Literature update on Botulism in cattle, sheep and goats from 2006 to 2019.

MICROBIOLOGICAL LITERATURE REVIEW

Abstract

The anaerobic bacterium *Clostridium botulinum* (*C. botulinum*) is found naturally in soil, vegetation and rotting carcasses and is able to produce spores resistant to a range of stresses. *C. botulinum* causes botulism in cattle, sheep and goats, through either ingestion of the potent neurotoxin *C. botulinum* produces, or ingestion of *C. botulinum* spores which then germinate and colonise the gut cause toxico-infection. Botulism in cattle, sheep and goats is often fatal. Poultry are carriers of *C. botulinum*; and the use of poultry litter in processes such as fertilising crops used for cattle feeds, has been associated with outbreaks of botulism in sheep and cattle. Foodborne cases of botulism in humans are caused mainly by ingestion of botulinum neurotoxin. Currently animals with botulism (or associated food products such as meat and milk) are prohibited from entering the food chain until 18 days after the cessation of clinical symptoms, however; asymptomatic animals from the same herd may still be used (ACMSF, 2009).

In 2006 the ACMSF produced a comprehensive report which assessed the risk to human health posed by botulism in cattle. In 2009 a further ACMSF report was produced which addressed botulism in sheep and goats. This report reviews the available literature produced since these reports covering the following areas:

1. *Clostridium botulinum* the organism;
2. Diagnosis and epidemiology of botulism in animals.
3. The link between poultry waste and botulism outbreaks in cattle, sheep and goats.
4. Contamination of food products through the transfer of spores, toxins or bacteria from groups of animals with botulism or suspected botulism.
5. The associated risk to public health from food products derived from these animals.

This information has been collected to assess whether there is new information which is relevant to the current ACMSF advice on botulism precautions.

List of acronyms

16s rRNA- 16 Svedberg ribosomal ribonucleic acid

ACMSF - Advisory Committee on the Microbiological Safety of Food

APHA - Animal and Plant Health Agency

BoNT - Botulinum NeuroToxin

DEFRA - Department for Environment Food and Rural Affairs

DNA - DeoxyriboNucleic Acid

EBSCO - Elton B. Stephens Company

ELISA - Enzyme-Linked Immunosorbent Assay

FSA - Food Standards Agency

NAATS- Nucleic Acid Amplification Tests

NTNH - NonToxin-NonHaemagglutinin

PHE- Public Health England

PCR - Polymerase Chain Reaction

qPCR - Quantitative Polymerase Chain Reaction (also referred to as Real time PCR)

UK- United Kingdom

VLA - Veterinary Laboratories Agency (now APHA)

WHO - World Health Organisation

Terms of Reference

The following terms of reference were provided by the Food Standards Agency Policy Directorate.

Details of the request

Have there been any updates in the literature on botulism in cattle, sheep or goats published since the 2006 and 2009 ACMSF reports?

Introduction

Background

The 2006 ACMSF report on botulism in cattle was produced in response to an increase in the number of suspected botulism cases in England, Wales and Northern Ireland between 2003 and 2006. In particular, it addresses the link between poultry litter and suspected botulism cases in cattle. The committee assessed the safety of cattle in proximity to poultry litter and the subsequent risk to human health from food chain issues linked to botulism in cattle. The 2006 report concluded that good practice in poultry litter management and disposal should be extended to cattle farmers and the FSA guidance on biosecurity should be extended to highlight the risks of disease transmission through poor management of carcass removal. The report also concluded that the risk to human health presented by toxin types C and D (the main toxin types to affect cattle) is low, and recommended that the voluntary restrictions on meat and milk from affected animals (for 14 days after the onset of the last clinical case or 17 days after removal of source of contamination) should be maintained, but indicated that in the absence of other signs, meat and milk products from healthy animals on farms where there have been clinically suspected cases of botulism do not need to be further restricted. In 2007 the ACMSF sub group met a further three times to discuss botulism in cattle, sheep and goats after the emergence of botulism in sheep. The committee produced a further report on botulism to assess whether the recommendations for cattle made in 2006 should also be applied to sheep and goats, where there have been suspected cases of botulism. The subsequent report published in 2009, concluded that although botulism outbreaks in sheep and goats in the UK are uncommon in comparison to cattle, the number of animals affected by each outbreak could potentially be higher. Poultry litter was also found to be a contributing factor to botulism in sheep and goats. The 2009 report concluded that there was a strong association between poultry litter and botulism in sheep in the UK between 1999 and 2007, highlighting that the advice on the management of poultry litter on farms should be extended to sheep and goat farmers. The risk to human health from consuming meat and milk from clinically healthy sheep and goats is negligible, and in the absence of other signs, meat and milk from healthy sheep on farms with suspected botulism should not be restricted. However, clinically affected sheep and goats should follow the same restrictions as those used for cattle.

In 2009, following the recommendations of the 2006 ACMSF report, the FSA amended advice on botulism to follow the recommendation of the committee. The voluntary restriction on meat and milk products from clinically affected cattle was unchanged, but the voluntary restriction on healthy cattle from farms with clinically suspected botulism was no longer necessary, with the recommendation that information should be reviewed if new evidence emerges of cases in cattle, sheep or goats caused by the toxin types A, B, E or F (human toxin types).

Clostridium botulinum

Clostridium botulinum (*C. botulinum)* is an anaerobic, spore-forming bacterium which is responsible for producing a potent family of neurotoxins (ACMSF, 2006). *C. botulinum* can be divided into four distinct taxonomic lineages (I-IV) with the botulinum neurotoxins (BoNT) produced being split into seven antigenically different lineages (A-G) (McLauchlin, Grant, and Little 2006, Rasetti-Escargueil et al. 2020). The organism is found in environments such as soil, rotting vegetation and carcasses (APHA, 2018). Spores are resistant to heat, desiccation, some chemicals and radiation, allowing the survival of the organism for long periods of time (ACMSF, 2006). However, *C. botulinum* is unable to grow in acidic conditions (pHs of 4.6 or lower), if spores are present in acidic conditions, they are unable to germinate and grow to produce BoNT (WHO, 2018).

Botulism in animals is often fatal, cows and sheep may become ill through the ingestion of BoNT or spores in contaminated grass and silage or through contact with poultry litter or carcasses (APHA, 2018). The majority of animal cases are thought to be intoxication caused by ingestion of the pre-formed BoNT. However, animals ingest the spores and in some cases it is possible that infection is caused by intestinal colonisation (ACMSF 2006). In animals, clinical signs include sudden onset ataxia, recumbency, and developing paralysis. Clinical signs can develop from 24 hours to three weeks after exposure (APHA, 2018). In the UK, botulism in animals is reported under a voluntary restriction, clinically affected animals may not be used for human consumption for 18 days after the cessation of clinical symptoms (ACMSF, 2009).

Human intoxication by *C. botulinum* can be foodborne and is potentially fatal. Infant botulism (two months to one year of age) can be caused by both the presence of pre-formed BoNT or *C. botulinum* bacteria. In infants and individuals with chronic gastro-intestinal conditions such as Crohn's disease, *C. botulinum* can colonise the intestine and produce toxin (Fox, Keet, and Strober 2005, Griffin et al. 1997, Sheppard et al. 2011). In adults, botulism is more frequently intoxication caused by the ingestion of the pre-formed BoNT; the *C. botulinum* bacteria are not usually able to colonise the intestine of adults as the resident intestinal flora outcompete *C. botulinum* (Roberts 2000, WHO 2018). In adults the onset of illness is between 12 to 36 hours after ingestion (McLauchlin, Grant, and Little 2006) with BoNT intoxication resulting in respiratory and muscular paralysis (ACMSF, 2006).

Table 1:Taxonomic lineages of *C. botulinum* with the strains most commonly affecting each organism, strain H is omitted (Collins and East 1998; McLauchlin, Grant, and Little 2006, Demarchi et al. 1958).

	Toxin type						
	A	B	C	D	E	F	G
Taxonomic Group	I	I or II	III	III	II	I	IV
Human	Yes	Yes	Very rarely	Very rarely	Yes	Yes	No
Cattle	Occasionally	No	Yes	Yes	No	No	No
Sheep	Occasionally	No	Yes	Yes	No	No	No
Goats	Occasionally	No	Yes	Yes	No	No	No
Poultry	Yes	No	Yes	Yes	No	No	No

Humans are generally affected by lineage I and II *C. botulinum* which produces toxin types A, B, E and F, with toxin types A and B being the most common foodborne types (Table 1) (McLauchlin, Grant, and Little 2006). Ruminants are more commonly affected by lineage III *C. botulinum* which produces toxin types C and D (APHA, 2018). The spores produced by all four taxonomic lineages of *C. botulinum* are very heat resistant and survive conventional pasteurisation conditions (Rasooly and Do 2010). Due to the heat tolerance of spores and fatal nature of the toxins it is essential that the risk to human health from potentially contaminated food products is carefully monitored.

<u>Reported botulism/suspected botulism incidents 2008-2018</u>

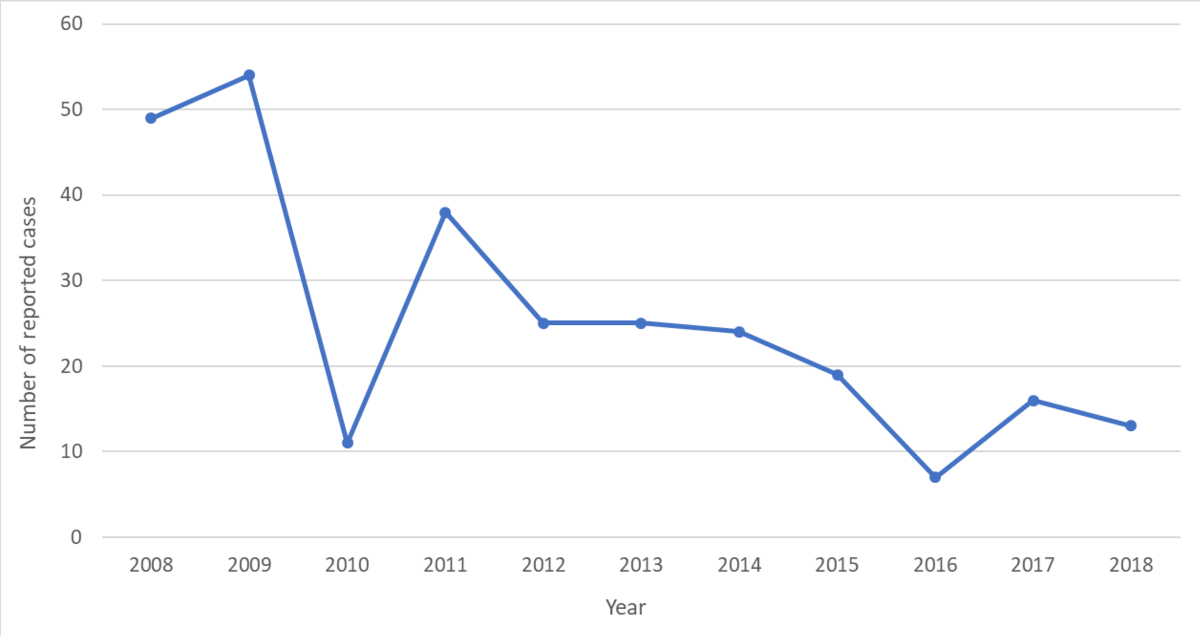

Figure 1: Reported botulism/suspected botulism incidents in UK animals and humans between 2008 and 2018.

The number of UK botulism/suspected botulism incidents in animals reported to the FSA/FSS between 2008 and 2018 are summarised in Figure 1. The number of botulism/suspected botulism incidents in animals since 2008 appear to have decreased. (Figures were prepared using raw data, a start date of 2008 was used as this was the earliest record on the current archive software. Botulism in the UK is not notifiable).

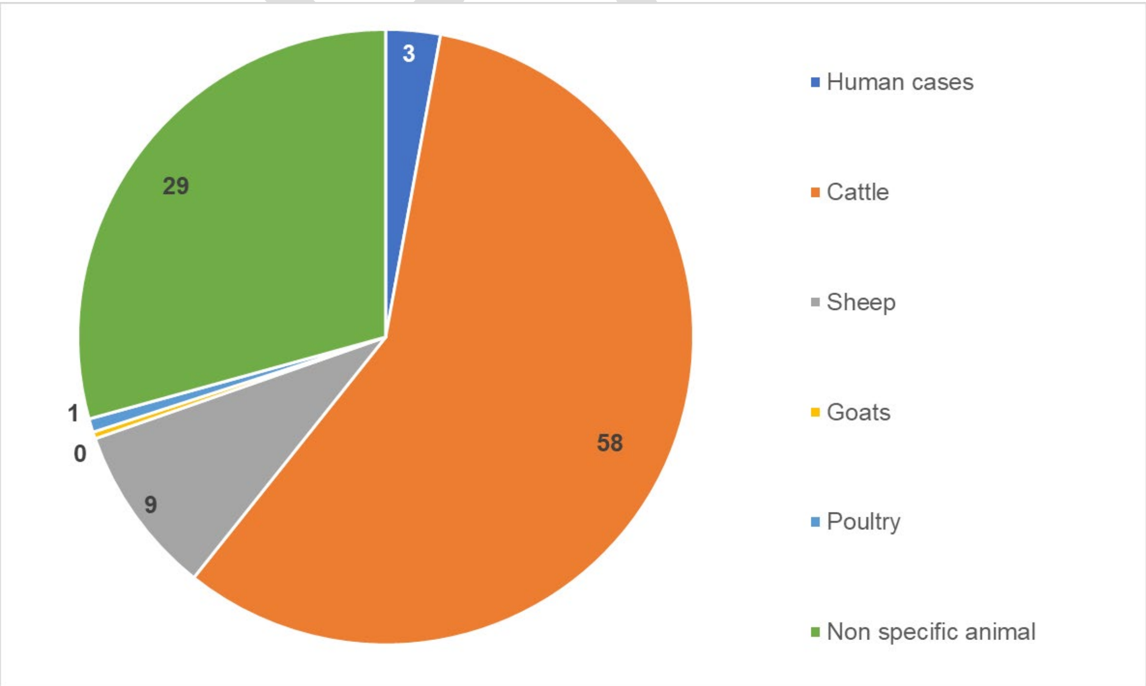

Figure 2:Percentage of reported botulism/suspected botulism incidents attributed to each animal species 2008-2018.

The distribution of cases across different animals is shown in Figure 2, with just over half of reported botulism cases in cattle. Non-specific animal refers to cases where suspected botulism was reported at a particular farm or location but no animal or case details were given and this accounted for 26%, sheep accounted for 9%, poultry 1% and goats 0% of cases.

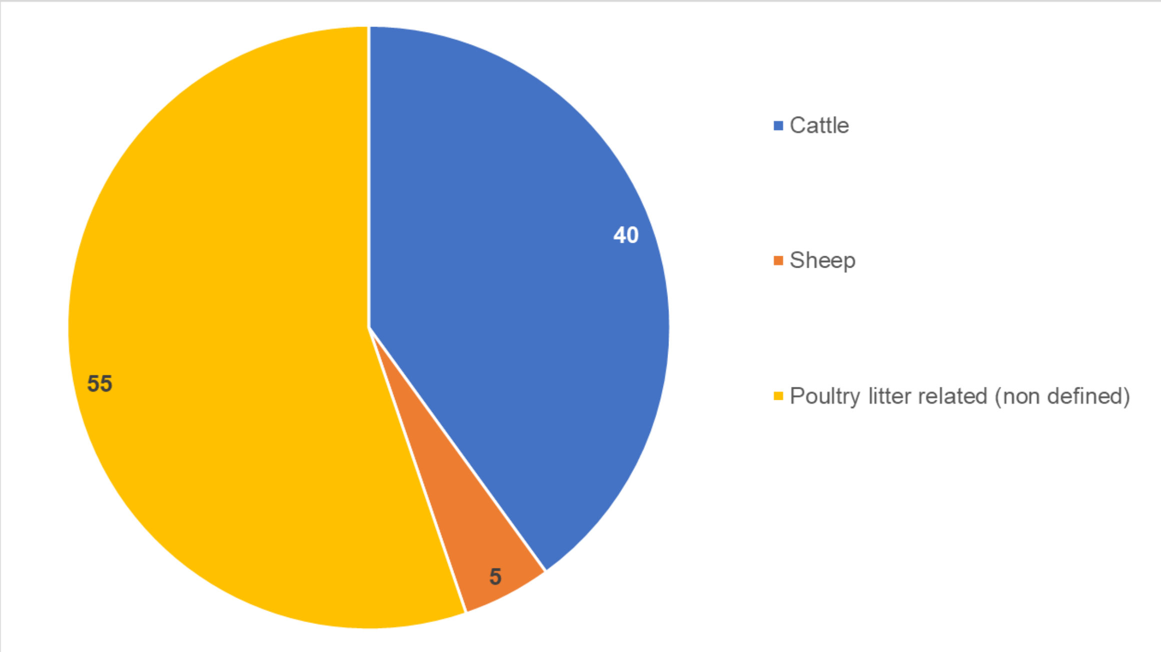

Figure 3 : Percentage of cases attributed to poultry litter as the cause.

Of the reported cases, 42% of cattle cases were attributed as being poultry litter related with only 5% of sheep cases linked to poultry litter. 58% of cases related to poultry litter and had no defined animal associated (Figure 3).

Objectives

The topics for each search were chosen to match the sub sections of the 2006 ACMSF report on botulism in cattle and subsequent 2009 ACMSF report.

A literature search was performed to obtain data on:

- *Clostridium botulinum* the organism
- Diagnosis and epidemiology of infection in animals
- Links with poultry waste and botulism outbreaks.
- *C. botulinum* infection of food products from cows, sheep or goats through the transfer of spores, toxin, bacteria.
- Updates on the risk to public health posed by food related botulism.

This review specifically considers data related to cows, sheep or goats and was conducted with a global reach.

Materials and Methods

The review was written following the principles of a systematic review methodology (Moher et al. 2009). This involved the following steps:

- defining review questions and developing the eligibility criteria
- literature searches
- screening studies for inclusion or exclusion
- data collation
- data presentation
- interpretation and conclusions

Review questions

The review questions were decided based on the original request from policy and the subsections of the 2006 ACMSF report the original request referred to. This resulted in five separate review questions as detailed below.

Review Question One

Have there been any peer reviewed publications detailing updates since 2006/ 2009 for *C. botulinum* and botulism including: the organism, pathogenesis and disease in humans, botulinum toxin, structure and processing, uptake of toxin, mode of action?

Review Question Two

Have there been any peer reviewed publications detailing updates since 2006/2009 for epidemiology and diagnosis of botulism including; occurrence, clinical signs, clinical diagnosis, laboratory diagnosis, immunology in cows, sheep or goats?

Review Question Three

Have there been any peer reviewed publications detailing updates since 2006/2009 concerning the risk to animals associated with poultry waste? With particular reference to: the definition of poultry waste, types of litter and manure, or practices and sources of risk to cattle, sheep or goats.

Review Question Four

Have there been any peer reviewed publications detailing updates since 2006/2009 concerning the risk to public health of products from transfer of botulinum toxin or vegetative spores of *C. botulinum* to milk and/ or meat from cows, sheep or goats?

Review Question Five

Have there been any peer reviewed publications detailing updates since 2006/2009 concerning public health advice/ risk to human health from the biological activity or availability of toxins in humans and milk/ meat products. Toxin availability in meat, human cases associated with consumption of foods produced from cows, sheep or goats? From both individuals with suspected botulism or healthy individuals from an affected herd.

Literature searches

Two databases were searched to retrieve relevant literature. These were PubMed and a database maintained by “EBSCO: The Food Science Source”. Resulting publications were exported directly and the finalised papers were added to the reference management software (Zotero 2.0.59, https://www.zotero.org/). There were no specific searches carried out for grey literature.

Search A

For Search A, which aimed to collate literature relevant to Review Question One, the keywords used are shown in Table 2. The searches were date limited from 1st January 2007 – 8th August 2019 to capture literature published after the previous evaluation.

Table 2: Keywords used in Search A grouped by element. * is a wildcard to include all search terms with this root

Botulism	Ilness/ Infection/ Intoxication	Animal	Exclusions
Botuli*	Toxin	Food	Botox
	Pathogen*	Animal	Drug
	Disease		Inject*
	Human		
	Classification		
	Toxin type		

This search included "NOT" terms to exclude papers on the clinical uses of botulinum toxin (botox) the excluded terms were "botox", "drug" and "inject".

The search string used for PubMed is shown below. Searches in other databases used similar strings but had minor syntax differences.

((botuli*[Title/Abstract]) AND ("toxin"[Title/Abstract] OR pathogen*[Title/Abstract] OR "disease"[Title/Abstract]) AND "human"[Title/Abstract] OR "classification"[Title/Abstract] OR "toxin type"[Title/abstract]) AND ("food"[title/abstract] OR "animal"[Title/Abstract]) NOT ("botox" [Title/Abstract] OR inject*[Title/Abstract] OR "drug"[Title/Abstract])) ("2007/01/01"[PDat] : "2019/08/08"[PDat])

Search B

For Search B, which aimed to collate literature relevant to Review Question Two, the keywords used are shown in Table 3: Keywords used in Search B grouped by element. * is a wildcard to include all search terms with this root. The searches were date limited from 1st January 2007 – 8th August 2019 to capture literature published after the previous evaluation.

Table 3: Keywords used in Search B grouped by element. * is a wildcard to include all search terms with this root

Botulism	Diagnosis	Animals
Botuli*	Clinical	Vet*
Botulism	Indica*	Sheep
Clostridium botulinum	Diagnos*	Cow
botulinum	Immun*	Goat
	Sign	Herd
		Dairy
		Cattle

		Ewe
		Ruminant
		Flock

The search string used for PubMed is shown below. Searches in other databases used similar strings but had minor syntax differences.

((“botulism”[Title/Abstract] OR “botulinum”[Title/Abstract] OR “clostridium botulinum”[Title/Abstract] OR botuli*[Title/Abstract]) AND (“clinical”[Title/Abstract] OR “indica*[Title/Abstract] OR diagnos*[Title/abstract] OR immun*[Title/Abstract] OR “sign”[Title/Abstract]) AND (Vet*[Title/Abstract] OR(“sheep”[Title/Abstract] OR “cow”[Title/Abstract] OR “goat”[Title/Abstract] OR “herd[Title/Abstract] OR “dairy”[Title/Abstract] OR “cattle”[Title/Abstract] OR “ewe”[Title/Abstract] OR “ruminant”[Title/Abstract] OR “flock”[title/Abstract])) AND ("2007/01/01"[PDat] : "2019/08/08"[PDat])

Search C

For Search C, which aimed to collate literature relevant to Review Question Three, the keywords used are shown in Table 4. The searches were date limited from 1st January 2007 – 8th August 2019 to capture literature published after the previous evaluation.

Table 4: Keywords used in Search C grouped by element. * is a wildcard to include all search terms with this root

Botulism	Poultry	Poultry litter
Botuli*	Broiler	Manure
Botulism	Chicken	Carcas*
Botulinum	Duck*	Litter
	Goose	
	Turkey	
	Geese	
	Fowl	
	Bird*	
	Avian	
	Poultry	

The search string used for PubMed is shown below. Searches in other databases used similar strings but had minor syntax differences.

((“broiler”[Title/Abstract] OR chicken*[Title/Abstract] OR "poultry"[Title/Abstract] OR duck*[Title/Abstract] OR “goose”[Title/Abstract] OR “turkey”[Title/Abstract] OR “geese”[Title/Abstract] OR “fowl”[Title/Abstract] OR bird*[Title/Abstract] or “avian”[Title/Abstract]) AND (“litter”[Title/Abstract] OR “manure”[Title/Abstract] OR carcas*[Title/Abstract]) AND (“botulism”[Title/Abstract] OR “botulinum”[Title/Abstract] or botuli*[Title/Abstract])) AND ("2007/01/01"[PDat] : "2019/08/08"[PDat])

Search D

For Search D, which aimed to collate literature relevant to Review Question Four, the keywords used are shown in Table 5. The searches were date limited from 1st

January 2007 – 8th August 2019 to capture literature published after the previous evaluation.

Table 5: Keywords used in Search D grouped by element. * is a wildcard to include all search terms with this root

Botulism	Type of C. botulinum	Contamination	Foods relating to animals of interest
Botuli*	Toxin	Transfer	Meat
Botulism	Spore	Contamin*	Food
Botulinum	Bacteria	Process*	Cheese
			Milk
			Dairy
			Cream
			Yoghurt
			Butter
			Lamb
			Mutton
			Beef
			Curds
			Whey

The search string used for PubMed is shown below. Searches in other databases used similar strings but had minor syntax differences.

((“Toxin”[Title/Abstract] OR “Spore”[Title/Abstract] OR “bacteria”[Title/Abstract]) AND (“botulism”[Title/Abstract] OR “botulinum”[Title/Abstract] or botuli*[Title/Abstract]) AND (“Transfer”[Title/Abstract] OR contamin*[Title/Abstract] OR process*[Title/Abstract]) AND (“meat”[Title/Abstract] OR “food”[Title/Abstract] OR “cheese”[Title/Abstract] OR “milk”[Title/Abstract] OR “dairy”[Title/Abstract] OR “cream”[Title/Abstract] OR “yoghurt”[Title/Abstract] OR “butter”[Title/Abstract] OR “lamb”[Title/Abstract] OR “mutton”[Title/Abstract] or “beef”[Title/Abstract] OR “curds”[Title/Abstract] OR “Whey”[Title/Abstract]) AND ("2007/01/01"[PDat] : "2019/08/08"[PDat])

Search E

For Search E, which aimed to collate literature relevant to Review Question Five, the keywords used are shown in Table 6. The searches were date limited from 1st January 2007 – 8th August 2019 to capture literature published after the previous evaluation.

Table 6: Keywords used in Search E grouped by element. * is a wildcard to include all search terms with this root

Botulism	Type of C. botulinum	Illness	Foods relating to animals of interest
Botuli*	Bacteri*	Human	Meat
Botulism	Toxin	Infect*	Food
Botulinum	Spore	Disease	Cheese
		Ill*	Milk

		Zoo*	Dairy
			Cream
			Yoghurt
			Butter
			Lamb
			Mutton
			Beef
			Curds
			Whey

The search string used for PubMed is shown below. Searches in other databases used similar strings but had minor syntax differences.

((“botulinum”[Title/Abstract] OR “botulism”[Title/Abstract] OR botuli*[Title/Abstract) AND (Bacteri*[Title/Abstract] OR “toxin”[Title/Abstract] OR “spore”[Title/Abstract]) AND (“human”[Title/Abstract]) AND (infect*[Title/Abstract] OR “disease”[Title/Abstract] OR ill*[Title/Abstract] OR zoo*[Title/Abstract]) AND (“meat”[Title/Abstract] OR “food”[Title/Abstract] OR “cheese”[Title/Abstract] OR “milk”[Title/Abstract] OR “dairy”[Title/Abstract] OR “cream”[Title/Abstract] OR “yoghurt”[Title/Abstract] OR “butter”[Title/Abstract] OR “lamb”[Title/Abstract] OR “mutton”[Title/Abstract] or “beef”[Title/Abstract] OR “curds”[Title/Abstract] OR “Whey”[Title/Abstract])) AND ("2007/01/01"[PDat] : "2019/08/08"[PDat])

Screening studies for inclusion or exclusion

Keyword title screening

For all searches, any titles that did not contain reference to botulism in some form were excluded. Papers that did not include the described inclusion terms were excluded.

For Search A, titles were screened to ensure that results focused on updates to *C. botulinum*, pathogenesis, classification and mode of action.

For Search B, to ensure that results were relevant, titles were screened to ensure that they included diagnosis of botulism in cows, sheep or goats and diagnosis including novel diagnosis methods.

For Search C, title screen terms were used to ensure that all search results included instances of botulism involving poultry.

For Search D titles were screened to ensure papers were relevant to *C. botulinum* in food products from cattle, sheep and goats.

For Search E search terms were used to ensure results were relevant to *C. botulinum* in food products from cattle, sheep and goats and associated risks to human health.

Keyword searches were performed as listed in Table 7. Duplicates were removed at this stage.

Table 7: Keywords used in title screening

Search A	Answers	Inclusion/Exclusion
Have there been any updates in: *C. botulinum* knowledge, *The organism, pathogenesis and disease in humans, botulinum toxin, structure and processing, uptake of toxin, mode of action.*	(botulism OR botulinum OR clostridium botulinum OR botuli*) AND (toxin OR pathogen OR disease OR classification OR toxin type)	Inclusion
Search B		
Have there been any updates in: Epidemiology and diagnosis of botulism *Occurrence, clinical signs, clinical diagnosis, lab diagnosis, immunology.*	(Botuli*) AND (sheep OR cow OR cattle OR herd OR goat OR Dairy OR ruminant OR ewe OR flock) AND (human OR health OR animal OR diagnos* OR vet*)	Inclusion
Search C		
Have there been any updates in: Poultry waste Definitions, *types of litter and manure, sources of risk.*	(Botuli*) AND (broiler OR chicken OR poultry OR avian OR flock OR goose OR geese) AND (litter OR carcas*)	Inclusion
Search D		
Have there been any updates in: Risk to public health of products from Transfer of botulinum toxin or vegetative spores of *C. botulinum* to milk and/ or meat.	(Botuli*) AND (toxin OR spore OR bacteria) AND (meat OR food OR cheese OR milk OR dairy OR cream OR yoghurt OR butter OR lamb OR mutton OR beef OR curds OR whey).	Inclusion
Search E		
Have there been any updates in: Public health advice/ risk to human health, Biological activity of toxins in humans and milk/ meat products. Toxin availability in meat, human cases associated with consumption	(Botuli*) AND (toxin OR spore OR bacteria OR infect OR disease OR zoo* OR ill*) AND (meat OR food OR cheese OR milk OR dairy OR cream OR yoghurt OR butter OR lamb OR mutton OR beef OR curds OR whey).	Inclusion

Keyword abstract screening

Following title screening, a more specific screen of the abstracts was performed using keywords. In all five searches, any abstracts which did not mention botulism were screened out.

Search A was narrowed to screen out all papers that did not refer to *C. botulinum*, botulism or botulinum toxin and human or animal infection.

Search B was refined by excluding results which did not mention *C. botulinum* or botulism and a relevant animal and a health or diagnosis related term.

Search C was refined by including the specific animals: sheep, cows or goats.

Search D was refined by excluding searches that did not include either *C. botulinum,* spores, bacteria or toxin, and the addition of the relevant animals to the inclusion terms.

Search E was also refined by the exclusion of abstracts that did not mention one of the forms of *C. botulinum* and one of the relevant animals. Screening strings are detailed in Table 8. Abstracts which met none of the exclusion criteria were taken forward, if the abstract did not refer to any of the exclusion or inclusion criteria the paper was taken to the full text stage and was appropriately screened for inclusion criteria.

Table 8: Screening strategies for abstracts

Questions	**Answers**	**Inclusion/Exclusion**
Review Question 1		
Does the abstract refer to *C. botulinum* or botulism?	Botuli*	Inclusion
	No	Exclusion
Does illness?	Toxin* OR pathogen* OR disease	Inclusion
Does it mention classification or toxin type?	Classification* OR toxin type	Inclusion
Does it include the relevant animals, foods or human disease?	Animal OR human OR food	inclusion
Review Question 2		
Does the abstract refer to *C. botulinum* or botulism?	Botuli*	Inclusion
	No	Exclusion
Does it mention relevant animals?	sheep OR cow OR cattle OR herd OR goat OR Dairy OR ruminant OR ewe OR flock	Inclusion
Does it mention human or animal diagnosis?	Human OR health OR animal OR diagnos* OR clinical	Inclusion
Review Question 3		
Does the abstract refer to *C. botulinum* or botulism?	Botuli*	Inclusion
	No	Exclusion
Does it mention poultry?	broiler OR chicken OR poultry OR avian OR flock OR goose OR geese	Inclusion
Does it mention poultry waste?	Litter OR carcas*	Inclusion

Does it mention the relevant animals	sheep OR cow OR cattle OR herd OR goat OR Dairy OR ruminant OR ewe OR flock	Inclusion
Review Question 4		
Does the abstract refer to *C. botulinum* or botulism?	Botuli*	Inclusion
	No	Exclusion
Does it mention any of the forms of botulism?	Toxin OR spore OR bacteria	Inclusion
	No	Exclusion
Does it include food products from relevant animals?	meat OR food OR cheese OR milk OR dairy OR cream OR yoghurt OR butter OR lamb OR mutton OR beef OR curds OR whey.	Inclusion
Does it include relevant animals?	sheep OR cow OR cattle OR herd OR goat OR Dairy OR ruminant OR ewe OR flock	Inclusion
Review Question 5		
Does the abstract refer to *C. botulinum* or botulism?	Botuli*	Inclusion
	No	Exclusion
Does it mention any of the forms of botulism?	Toxin OR spore OR bacteria	Inclusion
	No	Exclusion
Does it mention human infection?	Infect* OR disease OR zoo* OR ill*	Inclusion
Does it include food products from relevant animals?	meat OR food OR cheese OR milk OR dairy OR cream OR yoghurt OR butter OR lamb OR mutton OR beef OR curds OR whey.	Inclusion
Does it include relevant animals?	sheep OR cow OR cattle OR herd OR goat OR Dairy OR ruminant OR ewe OR flock	Inclusion

Manual screening

After keyword screening, the remaining results were manually screened by abstract to determine suitability for inclusion. This process was performed independently by two FSA researchers in line with good practice guidance for systematic literature reviews. Papers were excluded using the criteria listed in Table 9 based on reviewer interpretation. In the case of disagreements, papers were discussed until a consensus was achieved, with the default of continuing to include the paper in the next stage of the process. In search B there were a number of papers referring to vaccination of cattle against botulism, this was not part of the 2006 report however, they have been included due to the change in usage since the 2006 report was written.

Table 9: Categories for exclusion from manual sifting

Exclusion Category	**Explanation**

Botox	Concerning the use of botulinum toxin for clinical and aesthetic purposes such as botox (search A).
Host	Not concerning relevant animals of interest
Irrelevant	does not include botulism
Not available	Abstract unavailable
Medicinal	concerning drug production/ therapeutic use
Toxicological	Concerning toxicological risks
Question	Content not relevant to this question
Food	Concerning preserved foods or foods not of origin of the relevant animals
Clinical	concerning human diagnosis/ outbreak

Data collation

After screening was completed, the full text of the papers was examined and assessed. The data were extracted and collated using a standardised system independently by two FSA researchers. The methodology and categorisation for information extraction is listed in Table 10. Several papers which were written in a language other than English but had abstracts in English were discovered at this stage. The reviewers were unable to obtain the full texts of these papers in English, so they were excluded at this stage.

Table 10: Method for data extraction and collation

Questions	Answers	Inclusion/Exclusion
Is it a primary research paper?	Yes	Neutral
	No	Exclusion
Does the paper refer to the use of botulinum toxin eg. medical purposes.	Yes	Exclusion
	No	Neutral
Review Question One		
Does the text mention a specific toxin type?	Yes	Inclusion
	No	Neutral
Does the text include further information on botulism the organism?	Yes	Inclusion
	No	Neutral
Is uptake of the toxin mentioned?	Yes	Inclusion
	No	Neutral
Other information on botulism?	Yes	Neutral
	No	Neutral
Review Question Two		
Which animals are mentioned?	Cows	Inclusion
	Sheep	Inclusion
	Goats	Inclusion

Are clinical signs in animals mentioned?	Yes	Inclusion
	No	Neutral
Is a clinical diagnosis method mentioned?	Yes	Inclusion
	No	Neutral
Is toxin type mentioned?	Yes	Neutral
	No	Neutral
Other relevant information?	Yes	Neutral
	No	Neutral
Review Question 3		
Which animals are mentioned?	Cows	Inclusion
	Sheep	Inclusion
	Goats	Inclusion
	Other	Exclusion
Are poultry mentioned?	Yes	Neutral
	No	Exclusion
Does the text refer exclusively to poultry botulism infection?	Yes	Exclusion
	No	Inclusion
Does the text mention source of contamination or infection?	Yes	Inclusion
	No	Neutral
Is the source of risk to animals mentioned?	Yes	Inclusion
	No	Neutral
Other relevant information?	Yes	Inclusion
	No	Neutral
Review Question 4		
Which animals are mentioned?	Cows	Inclusion
	Sheep	Inclusion
	Goats	Inclusion
	Other	Exclusion
Affected food product	Meat	Inclusion
	Cheese	Inclusion
	Milk	Inclusion
	Dairy	Inclusion
	Cream	Inclusion
	Yoghurt	Inclusion
	Butter	Inclusion
	Lamb	Inclusion
	Mutton	Inclusion
	Beef	Inclusion
	Whey	Inclusion
	Curds	Inclusion
	Other product from relevant animals	Inclusion
	Non-related food product	Exclusion
Form of botulinum present?	Spores	Inclusion
	Toxin	Inclusion

	Bacteria	Inclusion
	Not mentioned	Exclusion
Is toxin type mentioned?	Yes	Neutral
	No	Neutral
Review Question 5		
Which animals are mentioned?	Cows	Inclusion
	Sheep	Inclusion
	Goats	Inclusion
	Other	Exclusion
Affected food product	Meat	Inclusion
	Cheese	Inclusion
	Milk	Inclusion
	Dairy	Inclusion
	Cream	Inclusion
	Yoghurt	Inclusion
	Butter	Inclusion
	Lamb	Inclusion
	Mutton	Inclusion
	Beef	Inclusion
	Whey	Inclusion
	Curds	Inclusion
	Other product from relevant animals	Inclusion
	Non-related food product	Exclusion
	Non-related food product plus related food product	Inclusion
Is the activity of botulinum in the food product mentioned?	Yes	Inclusion
	No	Neutral
Is risk to human health mentioned?	Yes	Inclusion
	No	Neutral

Results

Literature search and screening

Table 11 summarises the results of the literature searches and screening process. Search A, which concerned general updates *in C. botulinum* knowledge returned 4262 papers. Search B, which concerns botulism in cattle (cows) or sheep or goats returned 162 papers. Search C which concerns associations of poultry litter and botulism outbreaks in cattle, sheep or goats returned 29 papers. Search D which concerns transfer of *C. botulinum* bacteria or toxin or spores to milk and food products from infected animals, returned 131 papers. Search E which concerns the risk to human health from foods produced from animals with botulism returned 34 papers.

Table 11: Results of database searches and subsequent screens.

Database searches	Search A	Search B	Search C	Search D	Search E	Total
Records from PubMed	4262	82	29	131	34	4538
Records from Food Science Source	N/A	80	N/A	N/A	N/A	80
Post title screen and duplicate removal	54	64	16	69	13	216
Post abstract screen	53	57	7	21	13	151
Post manual abstract sift	17	37	5	3	2	64
Post full text analysis	10	23	4	2	1	40

The searches from the food science source which show as N/A had no search results for this database. After duplicates were removed, the papers were then automatically screened for relevant keywords in the title and abstract using excel formulas for the keywords described in the methods.

The abstracts of the papers were then manually sifted for relevance. At this point some of the papers were excluded from each section. Many of the papers excluded at this stage referred to clinical and medical uses of botulinum toxin, or on the chemical hazards of botulinum toxin. For search C a number were excluded as they discussed botulism in poultry and wild birds with no reference to transmission to cows, sheep or goats. Searches D and E contained a number of papers for food products that were not derived from cows, sheep or goats. During the full text analysis, a small number of papers were excluded due to the fact they were in a language other than English or that the paper was a review paper. The final number of papers was 40 with 10 in Search A, 23 in Search B, 4 in Search C, 2 in Search D and 1 in Search E.

Review Question One- *Clostridium botulinum* and botulism.

General information

Ten primary research papers contained relevant information on updates to *C. botulinum* or botulism knowledge. There were no relevant publications which contained novel information on *C. botulinum* the organism, pathogenesis of disease in humans or structure and processing. Five papers contained information on botulinum toxin types, contained information on uptake of toxin and mode of action.

Since the 2006 ACMSF report, a further *C. botulinum* type has been identified. There are eight antigenically different strains of *C. botulinum* A-H (Prathiviraj, Prisilla, and Chellapandi 2016). Type B *C. botulinum* produces two BoNT complexes, 16s and 12s toxins. The 16s toxin has higher toxicity as it is more stable in stomach acid conditions than the 12s. It was found that this increased toxicity is also due to the 16s toxin binding to secretory immunoglobin A which attenuates binding of the toxin to intestinal epithelial cells (Matsumura et al. 2007). Type B BoNT crosses the epithelial barrier of the human intestine using a hemagglutinin complex to disrupt the paracellular barrier of the intestinal epithelium. It was found that type A BoNT crosses the intestinal barrier in the same way as type B BoNT but is more potent. However, type C BoNT was unable to disrupt the paracellular barrier of human epithelial intestinal cells in culture and therefore had no effect (Fujinaga et al. 2009). Once intoxicated, it was previously thought that neuronal cells infected with BoNT could not be further infected by BoNT due to disruption of synaptic vesicle recycling. However, it was found that cultured neurons exposed to BoNT type A could still take up BoNT type E (Pellett et al. 2015).

In animals the most common toxin type is from group III. Animals are affected by toxin types C and D as well as a chimeric C/D strain consisting of two thirds C BoNT and one third D BoNT. The heavy chain receptor-binding domain of this mosaic strain was found to bind synaptosome membranes in cattle better than the D strain alone (Zhang et al. 2011). Group III C-type *C. botulinum* is able to produce a number of different C-type sub-strains, of which some are virulent and others, avirulent. The difference between the virulent and avirulent strains are single point mutations which induce minor structural changes, these avirulent C sub-strains identified serve as potential candidate strains for further vaccine production (Prathiviraj, Prisilla, and Chellapandi 2016).

One paper discusses the acquired thermal tolerance of *C. botulinum*: toxin type A *C. botulinum*, when exposed to prolonged high temperatures, which is reported to change gene expression away from sporulation, to pathways such as carbohydrate metabolism (Selby et al. 2017).

Four papers contained information on novel detection methods of BoNT which have been further developed since the 2006 ACMSF report. The first identified a novel method of detection designed to be used as a replacement for the mouse bioassay. As BoNTs block spontaneous neurotransmission, cultured neurons can be used as an in vitro method for detecting neuromuscular junction intoxication (Beske et al.

2016). Three papers discuss the use of qPCR for detection of BoNT genes. Primers or probes are designed to detect the NonToxin-NonHaemagglutinin (NTNH) regions of the BoNT genes. Four singleplex assays can be used to identify BoNT genes for the A, B, E, and F toxin types. These could be used to identify BoNT producing clostridia in food samples (Fach et al. 2009). This was then further developed into multiplex Taqman qPCR reactions to simultaneously detect A, B, E, F BoNT genes from human samples (Satterfield et al. 2010). This method was also used in a second paper which detailed the use of singleplex Taqman qPCR for the detection of neurotoxin-producing clostridia in food samples (Kirchner et al. 2010). Public Health England diagnostic methods for identifying *Clostridium* species, then differentiation of species and toxin types use a selective media-based anaerobic culture method to grow the bacteria combined with Gram staining and microscopy for colonial appearance, followed by nucleic acid amplification tests (NAATs) such as PCR, qPCR, 16s rRNA sequencing or whole genome sequencing (PHE, 2016).

Conclusion

Since the 2006 ACMSF report, there are now eight toxin types (A-H) and further characterisation of some toxin types such as the mosaic C/D strain has been carried out. Further mechanisms of intoxication have been identified. The use of NAATS in human animal and food diagnosis methods are now possible, and in vitro cell culture based methods as a replacement to mouse bioassays are being developed.

Review Question Two- epidemiology and diagnosis of botulism in animals.

General information

After the full text analysis twenty-two papers contained relevant information on the epidemiology and diagnosis of botulism in animals. Of these, all twenty-two referred to cattle, two papers referred to sheep (Payne et al. 2011; Abdel-Moein and Hamza 2016), two papers referred to goats (Abdel-Moein and Hamza 2016; Böhnel and Gessler 2013) and one paper referred to a human case potentially linked to cattle (Krüger et al. 2012).

Of the twenty-two papers, six were from Germany, three were from England and Wales and two were from France, the other papers were distributed across a range of countries as shown in Figure 4.

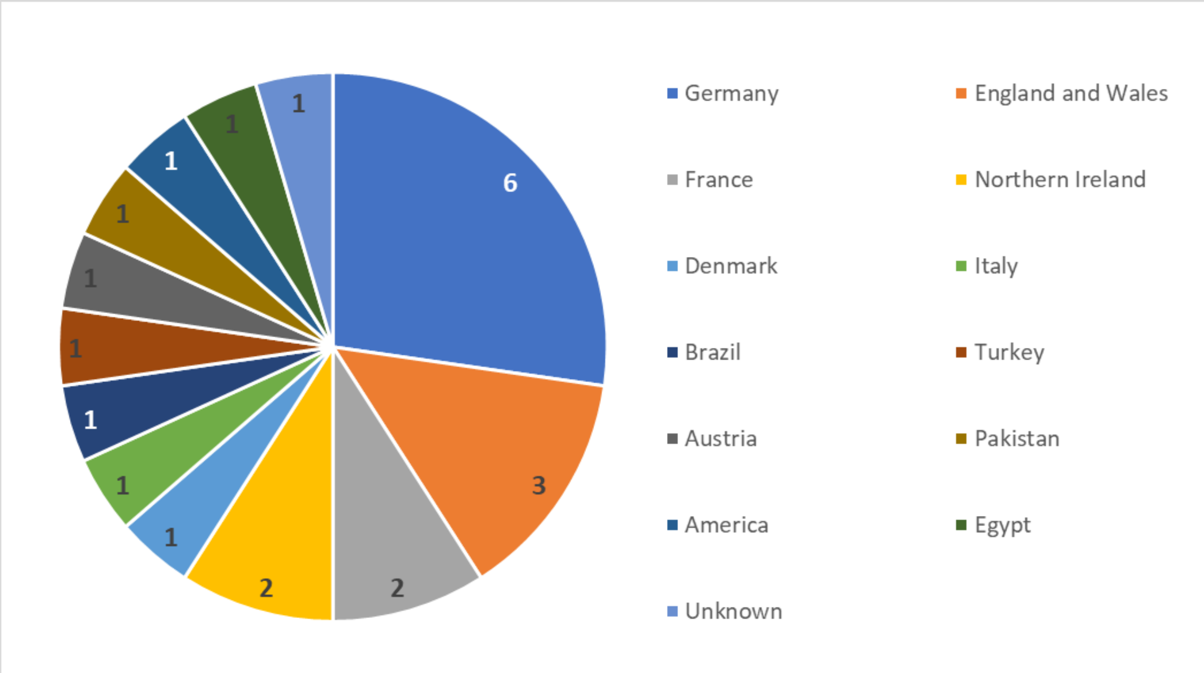

Figure 4: Geographical distribution of papers identified for question 2.

The number of animals assessed in each paper varied, the smallest number of cattle used was three and the largest was 1388 cattle. The two papers which included sheep used 52 and 15 sheep, and for goats 51 and 14. Two papers did not include the number of cattle used.

Eight papers contained information of clinical signs of botulism in cattle, three papers were studies on healthy or recovered animals (Souillard et al. 2015; Abdel-Moein and Hamza 2016; Krüger et al. 2013). Five contained clinical symptoms described as sudden onset: ataxia, recumbence, muscle weakness or stiffness, difficulty swallowing with a loss of tongue tone and excess salivation, loss of appetite and death (Relun et al. 2017; Kümmel et al. 2018; Krüger et al. 2013; Guizelini et al. 2019; Senturk and Cihan 2007). These clinical signs support the description of clinical signs in the 2006 ACMSF report.

Eighteen papers contained information on laboratory-based diagnosis of botulism (Figure 5). Four papers contained information on clinical diagnosis all as stool samples, (Souillard et al. 2017; Abdel-Moein and Hamza 2016; Krüger et al. 2012; Seyboldt et al. 2015), which are cultured on selective media for subsequent diagnostic tests.

The 2006 ACMSF report states that laboratory diagnosis is difficult particularly in distinguishing between toxin types. The accepted methods of diagnosis in 2006 were potential indicatory changes in urine of affected cattle and on occasion visible haemorrhage in the intestines. The most sensitive diagnostic test for *C. botulinum* toxin is the mouse bioassay method (ACMSF, 2006).

Of the eighteen papers identified; seven papers used ELISA as a detection method (Mawhinney et al. 2012; Krüger et al. 2012; 2013; 2014; Brooks et al. 2010; 2011;

Böhnel, Wagner, and Gessler 2008). Six papers used qPCR as a detection method (Souillard et al. 2017; Relun et al. 2017, Abdel-Moein and Hamza 2016; Fohler et al. 2016; Bano et al. 2015; Mariano et al. 2019). Seven papers used the mouse bioassay (Relun et al. 2017; Guizelini et al. 2019; Böhnel, Wagner, and Gessler 2008; Mariano et al. 2019; Kümmel et al. 2018, Brooks et al. 2010; 2011).

Of the papers that used ELISA, 1 paper used ELISA to detect *C. botulinum*, 5 papers used ELISA to detect the presence of BoNT and 2 papers used ELISA to detect spores (after pre-enrichment). ELISA's were found to be as sensitive as the mouse bioassay for identifying spores, BoNT or *C. botulinum*, provided that a heat and pre-enrichment step is used prior to the ELISA (Brooks et al. 2010). qPCR can be used to identify the presence of *C. botulinum* and differentiate between toxin types using primers or probes designed to the NTNH regions on BoNT genes as described by Kirchner et al. 2010. Laboratory methods that do not use live animals for diagnostic purposes are preferential and where possible alternative methods are used however, in some instances where diagnosis is difficult the mouse bioassay is still used supplementary to other diagnosis methods.

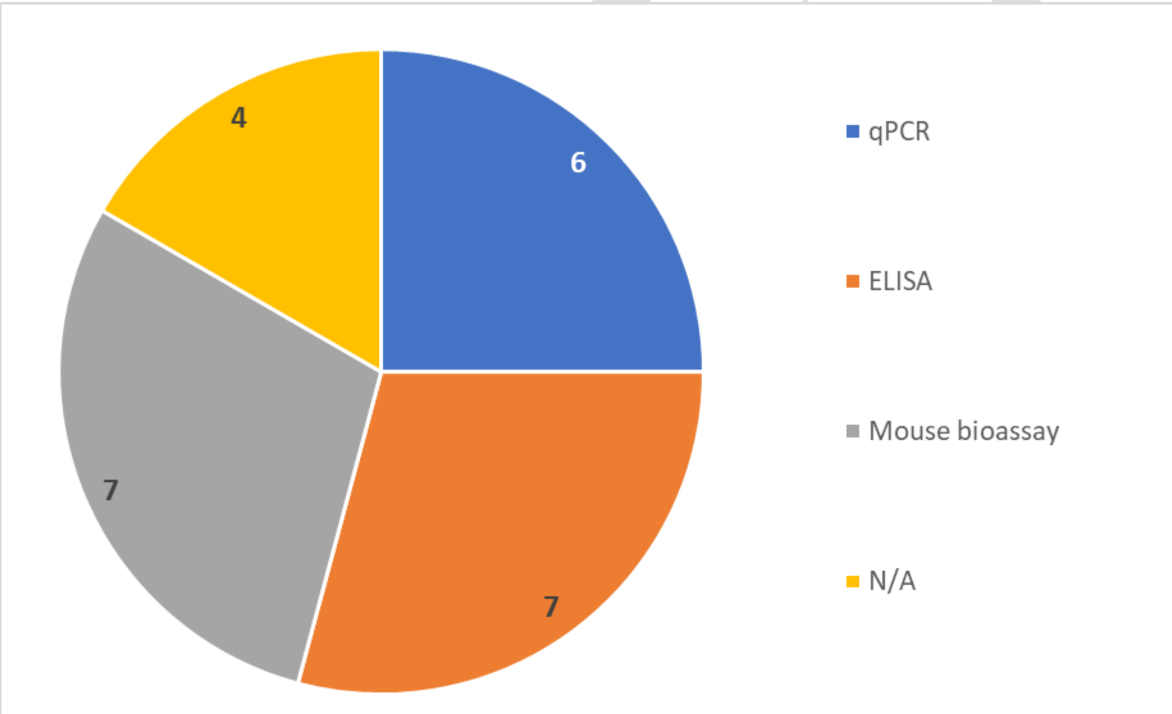

Figure 5; distribution of lab-based analysis methods used, for papers where more than one method was used have been counted for each method.

APHA diagnosis involves analysis of clinical symptoms and a possible cause such as proximity to poultry litter. If a cause such as proximity to poultry litter is not obvious, a post-mortem of the animal is carried out using visual inspection of organs and intestinal contents are tested tor the presence of BoNT by mouse bioassay and if negative, the presence of *C. botulinum*- associated toxin genes by PCR (APHA 2018). The Agri-food and Biosciences Institute in Northern Ireland, uses ELISA and PCR for diagnosis.

Nineteen papers contained information on toxin types (Table 12), eight referred to toxin type C, nine referred to toxin type D, one referred to the chimeric C/D type (Souillard et al. 2015) and four papers contained information on other toxin types.

Table 12: Summary of toxin types contained in literature, some papers refer to more than one toxin type.

	Toxin Type				
	C	D	C/D	D/C	Other
	(Souillard et al. 2015)	(Mawhinney et al. 2012)	(Souillard et al. 2015)	(Dlabola et al. 2015)	(Abdel-Moein and Hamza 2016)
	(Brooks et al. 2010)	(Brooks et al. 2010)		(Mariano et al. 2019)	(Krüger et al. 2012)
	(Brooks et al. 2011)	(Brooks et al. 2011)			(Seyboldt et al. 2015)
	(Krüger et al. 2014)	(Krüger et al. 2014)			(Böhnel, Wagner, and Gessler 2008)
	(Bano et al. 2015)	(Payne et al. 2011)			(Fohler et al. 2016)
	(Guizelini et al. 2019)	(Senturk and Cihan 2007)			
	(Senturk and Cihan 2007)	(Steinman et al. 2007)			
	(Kümmel et al. 2018)	(Kümmel et al. 2018)			
	(Mecitoğlu et al. 2015)	(Mecitoğlu et al. 2015)			
	(Moeller et al. 2009)				
	(Mawhinney et al. 2012)				
Total	**10**	**10**	**2**	**4**	

Four papers described detection of human pathogenic strains of *C. botulinum* in cows, sheep of goats (Table 13). Abdel-Moein and Hamza 2016 tested cattle sheep and goats for type A *C. botulinum*. The results, shown in Table 13, are the percentage of the 18.7% of animals that tested positive for the presence of type A *C. botulinum*. Krüger et al. 2012 tested cows on a farm where there where human cases of botulism. Of the cattle that tested positive 90.9% tested positive for type A *C. botulinum*, however the human cases had a higher incidence of type E BoNT, suggesting that the human cases where not from the cattle and originated from a different source. Fohler et al. 2016, tested for the incidence of BoNT genes from faeces of cattle, the results are summarised in Table 13. Whereas Böhnel and Gessler 2018 tested both cattle and goats for the presence of both human pathogenic strains of *C. botulinum* or the presence of pre formed BoNT defined as ABE, from a number of different organs in cattle. A fifth paper, Seyboldt et al. 2015 tested for the presence of BoNT in cattle faeces, the results showed no detection. These papers demonstrate that on occasion human pathogenic *C. botulinum* strains can be isolated from animals in Germany, alongside the more prevalent C, D and C/D forms.

Table 13: Non C or D toxin types described in the literature with the species these strains were isolated from, the percentage detection rate and method of testing used.

<table>
<tr><th>Paper</th><th>Species</th><th>Toxin Type</th><th colspan="2">Percentage detection rate</th><th>Tested for</th></tr>
<tr><td rowspan="3">(Abdel-Moein and Hamza 2016)</td><td>Cow</td><td>A</td><td colspan="2">2</td><td>C. botulinum</td></tr>
<tr><td>Sheep</td><td>A</td><td colspan="2">5.8</td><td>C. botulinum</td></tr>
<tr><td>Goat</td><td>A</td><td colspan="2">2</td><td>C. botulinum</td></tr>
<tr><td rowspan="2">(Krüger et al. 2012)</td><td>Cow</td><td>A</td><td colspan="2">90.9*</td><td>C. botulinum</td></tr>
<tr><td>Human</td><td>E</td><td colspan="2">64.7*</td><td>C. botulinum</td></tr>
<tr><td rowspan="4">(Fohler et al. 2016)</td><td rowspan="4">Cow</td><td>A</td><td colspan="2">1.7</td><td>BoNT genes</td></tr>
<tr><td>B</td><td colspan="2">2.2</td><td>BoNT genes</td></tr>
<tr><td>E</td><td colspan="2">0.7</td><td>BoNT genes</td></tr>
<tr><td>F</td><td colspan="2">2.2</td><td>BoNT genes</td></tr>
<tr><td rowspan="2">(Böhnel and Gessler 2013)</td><td>Cow</td><td>Human (ABE)</td><td>4</td><td>33</td><td>C. botulinum and BoNT</td></tr>
<tr><td>Goats</td><td>Human (ABE)</td><td>0</td><td>0</td><td>C. botulinum and BoNT</td></tr>
<tr><td>(Seyboldt et al. 2015)</td><td>Cow</td><td>A,B,E,F</td><td colspan="2">0</td><td>BoNT</td></tr>
</table>

*The results for Kruger et al. (2012) refer to a subset of animals. Of the 18.7% tests with positive results, 90.9% of the positive cow related cases were type A and 64.7% of human cases were type E.

Three papers contained information on healthy cattle as asymptomatic carriers of *C. botulinum*. The papers describe that *C. botulinum* can occasionally be found in the rumen and intestine of non-diseased cows (Fohler et al. 2016), particularly type C, *C. botulinum* which can be present without causing illness but induces seroconversion, leading to detectable levels of antibody (Bano et al. 2015). The third paper found that healthy asymptomatic cattle may be intermittent carriers of type C and D *C. botulinum*, particularly when tested following a botulism outbreak within a herd or neighbouring poultry farm. Healthy cattle tested positive for C/D and D/C *C. botulinum*. The prevalence of *C. botulinum* and spores in the environment mean that cattle contamination may occur through indirect contact with contaminated materials including from asymptomatic carriers, however when subsequent testing was carried out two months later no carriage was detected (Souillard et al. 2015). In accordance with the recommendations of the 2009 ACMSF report, clinically asymptomatic animals from farms with clinically suspected botulism are not restricted and products may be used for human consumption. From the papers identified, asymptomatic carriers can be defined as animals that are clinically healthy or asymptomatic but have *C. botulinum* present in their intestine, rumen or faeces. The faeces of asymptomatic carrier animals may test positive for BoNT, *C. botulinum*, or spores and blood tests may show increased antibody production against *C. botulinum* (Abdel-Moein et al. 2016).

Three papers in this search contained information on vaccination against *C. botulinum* type C and D in cattle. The first, tested both vaccinated and un-vaccinated cows for *C. botulinum*, types A, B, C, D, E in field conditions, as well as testing faeces for the presence of BoNT. The vaccinated cattle had elevated levels of antibodies against the C and D *C. botulinum* and a significantly reduced number of spores in their faeces (Krüger et al. 2013). The second paper vaccinated cattle from non-outbreak regions and cattle from outbreak regions that had positive antibodies against BoNT. In both groups the antibodies produced increased against BoNT C after each vaccination. The level of antibody against BoNT D increased after the first vaccination for both groups did not change between the second and third vaccination. There was no difference in the efficiency of the vaccination between the two groups meaning that the natural antibodies did not interfere with the vaccination process (Mecitoğlu et al. 2015). The third paper looked at the effectiveness of the vaccination strategy used. Two-week old calves were injected with a priming vaccination of C and D *C. botulinum*. This was followed by a 4-week booster vaccination. However, samples taken before the 1-year booster was given showed that only 15-30% of the calves were protected. The paper recommended giving an additional booster at 6 months (Steinman et al. 2007). The 2006 ACMSF report states that vaccination against *C. botulinum* is only permitted under a special treatment authorisation in the UK. Since 2010, three vaccines are available under special import to the UK which all prevent against botulism caused by *C. botulinum* C and D toxoids. “Botulism Vaccine” (Onderstepoort Biological Products, South Africa), “Singvac 3 year” (Virbac Australia) and “Ultravac Botulinum Vaccine” (Zoetis Australia). Ultravac is the vaccine referred to by (Krüger et al. 2013).

Conclusion

The ACMSF 2006 report on *C. botulinum* in cattle states that the toxin types most commonly associated with cattle infections are predominantly C and D. Since this report a new chimeric C/D toxin type has been identified and is associated with cattle outbreaks. Since 2006 there have been a small number of further instances of cattle outside of the UK testing positive for human pathogenic botulism strains (A, B, E, F). However no further human cases of C or D botulism have been identified. The most common human pathogenic strain to be identified in cattle and sheep is toxin type A followed by B and F for cattle. These animals were identified in Germany.

The 2006 ACMSF report describes the use of a mouse bioassay as the most informative method of *C. botulinum* detection. Since 2006 there has been a move away from using live animals for diagnostic purposes wherever possible. Testing for the presence of *C. botulinum*, BoNT or spores may now use pre-enrichment followed by ELISA based methods or qPCR. Mouse bioassay is still used for confirmation, or identification of *C.botulinum*, BoNT or spores in more difficult diagnoses. Both APHA and PHE use a mixture of laboratory-based methods including NAATS. Un-tested asymptomatic cattle may carry or test positive for the presence of *C. botulinum*, particularly type C *C. botulinum*, as type C *C. botulinum* produces avirulent strains it is possible that some of the strains carried by health animals are avirulent. It is not clear whether suspected clinically affected individuals are tested for the presence of all seven toxin types or just C and D type BoNT.

Since the 2006 ACMSF report two vaccinations against *C. botulinum* are used in the UK, however, available literature is still debating the efficiency of different vaccination regimes.

Review Question Three- poultry waste

General information

Four primary research papers contained information on incidents involving botulism outbreaks associated with poultry waste. All four papers referred to outbreaks involving cattle and two papers referred to outbreaks in sheep. No papers referred to the outbreaks in goats associated with poultry waste.

No papers were found for an updated definition of poultry waste, or the type of poultry litter and manure that caused the outbreaks.

Two papers detailed botulism in animals caused by proximity to broiler litter (VLA 2011). One paper detailed an outbreak in cattle due to grass silage from pastures alongside where poultry litter was stored and stacked. The silage was potentially contaminated by type D/C *C. botulinum* spores which were potentially spread through wind dispersal or run off from nearby poultry litter. The silage was insufficiently acidified, allowing the growth of *C. botulinum* and production of BoNT However, only the bacteria itself was detected not BoNT. Intestinal samples of one affected cow showed the presence of *C. botulinum* (Relun et al. 2017). One paper details positive *C. botulinum* test results for D/C *C. botulinum* from cattle in farms within a 1km proximity to asymptomatic poultry farms (Souillard et al. 2017).

Four papers detailed the source of risk to animals, two detailed the association of poultry litter with type C, D and D/C *C. botulinum* both cattle and sheep (VLA, 2011; Payne et al. 2011). Two detailed the potential risk of healthy poultry as a potential reservoir of type D and D/C, *C. botulinum* (Souillard et al. 2017; Relun et al. 2017).

Conclusion

Since the 2006 ACMSF report, a number of papers have looked at botulism in poultry and subsequent transmission to cattle. Poultry can be asymptomatic carriers of type C, D and D/C *C. botulinum* and cattle in proximity to seemingly uninfected poultry farms still pose a risk to cattle health. Disposal of poultry litter in or near fields used for growing crops for cattle or sheep feed poses a risk to the animal's health. The associations between poultry litter and botulism outbreaks support the conclusions of the 2006 ACMSF report advising careful management of poultry litter. However, new studies since 2006 have added additional information to this in that poultry may be asymptomatic carriers of C, D and C/D *C. botulinum*. Cross contamination may occur through proximity of cattle, sheep and goats or their feed to poultry farms rather than direct contact with poultry waste.

Review Question Four- risk to public health of products from transfer of botulinum toxin or vegetative spores of *C. botulinum* to milk and/ or meat.

General information

One primary research paper contained information on the risk of transfer of *C. botulinum* to food products and detailed the toxin types found.

The paper details both BoNT and bacteria as present in milk. In this study milk samples from 37 farms affected by botulism in Germany, were tested. Milk from three farms (8.1 per cent) contained BoNT, samples from two farms (5.4 per cent) contained *C botulinum*. Ten udder samples (19.6 per cent) contained toxin, with 7 (13.7 per cent) containing *C. botulinum*. One sample contained both *C. botulinum* and BoNT (Böhnel and Gessler 2013).

The toxin types responsible for human infection described as type A/B/E, identified that both spores and bacteria of this toxin type were present in three of the milk samples (Böhnel and Gessler 2013).

Conclusion

Since the 2006 ACMSF report there have been no reported instances of humans becoming ill from ingestion of contaminated meat or dairy products from cows, sheep or goats affected by botulism. However, one further study identified *C. botulinum* in milk from infected animals. This paper also identified a small number of samples that were type A/B/E human toxin forms of *C. botulinum*. No further papers were found on the heat tolerance of type C, D or C/D in milk (however, studies on the heat tolerance of type C and D were carried out prior to 2007). No further information was identified on the transmission of *C. botulinum* to meat, potentially as clinically suspected animals are prevented from entering the food chain and inspected at slaughter.

Review Question Five- public health advice/ risk to human health from the biological activity of toxins in humans and milk/ meat products.

General information

One primary research paper contained information on public health advice and the risk to human health from botulinum toxins in milk/ meat products. The paper details cows milk as the food product (Böhnel and Gessler 2013).The activity of toxins in milk or other food products is not mentioned. The paper found that in a small number of cases the udders of infected cows were colonised with *C. botulinum*. However, the origin of this infection is not known. It is suggested that the faeces and saliva of both healthy (asymptomatic) and infected cows may contain *C. botulinum* which may be a source of contamination. The recommended advice is to continue to avoid the use of milk and milk products from infected animals until fully recovered (Böhnel and Gessler 2013).

Conclusion

There was only one paper identified as detailing the risk to human health from food products produced from animals with clinically suspected botulism. This paper supports the recommendation of the 2006 ACMSF report which recommended that milk and meat from affected animals 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.

Overall conclusions

Since the 2006 and 2009 ACMSF reports, there are now eight recognised toxin types for *C. botulinum*, with some *C. botulinum* strains now further characterised for mechanism of virulence such as the mosaic C/D strain. Commonly used methods of identification of BoNT or clostridia from food, human and animal samples now include ELISA and NAATs to avoid the use of mice where possible however the mouse bioassay remains the most robust detection method.

Since the 2006 and 2009 ACMSF reports; there have been a small number of instances where human pathogenic strains of *C. botulinum* have been identified from animals outside of the UK. With the most commonly identified human pathogenic strain identified in cattle being toxin type A, followed by B and F, with sheep and goat occurrences of these toxin types being lower than cattle. (Potentially due to the smaller amount of research into botulism in sheep and goats).

Healthy cattle may be asymptomatic carriers of *C. botulinum*, particularly those within close proximity existing outbreaks or poultry farms. Poultry may be asymptomatic carriers of C, D, D/C type *C. botulinum* and there have been further instances of outbreaks of botulism in cattle and sheep associated with poultry litter, however there have been no outbreaks involving goats in association with poultry litter.

There have been few further papers published on the transmission of botulism from animals to food. One paper identified *C. botulinum* spores in milk identifying human toxin type (A/B/E) spores in milk. Type B spores are more heat resistant than type A spores which could cause an issue if the frequency of animal cases caused by type B *C. botulinum* were to increase, however the number of animal cases caused by type B C. botulinum are to date is very small (see question 2). The faeces and saliva of cows may contain *C. botulinum* spores, which is potentially be a source of contamination if good hygiene practices are not adhered to when milking. There was only one paper detailing the risk to human health from contaminated food (Böhnel and Gessler 2013); this paper supported the safety recommendations of the 2006 and 2009 ACMSF report update where milk and meat from affected animals is voluntarily withdrawn.

Uncertainties and future considerations

The ACMSF in 2009 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 is no requirement to restrict the slaughter of healthy cattle from herds where cases of confirmed or suspected botulism have occurred. However, the identified literature described that there have been identified cases of asymptomatic cattle still testing positive for *C. botulinum*. The strain of *C. botulinum* most commonly identified from these animals was strain C and avirulent strains of type C *C. botulinum* have been identified. However, it could not be established from the literature whether the asymptomatic animals were carrying a virulent or avirulent strain. This literature would be useful to establish whether asymptomatic carrier animals pose a risk to human health, particularly in the case of “raw” unpasteurised drinking milk.

The literature identified that sheep are more susceptible to type A botulism (a human pathogenic strain) than cattle, but the amount of published research on botulism in sheep is considerably less than cattle. The literature review identified gaps in literature around the instances of botulism in sheep, particularly whether *C. botulinum* can be detected in sheep milk. However, the literature identified that there are fewer instances of botulism in goats particularly type A botulism. No literature was identified that examined the presence of C. botulinum in goat milk.

From the literature identified, it is not clear exactly how *C. botulinum* spores get into milk. The literature has identified that *C. botulinum* cannot cross the blood-milk barrier, and that the suckling calves of adults do not also become ill. However, *C. botulinum* was isolated from udder swabs and milk samples. Further literature was not identified to detail how this contamination occurs, or the precise frequency of this occurrence.

Recommendations

The recommendations from both the 2006 and 2009 ACMSF reports have been included below, some of the recommended further work may have been completed or partially completed.

Recommendations from the 2006 ACMSF report

Epidemiology and diagnosis of botulism in cattle

- 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).
- Work should be undertaken to understand the diagnostic and clinical significance of finding botulinum toxins in gastrointestinal contents of cattle.
- 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.
- Samples collected during clinical investigations should be archived to assist with the development of further assay systems.

Poultry waste

- We recognise a need to reinforce DARDNI and VLA/DEFRA messages on the use and disposal of poultry litter (Annexes 4 and 5 in ACMSF 2006) 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.
- 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

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

- 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.
- 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

- 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.
- 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.
- 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.

Recommendations from the 2009 ACMSF report

- In the absence of other signs, there should be no requirement to restrict meat or milk from healthy sheep or goats from farms where there have been suspected cases of botulism.

- The incidence of toxin types other than C and D among sheep and goats should be monitored and the situation should be reviewed if there is evidence for the types associated with human disease.
- UK agriculture departments should reinforce their advice to farmers involved in the production, storage and spreading of poultry litter on measures for the prevention of on-farm botulism and the FSA should work closely with the poultry industry and enforcement bodies 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 sheep and goat farmers.
- UK veterinary authorities should continue to encourage sheep and goat farmers to report suspected cases of botulism

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