ROBERTS, T. A. (1977*a*). An outbreak of type C botulism in broiler chickens. *The Veterinary Record* **100**, 278–280.

SMART, J. L. & ROBERTS, T. A. (1977*b*). Bovine botulism. *The Veterinary Record* **101**, 201–202.

SMART, J. L., ROBERTS, T. A., MCCULLAGH, K. G., LUCKE, V. M. & PEARSON, H. (1980). An outbreak of type C botulism in captive monkeys. *The Veterinary Record* **107**, 445–446.

SPRINGELL, P. H. (1968). Red cell volume and blood volume in beef cattle. *Australian Journal of Agricultural Research* **19**, 145–160.

THEILER, A. (1927). Department of Agriculture, 11th and 12th Reports to the Director of Veterinary Education and Research. Pretoria, Union of South Africa.