Chapter 9

Spread and Control of Prion Diseases in the Food and Feed Chains

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/62118

Abstract

Prion diseases, or transmissible spongiform encephalopathies (TSEs), are a group of chronic, progressive, and fatal neurodegenerative disorders that affect a variety of mammalian species. This chapter discusses the issues raised by two foodborne prion diseases, namely bovine spongiform encephalopathy (BSE) and variant Creutzfeldt–Jakob disease (vCJD), particularly those related to their spread in cattle and humans, the contamination of specified risk material (SRM) in meat, the relevant regulations, and appropriate detection methods for surveillance.

Keywords: Bovine spongiform encephalopathy, variant Creutzfeldt–Jakob disease, specified risk material, central nervous system tissues, regulations, detection methods

1. Introduction

Prion diseases or transmissible spongiform encephalopathies (TSEs) are a group of chronic, progressive, and fatal neurodegenerative disorders that affect a variety of mammalian species. They include scrapie in sheep and goats, chronic wasting disease in deer and elk, bovine spongiform encephalopathy (BSE or so-called "mad cow disease") in cattle, and variant Creutzfeldt–Jakob disease (vCJD) in humans.[1] Since the term "prion" was coined by Dr. Stanley Prusiner in 1982,[2] the extensive research on prion diseases has investigated their molecular biology,[3] causes,[4] pathogenesis,[5] genetics,[6] types,[7] biochemical mechanisms,[8] and therapies,[9] among other aspects. A prion is a proteinaceous infectious particle that lacks nucleic acid.[7] Abnormal prions (PrP^{Sc}) are the pathologic isoforms of prions and are expressed in specified risk material (SRM) such as bovine central nervous system (CNS) tissue.[10] PrP^{Sc} has a high β-sheet content, is extremely resistant to heat and proteases, and is insoluble in non-denaturing detergents.[11, 12] Infection of prion diseases occurs naturally via

the oral route as well as by blood transfusion and maternal routes.[13] This chapter reviews the research into two foodborne prion diseases, BSE and vCJD, related to their spread in cattle and humans, the contamination of SRM in meat, the relevant regulations, and the detection methods available for surveillance.

2. Spread of BSE and vCJD

Research into clinical BSE cases has reported that 84.3% of the BSE infectivity is associated with bovine CNS tissue.[14] Another study found that the CNS tissue carries 89.7% of the infectivity.[15] With regard to the pathogenesis of BSE, animal by-products such as meat and bone meal (MBM), which have been used as nutritional supplements in livestock feedstuffs for more than 100 years, are major carriers of the infectious agent PrP^{Sc}.[16, 17] Significant changes in the manufacturing processes of feedstuffs that took place in the 1970s, including the introduction of mechanical systems that permitted continuous flow production and solvent extraction of fats, enabled PrP^{Sc} to enter the livestock feed chain.[18, 19] The recycling of animal by-products from BSE-infected animals caused a wide spread infection of BSE in cattle,[19] subsequently affecting the food chain through the consumption of the PrP^{Sc}-contaminated meat products.[20] To date, more than 190,000 BSE cases have been reported in 28 countries since it was first identified in the UK in around 1986,[21] with approximately 97% of the cases being reported in the UK (Figure 1).[22] It has been estimated that the total number of BSE-infected cattle is around one million, of which around 75% entered the human food chain during the 1980s and early to mid-1990s.[23, 24] BSE has had a huge impact on the beef industry worldwide.[25-30] In the USA, estimates of the total loss of beef and offal exports ranged from $3.2 billion to $4.7 billion in 2004 after the nation's first BSE case was confirmed at the end of 2003.[31] Although the link between BSE and vCJD through eating prion-contaminated meat has not been conclusively proven, the consumption of food of bovine origin contaminated with PrP^{Sc} has been strongly linked to the occurrence of vCJD in humans.[20] Both vCJD and BSE share the same infectious agent, PrP^{Sc}.[32, 33] A total of 229 vCJD patients have been identified in 12 countries since it was first discovered in the UK in 1996 (Figure 1),[34, 35] of whom about 77% were in the UK (Figure 1).[36] It should be noted that following the successful containment of the BSE epidemic through the imposition of strict feed controls for ruminant animals, the numbers of cases of both BSE and vCJD in the UK have declined significantly since 1992 and 2000, respectively (Figure 1).

3. Contamination of SRM in meat products

During the animal slaughter process, two major SRM, brain and spinal cord material, can easily contaminate meat products.[37-40] In general, there are four main pathways for the contamination of CNS-based SRM in meat products, which can occur at any stage during these processes: animal stunning, animal pithing, carcass dressing, and advanced meat recovery. The risks associated with each process have been studied and are discussed in turn below in

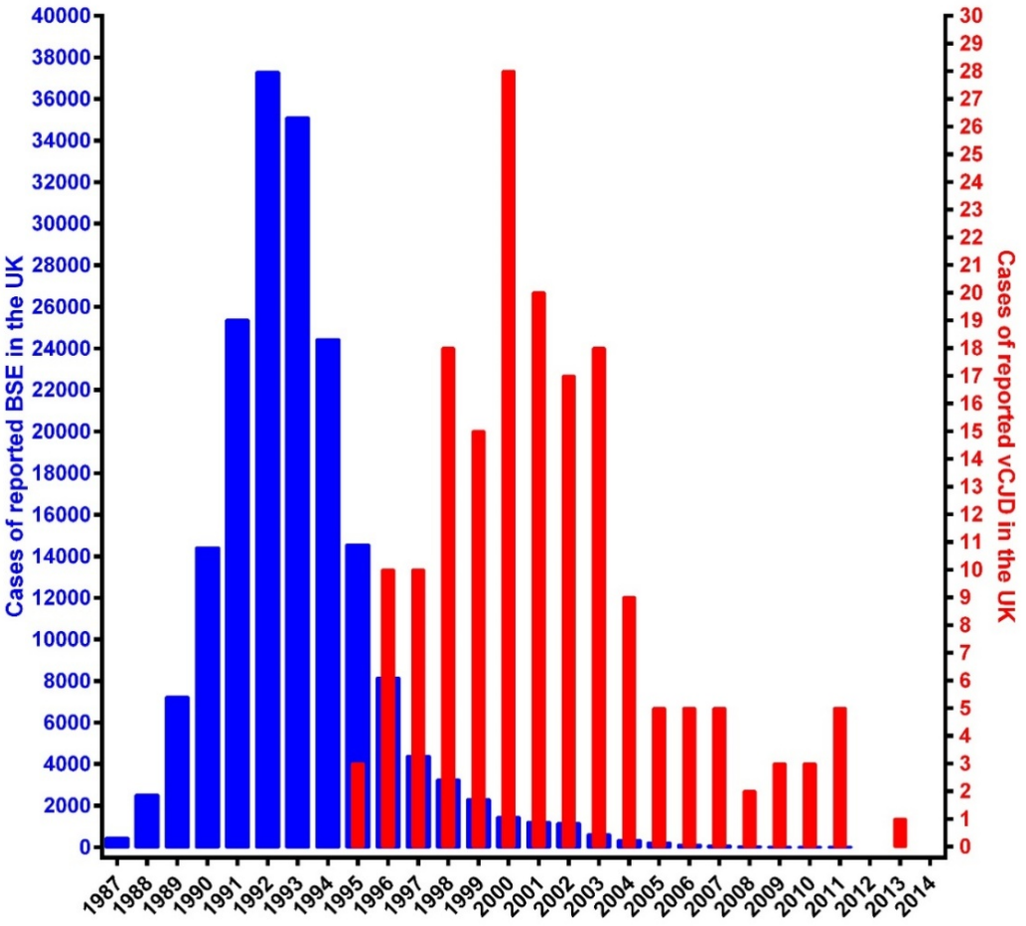

Figure 1. Number of reported BSE-cases (blue) and number of reported vCJD-cases (red) in the UK (1987-present)[22, 36]

the following sections. The major results of the relevant studies for each of these four pathways are summarized in Table 1.

3.1. Animal stunning

The stunning method has been widely used in many countries and areas of the world for some time as it slaughters the animals humanely. Stunning renders the livestock unconscious before slaughter, but because the heart of the stunned animal continues pumping for several minutes after stunning, during this time any CNS tissue that enters the jugular venous blood could still be spread throughout the body, contaminating both muscle and bone marrow via the blood circulation.[41] Several CNS markers that have a molecular weight similar to the PrP^{Sc} (MW: 30 kDa),[3] including syntaxin 1B and glial fibrillary acidic protein (GFAP),[42, 43] have been detected in non-CNS tissue after stunning. It thus seems likely that PrP^{Sc} could also be present in the edible carcass after stunning. Two types of captive bolt stunner, penetrative and non-penetrative, are widely used to stun domestic animals prior to bleeding (Figure 2). Most abattoirs prefer to use penetrative captive bolt stunners, either with or without air injection, to render cattle quickly and painlessly unconscious before slaughter.

Routes by which meat becomes contaminated by SRM			Location of SRM	SRM-contaminated samples (%)	Species		Detection methods					References
					Cattle	Sheep	Microscopy	Macroscopy	Immunoassay			
									ELISA[a]	IB[b]	IHC[c]	ICH[d]	
Animal stunning	Penetrative stunning with air injection		Left and right branches of the main pulmonary artery	2.5–5	√			√					[46]
			Right ventricle	33 (n^b=1050)	√			√					[51]
			Jugular vein	26.7 (n=15)	√		√		Syntaxin 1-B Annexin V			S100β	[48]
			Jugular vein	13.3 (n=15)		√	√		Syntaxin 1-B			S100β	[49]
			Aorta	18.2 (n=11)		√						NF[e] S100	[47]
			Heart, lung, liver and kidney					√					[52]
	Penetrative stunning without air injection	Pneumatic-powered stunning	Heart	12 (n=450)	√			√					[51]
		Cartridge-operated stunning	Heart	1 (n=480)	√			√					[51]
			Jugular vein	13.3 (n=15)		√						S100β	[49]
			Pulmonary artery and right ventricle	8.5 (n=412)				√					[56]
			Jugular vein	4 (n=100)	√		√		GFAP[f]			S100	[55]
			Jugular vein	1 (n=360)	√				GFAP				[178]
			Carcass surface	100 (n=30)	√				GFAP				[43]
	Non-penetrative stunning		Jugular vein	2 (n=100)	√		√		GFAP			S100	[55]
			Carcass surface	83.3 (n=30)	√				GFAP				[43]
Animal pithing			Jugular vein	6.3 (n=16)	√		√		Syntaxin 1-B Annexin V			S100β	[48]
			Pulmonary artery and right ventricle	4.1 (n=314)				√					[56]
Carcass dressing			Hand-held screen, tray, apron, and internal surfaces of the carcass along the cut vertebral surface		√				S100β				[69]
			Sawing water, areas of thoracal and cervical regions of the carcass		√					NSE[g]			[56]

http://dx.doi.org/10.5772/62118

Table 1. Summary of the results of SRM contamination through the four major routes during animal slaughtering and meat processing

Routes by which meat becomes contaminated by SRM	Location of SRM	SRM-contaminated samples (%)	Species		Detection methods						References
			Cattle	Sheep	Microscopy	Macroscopy	Immunoassay				
							ELISA[a]	IB[b]	IHC[c]	ICH[d]	
	Captive bolt, floor of the receiving platform, aprons, hands, knives, captive bolt aperture, head wash water, spinal cord channel, saw, table striploin, conveyor belt, and beef hide		√				Syntaxin 1B GFAP				[42]
	Captive bolt gun/hole, apron, hands, saw, spinal cord channel, and meat		√				Syntaxin 1B GFAP				[67]
	Carcasses, loin, forerib, minced meat, worktable surface, saw, and air samples		√				S100β GFAP				[179]
	Split vertebral face and saw		√				GFAP				[69]
AMRS[i]	Meat	35	√								[77]
	Meat	41.2 (*n*=17)	√						NF Synapto physin GFAP		[75]
	Meat	17.2 (*n*=279)	√						GFAP		[76]

[a]ELISA, enzyme-linked immunosorbent assay; [b]IB, immunoblotting; [c]IHC, immunohistochemistry; [d]ICH, immunocytochemistry; [e]NF, neurofilament; [f]GFAP, glial fibrillary acidic protein; [g]NSE, neuron-specific enolase; [h]*n*, sample size; [i]AMRS, advanced meat recovery system.

Note: NF is heteropolymer intermediate filaments that are composed of three neurofilament subunits (NF-L, NF-M and NF-H), which are expressed mainly in neurons.[180]

S100 protein (MW: 10.5 kDa) is a calcium-binding protein found predominantly in the vertebrate nervous system.[181] Syntaxin 1B (MW: 33 kDa) which is an integral membrane protein exclusively and abundantly expressed in nervous tissues.[182]

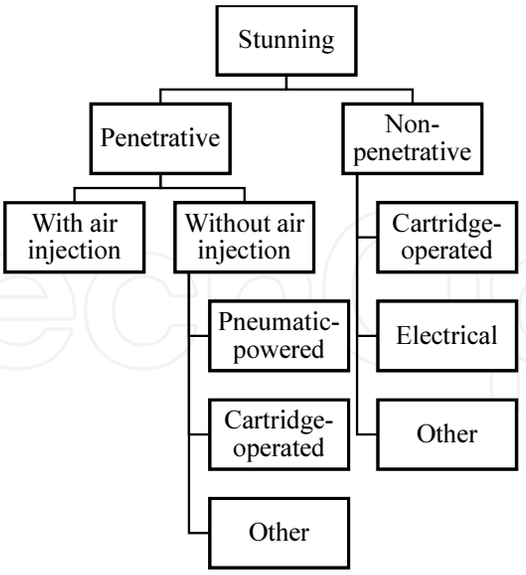

Figure 2. Classification of animal stunning techniques

3.1.1. Penetrative stunning with air injection

This stunning technique involves the injection of compressed air into the cranium of cattle to effectively disrupt the brain structure. In 2001, it was estimated that 15% of cattle were stunned using an air-injection stunner in the USA.[44] However, penetrative stunning with air injection poses a major risk of spreading the PrP^{Sc} from the BSE-infected CNS tissue to the edible carcass. Similarly, it is well known that severe penetrating injuries to the human brain, such as a gunshot wound, can cause acute brain damage and produce cerebral emboli that end up in the lung.[45]

For each BSE-infected animal stunned with this type of stunner, there is a 31.2, 16.3, 3.3, and 0.7% probability that a portion of the PrP^{Sc} would be transferred to the blood, heart, lung, and liver, respectively,[44] which is significant because it has been estimated that 60% of the liver, 50% of the heart, 25% of the kidney, and 5% of the blood are potentially available for human consumption.[44] Studies have also found that an air-injection stunner forced visible pieces of CNS tissue into the circulatory system of stunned cattle and sheep.[46, 47] This device has been shown to cause the formation of grossly visible brain tissue in the left and right branches of the main pulmonary artery in 2.5–5% of cattle,[46] in the jugular venous blood of cattle and sheep,[48-50] and in the aortic blood from sheep.[47] Particles of brain tissue as large as 20 μm in diameter can pass through the ovine pulmonary capillary network and enter the systemic arterial circulation during stunning, suggesting that this technique could easily disperse PrP^{Sc} throughout the edible carcass.[47] It has also been reported that CNS tissue has been observed in 33% of cattle hearts,[51] and in the kidneys of cattle stunned with air-injection stunners.[52]

Another factor to consider is that on occasion when operation of air-injection stunners fails, leading to an increase of CNS emboli formation and deposition, the probability of PrP^{Sc} transfer

could be nearly 50% higher in blood, twice as high for the heart, and 10 times higher for the lung and liver.[44] Furthermore, a slaughterer who uses this type of stunner for too long or uses it successively to immobilize the same cattle could significantly increase the CNS tissue dispersion in blood vessels of the carcass.[51] It is also common for this type of stunner to press brain material out of the brain cavity through the bolt hole in the animal skull and splash onto the operator's face.[41, 53] Based on these results, the use of this type of stunner has been prohibited in many BSE-reporting countries and areas.

3.1.2. Penetrative stunning without air injection

This type of captive bolt stunner has a sharp-rimmed steel bolt and is powered by either compressed air (pneumatic-powered) or a blank cartridge that produces sufficient penetration force to initiate trauma to the cortex. It is the preferred tool for stunning cattle in the EU; 79 and 96% of the slaughterhouses in Europe use this method for cattle and calves, respectively.[54] However, the use of pneumatic-powered stunners and cartridge-fired stunners produced visible CNS clots in 12 and 1% of the cattle heart samples, respectively.[51] In addition, after using this type of stunner, CNS tissue was detected in the jugular venous blood from sheep,[49, 50] in venous blood from cattle,[55] in bovine pulmonary arterial system,[56] and on the surface of beef carcasses.[43]

In a study designed to trace the spread of CNS tissue in the cattle body after stunning with a cartridge-fired stunner, a marker microorganism, *Pseudomonas fluorescens*, was inoculated into the bovine brain via the stunning procedure.[57] The researchers reported finding this bacterium in the animal's blood, spleen, liver, kidney, lymph node, lung, and spinal cord. Using a similar approach, two microorganisms, *Escherichia coli* and *P. fluorescens*, have been detected in the blood, liver, lung, spleen, lymph node, longissimus muscle, and on the carcass surface of stunned sheep.[58] These studies demonstrate that the use of penetrative stunning without air injection could still spread CNS tissue throughout other portions of the carcass.

As with air-injection stunning, multiple use of this type of stunner also poses a high risk of spreading PrP^{Sc} from the infected brain to the edible carcass. In 2004, as few as 71% of slaughterhouses in the USA using penetrating captive bolt stunning successfully stunned 99–100% of the cattle with one shot and only 50% of abattoirs in Canada achieved the same target.[59] In 2005, this percentage decreased to 55% in US beef plants.[60] Since then, the percentage has increased gradually in both countries, reaching 91% in the USA by 2011.[61] In the EU, about 4–6.6% of captive bolt stunning in cattle requires a second stun.[54] Such practices could increase the risk of CNS tissue contamination in meat products. Although this type of stunner exerts less impulsive force on the stunned animal compared with air-injection stunners, it remains a threat to meat safety because this type of stunner is still in use worldwide.

3.1.3. Non-penetrative stunning

Non-penetrative stunning devices include the non-penetrating cartridge-operated stunner and the electrical stunner. Several studies have reported that no CNS tissue was detected in either aortic or venous blood samples collected from cattle or sheep after using these two devices.[48-50] However, other studies have detected brain tissue in venous blood samples

from stunned cattle,[55] and on the surface of beef carcasses[43] after non-penetrating stunning. There also is an increased risk to operators as some animals may be inadequately stunned with non-penetrating stunning and recover their consciousness during slaughtering. Electrical stunning is the main alternative method for stunning cattle, but it is expensive to install and meat quality defects such as petechial hemorrhages have been linked to electrical stunning in sheep.[62]

3.2. Animal pithing

Pithing is the insertion of an elongated rod-shaped instrument into the cranial cavity of a stunned animal to further lacerate the CNS tissue.[63, 64] This operation prevents the animal stunned by a penetrating captive bolt from recovering permanently.[52] Pithing is considered by the industry to be a more effective technique for maintaining the safety of the operator and was used in 70% of UK abattoirs as recently as 1997.[50] However, in 2001, this technique, which has never been used in the USA,[52] was banned in the EU.[65] Brain tissue has been detected in venous blood[48, 50] and arterial blood samples[56] from cattle after pithing.

Based on scientific evidence of the dispersion of CNS-based SRM in response to various stunning and pithing techniques, the EU has ranked the stunning methods used in ruminants, from the most hazardous to the least dangerous, as: (1) penetrative stunning with air injection; (2) penetrative stunning without air injection; (3) captive bolt stunner with pithing; (4) captive bolt stunner without pithing and free bullet; (5) non-penetrative stunner and electro-narcosis.[66] Two techniques, air-injection stunning and pithing are now prohibited worldwide. However, there is still a 50% probability that emboli will be deposited in the blood of an animal stunned with a non-air-injection stunner.[44]

3.3. Carcass dressing

During animal slaughtering, the general sequence followed during the dressing procedure is stunning followed by exsanguination, dehiding, evisceration, splitting, washing, and chilling.[42] Of these steps, stunning is not the only point at which the carcass can potentially be contaminated with CNS tissue; other steps in the slaughtering procedure also have a high probability of dispersing CNS-based SRM across the carcass (Table 1). CNS tissue has been found in samples collected at a number of points along the slaughter line, including on the captive bolt gun, on the aprons worn by the operators, on their hands and the saws used, and in the longissimus muscle.[67]

In a study tracking two marker microorganisms, *E. coli* and *P. fluorescens*, both bacteria were transferred from the first stunned sheep to the stun wounds of the next 10 sheep stunned using the same contaminated penetrative cartridge-operated pistol.[58] These bacteria were also detected in the sheep's blood and on their carcass surfaces.[58] Perhaps most worryingly, the marker organisms were found in the air, and on the hands and aprons of the operator.[58] In another similar study,[57] the marker organism *P. fluorescens* was detected in the slaughter environment immediately after stunning and at each subsequent stage of the slaughter-dressing process, including on the hands of the operators, the slaughter equipment (captive bolt gun and knife), the cattle hide, and the carcass splitting saw.

Spinal cord material is easily spread to bovine carcasses during carcass splitting. The majority of the CNS contamination was found on the internal surfaces of the carcass and along the cut vertebral surface, with lesser levels of contamination inside the body cavity.[68] CNS tissue was also detected in the surroundings during splitting, including on the hand held screen, the tray and apron, the captive bolt, the captive bolt aperture, and the floor of the receiving platform.[42, 69] Spinal cord tissue can also be transferred to subsequent bovine carcasses during carcass splitting. The main risk of subsequent carcass contamination comes from the splitting saw.[68] Overall, these studies demonstrate that the practice of carcass dressing, especially splitting, can lead to the extensive spread of SRM within the abattoir environment, contaminating equipment, surfaces, operators, and edible carcasses. There are also grounds for concern regarding operator safety when dealing with CNS tissue, particularly due to the aerosol of cerebrospinal fluid and spinal cord produced during the splitting process.

3.4. Advanced meat recovery

Mechanical systems have been developed to separate meat from bone by scraping, shaving, or pressing the meat from the bone, for example, via advanced meat recovery systems (AMRS). AMRS enables processors to remove any remaining muscle tissue from beef carcasses without breaking the bones.[70] About 70% of fed cattle and hogs, and 60% of cows slaughtered in the USA were processed using AMRS in 1998[71] and around 5,000 metric tons of mechanically recovered meat (MRM) were produced yearly during the period 1980–95 in the UK.[72] Although AMRS makes deboning more efficient and considerably faster than using a knife, if spinal cord material is attached to the spinal column as it enters these machines, it can be incorporated into the meat product produced.

A number of studies have detected CNS tissue in MRM products (Table 1).[73-76] In 2002, about 35% of US bovine MRM contained CNS tissue[77] and other studies detected spinal cord tissue in 29%[77] and 33.3%[75] of bovine MRM samples. Dorsal root ganglia tissue was found in 10% of the MRM samples.[77] Schmidt *et al.*[74] reported that 50% of the bovine MRM samples from 14 slaughter plants that they tested were positive for CNS protein. In a later study by the same group,[76] 17.2% of the MRM samples tested were contaminated by CNS tissue.

By the 1990s, about 90% of the beef MRM produced was being used in burgers in the UK.[78] Around 75–80% of individuals surveyed in France consumed MRM products from burgers, and the consumption of burgers increased by 40% over the period 1980–1995.[79] Frequent consumption of beef and beef products, including burgers and meat pies, containing the MRM or head meat has been linked to an increased risk for vCJD;[80] infection with vCJD is thought to be predominantly due to exposure to BSE in beef MRM and head meat products.[81]

Looking at the results of the present review (Table 1), every stunning method except for electrical stunning could potentially contaminate meat products by dispersing CNS-based SRM, including any PrP^{Sc} present in sub-clinically infected cattle. Although the use of air-injection stunning, pithing, and AMRS has now been prohibited during animal slaughtering and meat processing (Table 2), penetrative stunning without air-injection and non-penetrative stunning are still in widespread legal use. Slaughter conditions and procedures such as carcass

dressing, especially the splitting process, can also result in widespread contamination within abattoirs, contaminating equipment, surfaces, operators, and carcasses destined for human consumption. CNS contamination of bovine carcasses simply cannot be eliminated by the slaughtering technology currently used.

4. The regulation of SRM

The contamination of animal carcass by SRM is not only a food and feed safety issue, but also of global economic importance, leading countries or areas such as the UK, the EU, Japan, Canada, and the USA to institute strict regulations to prevent SRM entering the human food/animal feed chain (Table 2). Although SRM has different definitions in different countries and areas, bovine CNS tissue (brain and spinal cord) tops every list of prohibitive materials due to its high prion infectivity.

Prohibited activity	UK	EU	Japan	USA	Canada
Air-injection stunning	April 1, 2001	April 1, 2001	N/A*	January 12, 2004	July 24, 2003
Pithing	April 1, 2001	January 1, 2001	N/A	Never be used	July 24, 2003
AMRS	December 15, 1995	October 1, 2000	N/A	January 12, 2004	July 24, 2003
SRM in food	December 17, 1997	January 1, 1998	July 4, 2002	January 12, 2004	August 23, 2003
SRM in feed	January 1, 1998	January 1, 2001	October 2001	April 27, 2009	July 12, 2007

* N/A, not available

Table 2. Legislation related to prion disease control

Given that it was the first and most seriously area affected, it is not surprising that the early legislation to control the spread of prion diseases came from the UK. In 1995, the UK banned the use of MRM from bovine vertebral column for human consumption[82] and in 1996, the "over thirty months" rule came into force, which automatically banned older cattle from entering the human food chain.[83] In 1997, the UK enacted a comprehensive set of SRM regulations,[84] which classified SRM into specified sheep or goat material and specified bovine material. It also emphasized that no person should use or sell any SRM, or any food containing SRM, for human consumption. To further prevent SRM from entering the human food chain, the UK prohibited the practice of pithing in 2001.[85] The EU was also quick to take action; since the consumption of BSE-infected feedstuffs by ruminants was rapidly identified to be the main BSE transmission channel, in 1994 the EU banned the use of proteins originating from mammalian tissues for feeding ruminants.[86] Largely as a result of this feed ban, the UK's BSE risk status dropped from high to low between 1996 and 2012. Since 2013, the regulations have been relaxed somewhat and cattle aged over 72 months (O72M) are now permitted to enter the food chain if they have tested negative for BSE.[87]

http://dx.doi.org/10.5772/62118

In 2001, the World Health Organization recommended that all countries should introduce risk management procedures such as the identification and removal of entire bovine heads and/or prohibiting the harvesting of all MRM. All tissues that have been shown to be capable of carrying BSE infectivity should be removed and destroyed. If the risk is high, additional precautions should be taken such as prohibiting cattle over a certain age from entering the food chain.[88] The World Organization for Animal Health suggests that when countries import beef products from a BSE risk country or area, meat processing should not use air-injection stunning or pithing.[89]

In the EU, the use of air-injection stunning has been banned for slaughtering cattle since 2001; [90] it has also prohibited pithing since 2001.[65] In 1997, the EU enacted strict regulations prohibiting the use of SRM,[91] including the skull, brain, eyes, tonsils and spinal cord of cattle, sheep, and goats aged over 12 months and the spleens of sheep and goats. This was later extended to include bovine intestines in 2000[92] and vertebral columns in 2001.[93] Since 2000, all the member states of the EU, including the UK, are prohibited from producing MRM from bones of the head and vertebral columns of bovine, ovine, and caprine animals,[65] and this was extended to include all types of bones from these three species in 2001.[93] From 2001 onwards, SRM was excluded from the feed chain as a result of an EU-wide ban on the feeding of processed animal protein to all farmed animals.[94] In 2013, BSE testing in the EU has been changed to the O72M rule.[87]

Among the Asian countries, Japan has been the most severely affected by TSE, with about 36 cases of BSE and one case of vCJD being confirmed since 2001.[21, 95] In 2002, Japan announced that bovine MBM could not be used as an ingredient in animal feedstuffs and prohibited the use of specified materials from cattle.[96] However, the bovine vertebral column can still be consumed as food if it is derived from cattle originating from a BSE-free country or zone.[97] Because the BSE risk status has moderated somewhat, the age of cattle subject to inspection for BSE in Japan was revised upward from zero months to 21 months in 2005.[98] In 2013, it was further revised and is now only required for cattle over 48 months of age.[99]

In Canada, the first cases of BSE and vCJD were reported in 2003 and 2002, respectively, triggering a food directorate policy on SRM in the food supply that was implemented in 2003. [100] This required the removal of SRM from all cattle during the slaughtering process and prohibited its sale or import for human consumption.[101] The vertebral column from all cattle aged 30 months or older must now be removed as an inedible product and cannot be used in the preparation of MRM.[102] Canada has also prohibited the use of air-injection stunning and the pithing technique for cattle.[102]

The USA banned the use of proteins originating from ruminant tissues for feeding ruminants in 1997.[103] Later, the USA issued a prohibition of SRM consumption as food in January 2004, adopting a definition for SRM that is similar to that used in Canada.[104, 105] To ensure that AMRS do not become a means of spreading CNS tissue into meat products, the USA has also prohibited the use of brain, spinal cord, dorsal root ganglia, trigeminal ganglia, and significant amounts of bone solids or marrow of all cattle, as well as the skull and vertebral column of cattle 30 months of age and older, in AMRS.[106] The National Animal Identification System, an efficient and effective animal identification program, has been implemented in the USA

since 2004 to support animal disease monitoring, surveillance, and eradication programs.[107] The USA has also prohibited the use of a number of cattle materials, including CNS-based SRM, in animal feedstuffs since 2009.[108] The USA currently prohibits the slaughter of cattle that are unable to stand or walk ("downer" cattle) when presented for pre-slaughter inspection[109] and in January 2004 also banned air-injection stunning of cattle; pithing has never been used.[52] From January 2004 to May 2005, the USDA performed inspection and verification procedures in about 6,000 meat and poultry establishments and found 1,036 procedures (< 1%) that were not in compliance with the regulations related to SRM.[110] In 2008, about 1.5% of the US companies handling materials prohibited from use in ruminant feed (n=7,876) committed technical violations, mostly consisting of minor recordkeeping lapses or conditions involving non-ruminant feeds.[111] However, in January 2006, Japan suspended all US beef imports after discovering SRM in beef products exported from the USA.[112] In South Korea, US beef imports did not resume until June 26, 2008, after having been suspended in 2003 after the first BSE case was reported in the USA.[113]

Based on statistical information related to MBM,[114] it is clear that the problems with SRM-contaminated feed should not be forgotten. In 2000, the total amount of mammalian MBM produced in the USA was about three million metric tons.[115, 116] Ruminant tissues have been prohibited in ruminant feed in the USA to prevent the spread of BSE since 1997[103] and about 41,520 metric tons of animal feed in the USA were recalled between 2006 and 2007 due to the omission of the cautionary BSE statement on the label or as a result of ruminant MBM contamination during the feed processing procedure.[117] As the data shown in Table 1, it is very easy for meat products to become contaminated by bovine CNS-based SRM when the animal is slaughtered and during meat processing. In 2012, about 53% of the red meat produced in the USA. was beef,[118] but fewer than 1.2% of the cattle slaughtered are tested for BSE each year.[119, 120] As part of the regulation enforcement, about 65,693 metric tons of ruminant SRM-contaminated cattle products in the USA have been recalled since 2003.[121]

5. Detection methods for surveillance of prion diseases

As international trade continues to increase, in order to successfully enforce food and feed safety regulations and avoid economic loss, many nonclinical methods for the detection of the contaminated products have been developed. Overall, these detection methods can be classified into two types: non-immunochemical detection (i.e., chromatography, spectroscopy, and polymerase chain reaction (PCR)) and immunochemical detection methods.

Among the non-immunochemical detection methods, chromatography is typically used to separate molecules based on differential absorption and elution, which involves the flow of a fluid carrier over an immobile absorbing phase. Using chromatography, brain-specific fatty acids such as lignoceric acid and the *cis*/*trans* isomers of nervonic acid[122, 123] had been used as the analyte for the detection of the presence of CNS tissue in meat products through their characteristic component patterns. Spectroscopic techniques are based on the unique absorbance profiles of the sample components at specific wavelengths of the electromagnetic spectrum.

Near-infrared spectroscopy[124] and attenuated total reflectance Fourier transform infrared spectroscopy[125] have both been used to analyze bovine spinal cord in ground beef. Some CNS fatty acids have also been used as markers in gas chromatography–mass spectrometry (GC–MS).[126, 127] However, these methods require expensive instruments and reagents and highly trained staff; their sophisticated nature and the laborious sample preparation involved have limited the utility of these instrumental methods for routine analysis. Alternatively, its ability to detect GFAP mRNA makes PCR a sensitive technique for the detection of bovine CNS tissue in meat products.[128-130] However, although PCR can achieve a very low detection limit, it suffers from serious drawbacks, once again requiring expensive instrumentation and reagents and expert technicians. PCR methods are also prone to contamination.[130-132]

Immunochemical detection methods based on the specific immunoreactions between an antibody and its target antigen have been extensively employed for the detection of CNS contamination in meat (Table 1), generally in the form of an enzyme-linked immunosorbent assay (ELISA),[76, 128, 133-143] immunoblotting[137-140, 144-149] or immunohistochemistry.[145, 146, 150-152] There are a number of advantages associated with using an immunoassay. For example, no serious instrumentation is required; it is easy to operate and it employs minimal reagents. In the case of ELISA, it not only has a large-scale screening and field test capability, but is also a rapid, specific, and sensitive technique. Several markers have been explored to detect bovine CNS tissue in meat products with immunoassays, either using monoclonal (mAb) or polyclonal (pAb) antibodies.

5.1. CNS markers for non-immunochemical detection methods

5.1.1. Cholesterol

Cholesterol (MW: 386.7 g/mol) is a sterol component of cell membranes, hormones, and bile acids. It has been reported that 85 g of brain tissue may contain as much as 2,640 mg of cholesterol, while the same amount of a meat sample will only contain up to 85 mg cholesterol.[153] Although cholesterol is not specific to CNS, it can serve as a useful marker for a screening test due to the low-cost and rapid procedures available for measuring it. Lucker *et al*.[146] pioneered the use of cholesterol to analyze CNS tissue in meat products, using it to detect the cholesterol content of 402 heat-treated meat products from different food markets in Germany. Sixteen field samples (4%) were identified as being possibly contaminated with CNS tissue using this procedure, but the presence of CNS tissue was confirmed by immunoblotting for both neuron-specific enolase (NSE) and GFAP in just 7 of these 16 meat products. This suggests that cholesterol is not a reliable marker for CNS tissue due to its non-tissue specificity. It can, however, be used to roughly screen for CNS residue in meat products even though the accuracy is much lower than that of markers such as GFAP and NSE.

5.1.2. Nervonic acid

Nervonic acid (15-tetracosenoic acid, MW: 366.6 g/mol), a monounsaturated long chain fatty acid (C 24:1), is enriched in nervous tissue and is mainly present in the sphingolipids of the brain. As it is seldom found in non-CNS tissue, nervonic acid has been used as a marker

indicating the presence of CNS for the detection of SRM in meat products by chromatographic techniques. Based on the different ratios of *cis*/*trans* isomers of nervonic acid in different animal species, nervonic acid has also been used to differentiate CNS tissue from various animal species in meat products using an on-line liquid chromatography–gas chromatography (LC–GC) method developed by Barcarolo *et al*.[123] The authors reported that the ratio of the *cis*/*trans* isomers can be used to provide a rough estimate of the age of the animal but may not be an accurate method for the detection of CNS adulterant. Biedermann *et al*.[154] analyzed the concentrations of fatty acids typical in bovine CNS, including docosahexaenoic acid (C22:6), lignoceric acid (C24), nervonic acid, and cerebronic acid ($C_{24}OH$), using GC–MS to determine the CNS content The detection limit for CNS using this GC–MS method was 0.01%. In 2003, Agazzi *et al*.[122] reported an enhanced GC method for the detection of isomers of nervonic acid in meat products, reporting that the ratio of the *cis*/*trans* isomers of nervonic acid not only exhibit species specificity but also show a significant difference between cow and calf, and between pig and piglet.

Biedermann *et al*.[127] went on to study the structural characterization of nervonic acid as a marker for SRM using GC–MS and found that the ratio of *cis*/*trans* isomers used previously should be replaced by the ratio of positional isomers, i.e., the ratio of ω9/ω7-nervonic acid (15c-C24:1/17c-C24:1). Overall, although nervonic acid may be a specific and stable marker, the GC–MS-based CNS method is costly, requires a lengthy analysis time, and the data interpretation is highly complex. In addition, the lack of species and age specificity of nervonic acid for the detection of CNS disqualifies the GC–MS-based method as an effective method.

5.1.3. Glial Fibrillary Acidic Protein (GFAP) mRNA

In the CNS, GFAP (MW: about 50 kDa) is the major component of the filaments found in the astrocytes that support the functions of nerve cells.[155] In the peripheral nervous system (PNS), GFAP is mainly expressed by Schwann cells. The PCR method has been used in several studies to detect GFAP mRNA as a potential marker of CNS tissue contamination in meat products.

Seyboldt *et al*.[130] developed a reverse transcription-PCR (RT-PCR) assay coupled with restriction fragment length polymorphism for the detection of GFAP mRNA from bovine CNS tissue. Although this approach suffered from cross-reactions with unheated heart and skeletal muscle tissues, heat treatment (70 °C for 20 min) prior to RNA extraction was found to reduce the cross-reaction with a detection limit of 0.5% (g/g) heated bovine brain in bovine minced meat. In another study[143], a similar method was able to detect 0.25% (g/g) bovine CNS tissue in pork liver sausage (after a heat treatment of 80 °C for 80 min) that had been stored up to 28 days at 4 °C, although native peripheral nerves showed positive results.

Abdulmawjood and co-workers[129, 131, 156] developed a quantitative real-time RT-PCR to detect GFAP mRNA. Their system was capable of detecting down to 0.1% of CNS tissue in strongly heated (120 °C for 15 min) or medium-heated (80 °C for 90 min) meat samples (50% pork and 50% beef), and 0.2% of CNS tissue in pork liver sausages (80 °C for 1 h). However, low levels of GFAP could still be detected in peripheral nerves (sciatic and axillary nerves) and non-neuronal tissues (parotid gland, pancreas and adrenal gland).

5.2. CNS Markers for immunochemical detection

5.2.1. GFAP

The immunochemical differences between CNS-GFAP and PNS-GFAP are well known [157], and GFAP was first exploited to indicate the presence of brain or spinal cord tissues in meat as early as 1999 [134]. It has since been utilized as a marker for the detection of CNS tissue in meat products in a number of immunoassay systems [74, 76, 133, 141, 158]. Schmidt *et al*. were the first to develop a colorimetric sandwich ELISA in 1999 [134] and went on to develop an improved fluorescent sandwich ELISA in 2001 [135]. Although this fluorescent method was capable of detecting the presence of as little as 0.05% (g/g) bovine brain and spinal cord in beef, once again this assay suffers from a cross-reaction with PNS-GFAP, which is present in sciatic nerve and cervical ganglion tissue and is not considered an SRM [135]. These results suggest that the anti-GFAP pAb used in their assay was not actually specific to the CNS tissue.

GFAP has also been used as a CNS marker in a number of immunoblotting techniques [137, 145-147]. In a study by Lucker *et al.* [146], the immunoresponse of heat-treated samples was reduced significantly, however, and the sensitivity of the assay was inadequate when intensively heat-treated meat products were analyzed. Several other studies have also shown that the ability to detect GFAP is diminished when meat samples are subjected to severe heat treatment [133, 135].

5.2.2. Neuron-Specific Enolase (NSE)

NSE (MW: 48 kDa) is an enzyme that is found in both neurons and neuroendocrine cells. The immunological CNS specificity of $\gamma\gamma$-enolase has been used in clinical research for a long time [159], and in 1999 Lucker *et al*. were the first to adapt this clinical method to permit the use of NSE as a marker for the detection of CNS tissue in meat products using immunoblotting [148]. The detection limit of this assay was 1% (g/g) spiked brain in cooked (100 °C for 1 h) sausages of the emulsion type. They suggested that the sensitivity of the assay could be dramatically improved by removing the sample's fat content (30 to 40%) using Soxhlet extraction, although this is exceptionally time-consuming, requiring 8 h to extract the fat.

Several commercial immunochemical test kits have been developed [160]. Hughson *et al*. [140] compared the performance of two commercial kits, the RIDASCREEN GFAP kit and the Brainostic NSE kit (Table 3), for the detection of CNS tissue in meat products; these kits were based on the methods proposed by Schmidt *et al*. [134] and Lucker *et al*. [148], respectively. Hughson *et al*. [140] reported that levels down to 0.1% (g/g) of ovine spinal cord tissues could be detected in raw lamb meat using the GFAP kit, while 1.0% (g/g) was detected by the NSE kit. The detection limits of the GFAP and NSE methods for cooked samples (80 °C for 40 min) were 0.2% (g/g) bovine brain in pork and 0.1% (g/g) bovine spinal tissue in pork, respectively. They noted that the GFAP assay was easier to use and to interpret the data than the NSE assay. In a similar study by Agazzi *et al*. [138], the sensitivity of the NSE test kit was reported as being 0.5% (g/g) CNS tissue in raw and medium-heated (80 °C for 20 min) meat samples, rising to 2.0% (g/g) in strongly heated (120 °C for 20 min) meat materials. Another study [146] also found that the NSE immunoreactivity in samples of pure brain and brain muscle dropped signifi-

cantly with time at 100 °C, almost disappearing after a 2 h heat treatment. The properties of these commercial kits are summarized in Table 3.

Table 3. Commercial immunoassay kits for the detection of prohibited CNS and ruminant tissue contaminants in meat and feed products

Company	**ScheBo Biotech AG**	**R-Biopharm**		**ELISA Technologies**	**Neogen**	
Commercial kit name	Brainostic Test (GFAP-ELISA)	RIDASCREEN Risk Material	RIDASCREEN Risk Material 10/5	MELISA-TEK Ruminant Kit	Reveal for Ruminant in Feed	Reveal for Ruminant in MBM
Detection method	sELISA*	sELISA	sELISA	sELISA	LFA*	LFA
LOD (%)	0.1	0.2	0.1	0.5	1	2
Target products	Raw, processed (heated) meat and on contaminated surfaces	Processed (heated) meat and sausages	Raw meat, meat products and on contaminated surfaces	MBM	Ruminant	Ruminant
Sample preparation	Homogenization, extraction, swabs, dilution	Homogenization, extraction, centrifugation, dilution	Place the sampling swab into the sample dilution buffer	Extraction	Extraction	Extraction
Species selectivity	Unknown	Cattle, veal, sheep, goat, horse, poultry and pig	Cattle, veal, sheep, goat, horse, poultry and pig	Beef, sheep and water buffalo	N/A*	N/A
Tissue specificity	Unknown	No	No	Muscle	Muscle	Muscle
Minimum sample amount	50 mg	2 g	50 mg	5 g	10 g	10 g
Antigen#	GFAP*	GFAP	GFAP	Troponin-I	Troponin-I	Troponin-I
Test implementation	45 min	1 h	15 min	1 h	10 min	15 min
Tests possible per kit	42 (each in duplicate)	96 (including standards)	96 (including standards)	16 (each in triplicate)	25	25
Kit store temperature	4–8 °C	2–8 °C	2–8 °C	4–8°C	18–30 °C	18 – 30°C
Standards provided	0.1, 0.2, 0.4 and 1.0%	0, 0.2, 1.0 and 2.0%	0, 0.1, 0.2 and 0.4%	No	No	No

*sELISA: sandwich ELISA; GFAP: glial fibrillary acidic protein; LFA: immunochromatographic lateral flow assay; N/A: not available

5.2.3. Myelin proteolipid protein

In the CNS, myelin proteolipid protein (MPP, MW: 30 kDa) makes up approximately 50% of the myelin proteins [161]. This protein can be readily extracted from brain white matter using organic solvents [162]. Although MPP is the most abundant myelin protein in the CNS, mRNA for PLP is also expressed in the PNS and small amounts of MPP are synthesized [162]. This protein has also been used as a marker for the detection of CNS tissue in meat products [163]. The detection limit for CNS in raw meat can be as low as 0.025% using Western blot [164], and 0.01% bovine brain in raw minced beef using dot blot [165]. However, the antibody used in these studies was not species-specific and was unable to differentiate between mammalian species. Another problem is that MPP is not thermostable; the reaction signal decreased significantly when the heating time was increased to 95 °C. MPP was not detectable after 3 h of heat treatment at this temperature using dot blot [164].

5.2.4. Myelin Basic Protein (MBP)

MBP is located in CNS on the cytoplasmic face of myelin membranes in the white matter corresponding to the major dense line. MBP makes up about 30% of the total protein in the mammalian CNS myelin. It is the only structural protein found so far that is essential for the formation of CNS myelin [166]. As a water-soluble protein, MBP can be extracted with either acid or salt solutions [162]. There are four isoforms of MBP, each with a different molecular weight: 21 kDa, 18.5 kDa, 17 kDa and 14 kDa [162]. Different animal species have different isoforms. In particular, 18.5-kDa MBP, the major isoform protein in the CNS, is highly unfolded with essentially no tertiary structure in solution and the amino acid sequence of this major basic protein is similar in a number of animal species [162]. The sequence identity of the 18.5-kDa MBP in bovines and pigs is 93% homologous [167]. It has been reported that bovine 18.5-kDa MBP is not only very stable in solution at neutral pH (pH 7.2), but also highly thermostable [168]. Its immunoreactivity has been shown to remain clearly detectable even after severe heat treatment at 133 °C for 30 min [168].

MBP has been used as a marker for the detection of CNS contamination using immunoassays in several studies.[136, 143, 145, 147, 151] Levels as low as 1% (g/g) of bovine brain have been detected in a heated luncheon meat-type product (115 °C for 1 h) using a pAb in immunohistochemistry[151] and a mAb in immunoblotting.[147] In another study,[136] a detection limit of 10% was recorded for CNS tissue using a mAb in indirect competitive ELISA. Using an improved MBP extraction method,[143] compared with the detection limit reported using the same antibody and the same ELISA method,[136] the detection limit for brain tissue could be enhanced from 10 to 0.05% (~200 times). This improved extraction method will provide a useful analytical basis for the subsequent development of a specific immunoassay for the detection of bovine CNT in processed meat and feed products.

5.3. Detection methods for the control of prion diseases in feed products

To safeguard livestock, a number of non-immunochemical and immunochemical detection methods have been developed to detect the presence of banned animal materials in feeds. A

high-performance liquid chromatography method has been developed that utilizes dipeptide carnosine and related dipeptides as the markers,[169] and several other studies have used near-infrared reflectance spectroscopy (NIRS) to predict the amino acid content in animal feeds. [170-172] PCR has also been used for the detection of animal tissue in feeds. For example, Gao *et al.*[173] amplified a highly conserved eukaryotic DNA region of the 18S ribosomal gene using multiplex PCR (MPCR) and were able to detect levels as low as 0.02% bovine MBM in feedstuffs. Bellagamba *et al.*[174] detected 0.25% ruminant or pig adulterants in fish meal by MPCR.

A number of immunoassays have been developed to detect prohibited ruminant animal materials in feed. In our laboratory, a panel of species-specific mAbs have been produced for the detection of animal tissues in both food and feed products. [175-177] A skeletal muscle protein, troponin I (sTnI, 24 kDa), was first identified as a thermal-stable species marker protein; sTnI maintains its solubility and antigenicity even after undergoing severe heat treatment (126 °C for 120 min).[177] Given its even distribution in skeletal muscles, sTnI appears to be an ideal marker, allowing antibodies to be developed for the detection of animal tissues in severely heat-treated samples such as MBM. sTnI-specific mAbs have demonstrated their usefulness in an indirect non-competitive ELISA for the detection of rendered muscle tissues in animal feedstuffs, with detection limits of the mammalian and ruminant assays being between 0.3 and 2%.[175] In 2004, a sandwich ELISA was reported for the detection of ruminant proteins in feedstuffs.[176] This assay used a capture mAb specific to bovine and ovine sTnI and a biotin-conjugated detection mAb that reacts to all animal sTnI. The optimized assay achieved detection limits for bovine and ovine sTnI as low as 5.0 and 4.0 ng/ml, respectively. [176] Based on the detection of sTnI, several commercial immunoassay kits are now available for the enforcement of ruminant feed ban regulations (Table 3).

6. Conclusions

In order to maximize the economic profit from animal food production, humans made livestock animal's cannibals in the 1970s. Only a few years later, this backfired spectacularly, resulting in the rapid spread of infectious PrP^{Sc} through both the feed and food chains. These practices created a huge man-made disaster, mad cow disease, which went on to enter the human food chain through poor animal slaughtering and meat processing practices. The devastating impact of BSE on cattle has been called "a punishment from God" from which the global beef industry is only now beginning to recover. Although the negative impacts of the human version of mad cow disease, vCJD, are less significant, they continue to receive a great deal of publicity and the risk of recurrence of the disaster cannot be ignored. Most countries and areas have now enacted legislation to prohibit the use of SRM in the food and feed chain, and the goal of everyone concerned is to eventually eliminate the threat of BSE completely. Currently, through the strict implementation of feed bans and the enforcement of the regulations, both types of foodborne prion diseases appear to be under control. However, the prevention of prion diseases is not simply a matter of food or feed safety, but is also a significant factor affecting a number of political and economic issues. Effective detection methods for

ruminant SRM in rendered feedstuffs are still lacking, as currently none of the available assays can effectively differentiate ruminant CNS tissue from that of non-ruminant animal species, or detect bovine CNS tissue in excessively processed (e.g., 133 °C for 20 min) meat and feed products. It is therefore vital to continue to conduct scientific research in this area if we are to gain a better understanding of these destructive prion diseases and develop more effective surveillance techniques for this disease in both humans and animals. We must take to heart this solemn lesson if we are to avoid another such punishment from God.

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References

[1] Doherr, M. G., Brief review on the epidemiology of transmissible spongiform encephalopathies transmissible (TSE). *Vaccine* 2007, *25*, 5619-5624.

[2] Prusiner, S. B., Novel proteinaceous infectious particles cause scrapie. *Science* 1982, *216*, 136-144.

[3] Prusiner, S. B., Molecular biology of prion diseases. *Science* 1991, *252*, 1515-1522.

[4] Collinge, J., Prion diseases of humans and animals: Their causes and molecular basis. *Annual Review of Neuroscience* 2001, *24*, 519-550.

[5] Prusiner, S. B., Molecular biology and pathogenesis of prion diseases. *Trends in Biochemical Sciences* 1996, *21*, 482-487.

[6] Mead, S., Prion disease genetics. *European Journal of Human Genetics* 2006, *14*, 273-281.

[7] Prusiner, S. B., Prion diseases and the BSE crisis. *Science* 1997, *278*, 245-251.

[8] Cobb, N. J.; Surewicz, W. K., Prion diseases and their biochemical mechanisms. *Biochemistry* 2009, *48*, 2574-2585.

[9] Cashman, N. R.; Caughey, B., Prion diseases - Close to effective therapy? *Nature Reviews Drug Discovery* 2004, *3*, 874-884.

[10] Safar, J.; Roller, P. P.; Gajdusek, D. C.; Gibbs, C. J., Thermal-stability and conformational transitions of scrapie amyloid (prion) protein correlate with infectivity. *Protein Science* 1993, *2*, 2206-2216.

[11] Appel, T.; Wolff, M.; von Rheinbaben, F.; Heinzel, M.; Riesner, D., Heat stability of prion rods and recombinant prion protein in water, lipid and lipid-water mixtures. *Journal of General Virology* 2001, *82*, 465-473.

[12] Glatzel, M.; Stoeck, K.; Seeger, H.; Luhrs, T.; Aguzzi, A., Human prion diseases: molecular and clinical aspects. *Archives of Neurology* 2005, *62*, 545-52.

[13] McKenzie, D.; Aiken, J., Prion diseases. In *Guide to Foodborne Pathogens*, John Wiley & Sons: Chichester, West Sussex, UK, 2013; pp 399-413.

[14] Comer, P. J.; Huntly, P. J., Exposure of the human population to BSE infectivity over the course of the BSE epidemic in Great Britain and the impact of changes to the Over Thirty Month Rule. *Journal of Risk Research* 2004, *7*, 523-543.

[15] European Commission's Scientific Steering Committee. Opinion of the scientific steering committee on the human exposure risk (HER) via food with respect to BSE. http://ec.europa.eu/food/fs/sc/ssc/out67_en.pdf. (Access: October 19, 2015).

[16] Wilesmith, J. W.; Ryan, J. B. M.; Atkinson, M. J., Bovine spongiform encephalopathy - epidemiologic studies on the origin. *Veterinary Record* 1991, *128*, 199-203.

[17] Smith, P. G.; Bradley, R., Bovine spongiform encephalopathy (BSE) and its epidemiology. *British Medical Bulletin* 2003, *66*, 185-198.

[18] Taylor, D. M.; Woodgate, S. L.; Fleetwood, A. J.; Cawthorne, R. J. G., Effect of rendering procedures on the scrapie agent. *Veterinary Record* 1997, *141*, 643-649.

[19] Collee, J. G.; Bradley, R., BSE: a decade on - part I. *Lancet* 1997, *349*, 636-641.

[20] Almond, J.; Pattison, J., Human BSE. *Nature* 1997, *389*, 437-438.

[21] World Organisation for Animal Health. Number of reported cases of bovine spongiform encephalopathy (BSE) in farmed cattle worldwide. http://www.oie.int/?id=505. (Access: May 17, 2015).

[22] World Organisation for Animal Health. Number of cases of bovine spongiform encephalopathy (BSE) reported in the United Kingdom. http://www.oie.int/en/animal-health-in-the-world/bse-specific-data/number-of-cases-in-the-united-kingdom/. (Access: May 17, 2015).

[23] Ferguson, N. M.; Donnelly, C. A.; Woolhouse, M. E. J.; Anderson, R. M., The epidemiology of BSE in cattle herds in Great Britain.2. Model construction and analysis of transmission dynamics. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* 1997, *352*, 803-838.

[24] Anderson, R. M.; Donnelly, C. A.; Ferguson, N. M.; Woolhouse, M. E. J.; Watt, C. J.; Udy, H. J.; MaWhinney, S.; Dunstan, S. P.; Southwood, T. R. E.; Wilesmith, J. W.;

Ryan, J. B. M.; Hoinville, L. J.; Hillerton, J. E.; Austin, A. R.; Wells, G. A. H., Transmission dynamics and epidemiology of BSE in British cattle. *Nature* 1996, *382*, 779-788.

[25] Wigle, R.; Weerahewa, J.; Bredahl, M.; Samarajeewa, S., Impacts of BSE on world trade in cattle and beef: Implications for the Canadian economy. *Canadian Journal of Agricultural Economics* 2007, *55*, 535-549.

[26] Miran, B.; Akgungor, S., The effect of mad cow (BSE) scare on beef demand and sales loss: The case of Izmir. *Turkish Journal of Veterinary & Animal Sciences* 2005, *29*, 225-231.

[27] Jin, H. J.; Koo, W. W., The effect of the BSE outbreak in Japan on consumers' preferences. *European Review of Agricultural Economics* 2003, *30*, 173-192.

[28] Iraizoz, B.; Bardaji, I.; Rapun, M., The Spanish beef sector in the 1990s: impact of the BSE crisis on efficiency and profitability. *Applied Economics* 2005, *37*, 473-484.

[29] Herrmann, R., Economic consequences of BSE in Germany. *Deutsche Tierarztliche Wochenschrift* 2002, *109*, 358-361.

[30] CNN. Clashes over U.S. beef in S. Korea. http://www.cnn.com/2008/WORLD/asiapcf/06/01/skorea.usbeef/index.html?iref=newssearch#cnnSTCText. (Access: June 6, 2015).

[31] Coffey, B.; Mintert, J.; Fox, S.; Schroeder, T.; Valentin, L. The economic impact of BSE on the U.S. beef industry: product value losses, regulatory costs, and consumer reactions. http://www.ksre.ksu.edu/bookstore/pubs/MF2678.pdf. (Access: June 6, 2015).

[32] Brown, D. A.; Bruce, M. E.; Fraser, J. R., Comparison of the neuropathological characteristics of bovine spongiform encephalopathy (BSE) and variant Creutzfeldt-Jakob disease (vCJD) in mice. *Neuropathology and Applied Neurobiology* 2003, *29*, 262-272.

[33] Hill, A. F.; Desbruslais, M.; Joiner, S.; Sidle, K. C. L.; Gowland, I.; Collinge, J.; Doey, L. J.; Lantos, P., The same prion strain causes vCJD and BSE. *Nature* 1997, *389*, 448-450.

[34] U.S. Centers for Disease Control and Prevention. Fact sheet: variant Creutzfeldt-Jakob disease. http://www.cdc.gov/ncidod/dvrd/vcjd/factsheet_nvcjd.htm. (Access: May 17, 2015).

[35] Will, R. G.; Ironside, J. W.; Zeidler, M.; Cousens, S. N.; Estibeiro, K.; Alperovitch, A.; Poser, S.; Pocchiari, M.; Hofman, A.; Smith, P. G., A new variant of Creutzfeldt-Jakob disease in the UK. *Lancet* 1996, *347*, 921-925.

[36] UK National Creutzfeldt-Jakob Disease Surveillance Unit. Creutzfeldt-Jakob disease in the UK. http://www.cjd.ed.ac.uk/documents/figs.pdf. (Access: June 7, 2015).

[37] Munro, R., Neural tissue embolism in cattle. *Veterinary Record* 1997, *140*, 536.

[38] Coore, R. R.; Barr, F. J.; McKinstry, J. L.; Anil, M. H., Neural embolism and cerebral venous drainage at stunning and slaughter. *Veterinary Record* 2004, *155*, 86-87.

[39] Anil, M. H.; Harbour, D. A., Current stunning and slaughter methods in cattle and sheep - Potential for carcass contamination with central nervous tissue and microorganisms. *Fleischwirtschaft* 2001, *81*, 123-124.

[40] Love, S.; Helps, C. R.; Williams, S.; Shand, A.; McKinstry, J. L.; Brown, S. N.; Harbour, D. A.; Anil, M. H., Methods for detection of haematogenous dissemination of brain tissue after stunning of cattle with captive bolt guns. *Journal of Neuroscience Methods* 2000, *99*, 53-58.

[41] Gregory, N. G., Recent concerns about stunning and slaughter. *Meat Science* 2005, *70*, 481-491.

[42] Prendergast, D. M.; Sheridan, J. J.; Daly, D. J.; McDowell, D. A.; Blair, I. S., Dissemination of central nervous system tissue from the brain and spinal cord of cattle after captive bolt stunning and carcass splitting. *Meat Science* 2003, *65*, 1201-1209.

[43] Lim, D. G.; Erwanto, Y.; Lee, M., Comparison of stunning methods in the dissemination of central nervous system tissue on the beef carcass surface. *Meat Science* 2007, *75*, 622-627.

[44] Cohen, J. T.; Duggar, K.; Gray, G. M.; Kreindel, S. Evaluation of the potential for bovine spongiform encephalopathy in the United States. http://www.aphis.usda.gov/newsroom/hot_issues/bse/background/documents/appendix1.pdf. (Access: June 7, 2015).

[45] Miyaishi, S.; Moriya, F.; Yamamoto, Y.; Ishizu, H., Massive pulmonary embolization with cerebral tissue due to gunshot wound to the head. *Brain Injury* 1994, *8*, 559-564.

[46] Garland, T.; Bauer, N.; Bailey, M., Brain emboli in the lungs of cattle after stunning. *Lancet* 1996, *348*, 610-610.

[47] Coore, R. R.; Love, S.; McKinstry, J. L.; Weaver, H. R.; Phillips, A.; Hillman, T.; Hiles, M. J.; Shand, A.; Helps, C. R.; Anil, M. H., Dissemination of brain emboli following captive bolt stunning of sheep: Capacity for entry into the systemic arterial circulation. *Journal of Food Protection* 2004, *67*, 1050-1052.

[48] Anil, M. H.; Love, S.; Williams, S.; Shand, A.; McKinstry, J. L.; Helps, C. R.; Waterman-Pearson, A.; Seghatchian, J.; Harbour, D. A., Potential contamination of beef carcases with brain tissue at slaughter. *Veterinary Record* 1999, *145*, 460-462.

[49] Anil, M. H.; Love, S.; Helps, C. R.; McKinstry, J. L.; Brown, S. N.; Philips, A.; Williams, S.; Shand, A.; Bakirel, T.; Harbour, D., Jugular venous emboli of brain tissue induced in sheep by the use of captive bolt guns. *Veterinary Record* 2001, *148*, 619-620.

[50] Anil, M. H.; Love, S.; Helps, C. R.; Harbour, D. A., Potential for carcass contamination with brain tissue following stunning and slaughter in cattle and sheep. *Food Control* 2002, *13*, 431-436.

[51] Schmidt, G. R.; Hossner, K. L.; Yemm, R. S.; Gould, D. H., Potential for disruption of central nervous system tissue in beef cattle by different types of captive bolt stunners. *Journal of Food Protection* 1999, *62*, 390-393.

[52] U.S. Department of Agriculture., 9 CFR Parts 310 and 313 Prohibition of the use of certain stunning devices used to immobilize cattle during slaughter. *Federal Register* 2004, *69*, 1885-1891.

[53] Taylor, K. C., Brain emboli in the lungs of cattle. *Lancet* 1996, *348*, 749-749.

[54] EU Civic Consulting. Study on the stunning/killing practices in slaughterhouses and their economic, social and environmental consequences-final report part I: red meat. http://ec.europa.eu/food/animal/welfare/slaughter/docs/report_parti_en.pdf. (Access: June 7, 2015).

[55] Coore, R. R.; Love, S.; McKinstry, J. L.; Weaver, H. R.; Philips, A.; Hillman, T.; Hiles, M.; Helps, C. R.; Anil, M. H., Brain tissue fragments in jugular vein blood of cattle stunned by use of penetrating or nonpenetrating captive bolt guns. *Journal of Food Protection* 2005, *68*, 882-884.

[56] Lucker, E.; Schlottermuller, B.; Martin, A., Studies on contamination of beef with tissues of the central nervous system (CNS) as pertaining to slaughtering technology and human BSE-exposure risk. *Berliner und Munchener Tierarztliche Wochenschrift* 2002, *115*, 118-121.

[57] Daly, D. J.; Prendergast, D. M.; Sheridan, J. J.; Blair, I. S.; McDowell, D. A., Use of a marker organism to model the spread of central nervous system tissue in cattle and the abattoir environment during commercial stunning and carcass dressing. *Applied and Environmental Microbiology* 2002, *68*, 791-798.

[58] Buncic, S.; McKinstry, J.; Reid, C. A.; Anil, M. H., Spread of microbial contamination associated with penetrative captive bolt stunning of food animals. *Food Control* 2002, *13*, 425-430.

[59] Grandin, T. 2004 Restaurant animal welfare audits of stunning and handling in federally inspected U.S. and Canadian beef, veal, pork, lamb, and poultry slaughter plants. http://www.grandin.com/survey/2004.restaurant.audits.html. (Access: June 7, 2015).

[60] Grandin, T. 2005 Restaurant animal welfare audits of federally inspected beef and pork slaughter plants. http://www.grandin.com/survey/2005.restaurant.audits.html. (Access: June 7, 2015).

[61] Grandin, T. 2011 Restaurant animal welfare and humane slaughter audits in u.S. Federally inspected beef and pork slaughter plants. http://www.grandin.com/survey/2011.restaurant.audits.html. (Access: June 7, 2015).

[62] Gilbert, K. V.; Devine, C. E., Effect of electrical stunning methods on petechial haemorrhages and on the blood pressure of lambs. *Meat Science* 1982, *7*, 197-207.

[63] Gracey, J., *Meat plant operations*. Chalcombe Publications: Southampton, UK, 1998.

[64] Geering, W. A.; Penrith, M.-L.; Nyakahuma, D. Methods of destruction (Part 1: Destruction of animals). http://www.fao.org/docrep/004/Y0660E/Y0660E01.htm#ch1.3. (Access: 16 April 2009).

[65] European Commission, Commission Decision of 29 June 2000 regulating the use of material presenting risks as regards transmissible spongiform encephalopathies and amending Decision 94/474/EC. *Official Journal of the European Communities* 2000, *L158*, 76-82.

[66] European Commission. Scientific report on stunning methods and BSE risks. http://ec.europa.eu/food/fs/sc/ssc/out247_en.pdf. (Access: June 7, 2015).

[67] Prendergast, D. M.; Sheridan, J. J.; Daly, D. J.; McDowell, D. A.; Blair, I. S., Dissemination of central nervous system tissue during the slaughter of cattle in three Irish abattoirs. *Veterinary Record* 2004, *154*, 21-24.

[68] Helps, C. R.; Fisher, A. V.; Harbour, D. A.; O'Neill, D. H.; Knight, A. C., Transfer of spinal cord material to subsequent bovine carcasses at splitting. *Journal of Food Protection* 2004, *67*, 1921-1926.

[69] Helps, C. R.; Hindell, P.; Hillman, T. J.; Fisher, A. V.; Anil, H.; Knight, A. C.; Whyte, R. T.; O'Niell, D. H.; Knowles, T. G.; Harbour, D. A., Contamination of beef carcasses by spinal cord tissue during splitting. *Food Control* 2002, *13*, 417-423.

[70] U.S. Department of Agriculture. Revised directive for advanced meat recovery systems. http://www.fsis.usda.gov/Frame/FrameRedirect.asp?main=http://www.fsis.usda.gov/Oa/background/amrdirec.htm. (Access: June 7, 2015).

[71] Sparks Companies Inc. Advanced meat recovery systems - an economic analysis of the proposed USDA regulation. http://www.fsis.usda.gov/OPPDE/rdad/FRPubs/03-038IF/AMRSparksAnalysis.pdf. (Access: June 7, 2015).

[72] UK Spongiform Encephalopathy Advisory Committee. Sources of BSE infection: historical uses of mechanically recovered meat. http://www.seac.gov.uk/papers/seac76_4.pdf. (Access: 30 March 2009).

[73] Hajmeer, M. N.; Cliver, D. O.; Marsden, J. L., Central nervous system tissue detection in meat from advanced meat recovery systems. *Meat Science* 2006, *72*, 656-659.

[74] Schmidt, G. R.; Yemm, R. S.; Childs, K. D.; O'Callaghan, J. P.; Hossner, K. L., Verification of different glial fibrillary acidic protein (GFAP) analyses as accurate detectors of

central nervous system tissue in advanced meat recovery (AMR) products. *Meat Science* 2002, *62*, 79-84.

[75] Kelley, L. C.; Hafner, S.; McCaskey, P. C.; Sutton, M. T.; Langheinrich, K. A., An evaluation of methods for the detection of spinal cord in product derived from advanced meat recovery systems. *Journal of Food Protection* 2000, *63*, 1107-1112.

[76] Hossner, K. L.; Yemm, R. S.; Sonnenshein, S. E.; Mason, G. L.; Cummings, B. A.; Reddy, M. C. S.; Sofos, J. N.; Scanga, J. A.; Tatum, J. D.; Smith, G. C.; Belk, K. E., Comparison of immunochemical (enzyme-linked immunosorbent assay) and immunohistochemical methods for the detection of central nervous system tissue in meat products. *Journal of Food Protection* 2006, *69*, 644-650.

[77] U.S. Department of Agriculture. Analysis of 2002 FSIS bovine AMR products survey results. http://www.fsis.usda.gov/oa/topics/AMRAnalysis.pdf. (Access: June 7, 2015).

[78] Cooper, J. D.; Bird, S. M., UK dietary exposure to BSE in beef mechanically recovered meat: by birth cohort and gender. *Journal of Cancer Epidemiology and Prevention* 2002, *7*, 59-70.

[79] Chadeau-Hyam, M.; Tard, A.; Bird, S.; Le Guennec, S.; Bemrah, N.; Volatier, J. L.; Alperovitch, A., Estimation of the exposure of the French population to the BSE agent: comparison of the 1980-95 consumption of beef products containing mechanically recovered meat in France and the UK, by birth cohort and gender. *Statistical Methods in Medical Research* 2003, *12*, 247-260.

[80] Ward, H. J. T.; Everington, D.; Cousens, S. N.; Smith-Bathgate, B.; Leitch, M.; Cooper, S.; Heath, C.; Knight, R. S.; Smith, P. G.; Will, R. G., Risk factors for variant Creutzfeldt-Jakob disease: A case-control study. *Annals of Neurology* 2006, *59*, 111-120.

[81] Cooper, J. D.; Bird, S. M., Predicting incidence of variant Creutzfeldt-Jakob disease from UK dietary exposure to bovine spongiform encephalopathy for the 1940 to 1969 and post-1969 birth cohorts. *International Journal of Epidemiology* 2003, *32*, 784-791.

[82] UK Minister of Agriculture Fisheries and Food. Statutory Instrument 1995 No. 3246: The specified bovine offal (amendment) order 1995. http://www.opsi.gov.uk/si/si1995/Uksi_19953246_en_1.htm. (Access: June 7, 2015).

[83] UK Minister of Agriculture Fisheries and Food. Statutory Instrument 1996 No. 2097: The fresh meat (beef controls) (No. 2) regulations 1996. http://www.opsi.gov.uk/si/si1996/Uksi_19962097_en_1.htm. (Access: June 7, 2015).

[84] UK Minister of Agriculture Fisheries and Food. Statutory Instrument 1997 No. 2965: The specified risk material regulations 1997. http://www.opsi.gov.uk/si/si1997/19972965.htm. (Access: June 7, 2015).

[85] UK Minister of Agriculture Fisheries and Food. Statutory Instrument 2001 No. 447: The restriction on pithing (England) regulations 2001. http://www.opsi.gov.uk/si/si2001/20010447.htm. (Access: June 7, 2015).

[86] European Commission, 94/381/EC: Commission Decision of 27 June 1994 concerning certain protection measures with regard to bovine spongiform encephalopathy and the feeding of mammalian derived protein. *Official Journal of the European Communities* 1994, *L172*, 23-24.

[87] European Commission, Commission implementing decision of 4 February 2013 amending Decision 2009/719/EC authorising certain Member States to revise their annual BSE monitoring programmes. *Official Journal of the European Communities* 2013, *L35*, 6-7.

[88] World Organisation for Animal Health. Joint WHO/FAO/OIE technical consultation on BSE: public health, animal health and trade. http://whqlibdoc.who.int/publications/2001/9290445556.pdf. (Access: June 7, 2015).

[89] World Organisation for Animal Health. Terrestrial animal health code - 2014 Chapter 11.4. Bovine spongiform encephalopathy. http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_bse.htm. (Access: June 7, 2015).

[90] European Commission, Regulation (EC) No 999/2001 of the European Parliament and of the Council of 22 May 2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies. *Official Journal of the European Communities* 2001, *L147*, 1-40.

[91] European Commission, Commission Decision of 30 July 1997 on the prohibition of the use of material presenting risks as regards transmissible spongiform encephalopathies. *Official Journal of the European Communities* 1997, *L216*, 95-98.

[92] European Commission, Commission Decision of 27 December 2000 amending Decision 2000/418/EC regulating the use of material presenting risks as regards transmissible spongiform encephalopathies. *Official Journal of the European Communities* 2001, *L1*, 21-22.

[93] European Commission, Commission Decision of 14 March 2001 amending Decision 2000/418/EC as regards mechanically recovered meat and bovine vertebral column. *Official Journal of the European Communities* 2001, *L84*, 59-61.

[94] European Commission, Council Decision of 4 December 2000 concerning certain protection measures with regard to transmissible spongiform encephalopathies and the feeding of animal protein. *Official Journal of the European Communities* 2000, *L306*, 32-33.

[95] World Health Organization. Variant Creutzfeldt-Jakob disease. http://www.who.int/mediacentre/factsheets/fs180/en/. (Access: June 6, 2015).

[96] Japan Ministry of Health Labour and Welfare. Law on special measures against bovine spongiform encephalopathy. http://www.mhlw.go.jp/english/topics/foodsafety/bse/dl/2-1.pdf. (Access: June 7, 2015).

[97] Japan Ministry of Health Labour and Welfare. Standards for foods, food additives, etc. under the Japanese Food Sanitation Law total ban on the food use of bovine ver-

tebral column from BSE countries or zones. http://www.mhlw.go.jp/english/topics/foodsafety/vertebral.html. (Access: June 7, 2015).

[98] Japan Ministry of Health Labour and Welfare. About the partial revision of the MHLW-related implementation rules for the law on special measures against bovine spongiform encephalopathy. http://www.mhlw.go.jp/english/topics/foodsafety/bse/dl/1-4.pdf. (Access: June 7, 2015).

[99] Ministry of Health Labour and Welfare. Measures to ensure food safety. http://www.mhlw.go.jp/english/policy/health-medical/food/dl/pamphlet.pdf. (Access: June 6, 2015).

[100] Health Canada. Food directorate policy on specified risk material (SRM) in the food supply. http://www.hc-sc.gc.ca/fn-an/securit/animal/bse-esb/policy_srm-politique_mrs-eng.php. (Access: June 7, 2015).

[101] Canada Gazette. Statutory Instruments 2003 SOR/2003-265 Regulations amending the food and drug regulations (1389 — specified risk material). http://publications.gc.ca/gazette/archives/p2/2003/2003-08-13/pdf/g2-13717.pdf. (Access: June 6, 2015).

[102] Canadian Food Inspection Agency. Meat hygiene manual of procedures, chapter 17 - ante and post-mortem procedures, dispositions, monitoring and controls - meat species, ostriches, rheas and emus, annex D - specified risk materials. http://www.inspection.gc.ca/food/meat-and-poultry-products/manual-of-procedures/chapter-17/annex-d/eng/1369768468665/1369768518427. (Access: June 6, 2015).

[103] U.S. Food and Drug Administration, 21 CFR Part 589 Substances prohibited from use in animal food or feed; animal proteins prohibited in ruminant feed; final rule. *Federal Register* 1997, *62*, 30936-30978.

[104] U.S. Department of Agriculture, 9 CFR Parts 309, 310, 311, 318, and 319 Prohibition of the use of specified risk materials for human food and requirements for the disposition of non-ambulatory disabled cattle. *Federal Register* 2004, *67*, 1862-1874.

[105] U.S. Department of Agriculture, 9 CFR Parts 310 and 318 Prohibition of the use of specified risk materials for human food and requirements for the disposition of non-ambulatory disabled cattle. *Federal Register* 2005, *70*, 53043-53050.

[106] U.S. Department of Agriculture, 9 CFR Parts 301, 318, and 320 Meat produced by advanced meat/bone separation machinery and meat recovery (AMR) systems. *Federal Register* 2004, *69*, 1874-1885.

[107] U.S. Department of Agriculture, National animal identification system; user guide and technical documents. *Federal Register* 2007, *72*, 4680-4681.

[108] U.S. Food and Drug Administration, 21 CFR Part 589 Substances prohibited from use in animal food or feed; final rule. *Federal Register* 2008, *73*, 22720-22758.

[109] USDA, 9 CFR Parts 309, 310 and 318 Prohibition of the use of specified risk materials for human food and requirements for the disposition of non-ambulatory disabled cattle; prohibition of the use of certain stunning devices used to immobilize cattle during slaughter; rule. *Federal Register* 2007, *72*, 38699-38730.

[110] U.S. Department of Agriculture. BSE rules being strictly enforced. http://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/production-and-inspection/bse-rules-being-strictly-enforced/bse-rules-being-strictly-enforced. (Access: June 7, 2015).

[111] U.S. Food and Drug Administration. CVM reports BSE inspection figures as of November 15, 2008. http://madcowfeed.blogspot.com/2008_11_01_archive.html. (Access: June 8, 2015).

[112] Hanrahan, C. E.; Becker, G. S. Mad cow disease and U.S. beef trade. http://nationalaglawcenter.org/wp-content/uploads/assets/crs/RS21709.pdf. (Access: June 7, 2015).

[113] Kim, J. South Korea to allow return of US beef imports. http://www.reuters.com/article/latestCrisis/idUSSP212539. (Access: June 7, 2015).

[114] World Organisation for Animal Health. Terrestrial animal health code: glossary. http://www.oie.int/index.php?id=169&L=0&htmfile=glossaire.htm. (Access: June 7, 2015).

[115] Sparks Companies Inc. *The rendering industry: economic impact of future feeding regulations*; National Renderers Association: Alexandria, VA, 2001.

[116] Becker, G. S. Animal rendering: economics and policy. http://nationalaglawcenter.org/wp-content/uploads/assets/crs/RS21771.pdf. (Access: June 6, 2015).

[117] U.S. FDA Animal and veterinary recalls archive. http://www.fda.gov/AnimalVeterinary/SafetyHealth/RecallsWithdrawals/ucm393160.htm. (Access: October 19, 2015).

[118] U.S. Department of Agriculture. Agricultural statistics 2013. http://www.nass.usda.gov/Publications/Ag_Statistics/2013/Agricultural_Statistics_2013.pdf. (Access: June 7, 2015).

[119] U.S. Department of Agriculture. BSE surveillance in the United States. http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/animalhealth/sa_animal_disease_information/sa_cattle_health/sa_bse/ct_surv_in_usa/!ut/p/a0/04_Sj9CPykssy0xPLMnMz0vMAfGjzOK9_D2MDJ0MjDzdgy1dDTz9wtx8LXzMjf09TPQLsh0VAZdihIg!/. (Access: June 7, 2015).

[120] U.S. Department of Agriculture. Agricultural statistics annual. http://www.nass.usda.gov/Publications/Ag_Statistics/. (Access: June 7, 2015).

[121] U.S. Department of Agriculture. Recall case archive. http://www.fsis.usda.gov/wps/portal/fsis/topics/recalls-and-public-health-alerts/recall-case-archive. (Access: June 7, 2015).

[122] Agazzi, M. E.; Bau, A.; Barcarolo, R.; Lucker, E.; Barrero-Moreno, J.; Anklam, E., In-house validation of an improved sample extraction and clean-up method for GC determination of isomers of nervonic acid in meat products. *Analytical and Bioanalytical Chemistry* 2003, *376*, 360-365.

[123] Barcarolo, R.; Bau, A.; Moreno, J. B.; Dimitrova, B.; Anklam, E., On-line LC-GC method for determination of isomers of nervonic acid in meat-derived food. *Journal of Separation Science* 2003, *26*, 1347-1352.

[124] Gangidi, R. R.; Proctor, A.; Pohlman, F. W.; Meullenet, J. F., Rapid determination of spinal cord content in ground beef by near-infrared spectroscopy. *Journal of Food Science* 2005, *70*, C397-C400.

[125] Gangidi, R. R.; Proctor, A.; Pohlman, F. W., Rapid determination of spinal cord content in ground beef by attenuated total reflectance Fourier transform infrared spectroscopy. *Journal of Food Science* 2003, *68*, 124-127.

[126] Lucker, E.; Biedermann, W.; Lachhab, S.; Truyen, U.; Hensel, A., GC-MS detection of central nervous tissues as TSE risk material in meat products: analytical quality and strategy. *Analytical and Bioanalytical Chemistry* 2004, *380*, 866-870.

[127] Biedermann, W.; Lucker, E.; Porschmann, J.; Lachhab, S.; Truyen, U.; Hensel, A., Structural characterisation of some fatty acids from the brain as biomarkers of BSE risk material. *Analytical and Bioanalytical Chemistry* 2004, *379*, 1031-1038.

[128] Nowak, B.; von Mueffling, T.; Kuefen, A.; Ganseforth, K.; Seyboldt, C., Detection of bovine central nervous system tissue in liver sausages using a reverse transcriptase PCR technique and a commercial enzyme-linked immunosorbent assay. *Journal of Food Protection* 2005, *68*, 2178-2183.

[129] Abdulmawjood, A.; Schonenbrocher, H.; Bulte, M., Novel molecular method for detection of bovine-specific central nervous system tissues as bovine spongiform encephalopathy risk material in meat and meat products. *Journal of Molecular Diagnostics* 2005, *7*, 368-374.

[130] Seyboldt, C.; John, A.; von Mueffling, T.; Nowak, B.; Wenzel, S., Reverse transcription-polymerase chain reaction assay for species-specific detection of bovine central nervous system tissue in meat and meat products. *Journal of Food Protection* 2003, *66*, 644-651.

[131] Schoenbrucher, H.; Abdulmawjood, A.; Gobel, K. A.; Bulte, M., Detection of central nervous system. tissues in meat products: Validation and standardization of a real-time PCR-based detection system. *Veterinary Microbiology* 2007, *123*, 336-345.

[132] Shi, X. J.; Ma, G. P.; Li, B. L.; Yang, J. L.; Wang, Y.; Li, Y. X.; Liu, X. H.; Liu, Q. G., Detection of bovine central nervous system tissue as bovine spongiform encephalop-

athy risk material by real-time reverse transcriptase-PCR in raw and cooked beef products. *Animal Biotechnology* 2008, *19*, 225-230.

[133] Agazzi, M. E.; Moreno, J. B.; von Holst, C.; Lucker, E.; Anklam, E., Quantitative analysis of tissues of the central nervous system in food products by GFAP-ELISA test kit. Results of an interlaboratory study. *Food Control* 2004, *15*, 297-301.

[134] Schmidt, G. R.; Hossner, K. L.; Yemm, R. S.; Gould, D. H.; O'Callaghan, J. P., An enzyme-linked immunosorbent assay for glial fibrillary acidic protein as an indicator of the presence of brain or spinal cord in meat. *Journal of Food Protection* 1999, *62*, 394-397.

[135] Schmidt, G. R.; Yemm, R. S.; Childs, K. D.; O'Callaghan, J. P.; Hossner, K. L., The detection of central nervous system tissue on beef carcasses and in comminuted beef. *Journal of Food Protection* 2001, *64*, 2047-2052.

[136] Rencova, E., Comparison of commercially available antibodies for the detection of central nervous system tissue in meat products by enzyme-linked immunosorbent assay. *Journal of Food Protection* 2005, *68*, 630-632.

[137] Lucker, E.; Hardt, M.; Groschup, M. H., Detection of CNS and PrP^{Sc} in meat products. *Berliner und Munchener Tierarztliche Wochenschrift* 2002, *115*, 111-117.

[138] Agazzi, M. E.; Barrero-Moreno, J.; Lucker, E.; von Holst, C.; Anklam, E., Performance comparison of two analytical methods for the detection of tissues of the central nervous system in sausages: results of an interlaboratory study. *European Food Research and Technology* 2002, *215*, 334-339.

[139] Hajmeer, M.; Cliver, D. O.; Provost, R., Spinal cord tissue detection in comminuted beef: comparison of two immunological methods. *Meat Science* 2003, *65*, 757-763.

[140] Hughson, E.; Reece, P.; Dennis, M. J.; Oehlschlager, S., Comparative evaluation of the performance of two commercial kits for the detection of central nervous system tissue in meat. *Food Additives and Contaminants* 2003, *20*, 1034-1043.

[141] Yesilbag, K.; Kalkan, A., Detection of central nervous system tissues as BSE specified risk material in meat products in Turkey. *Food Control* 2005, *16*, 11-13.

[142] Reddy, M. C. S.; Hossner, K. L.; Belk, K. E.; Scanga, J. A.; Yemm, R. S.; Sofos, J. N.; Smith, G. C., Detection of central nervous system tissue on meat and carcass-splitting band saw blade surfaces using modified fluorescent glial fibrillary acidic protein enzyme-linked Immunosorbent assay sampling and extraction procedures. *Journal of Food Protection* 2006, *69*, 1966-1970.

[143] Rao, Q. C.; Hsieh, Y.-H. P., Enhanced immunodetection of bovine central nervous tissue using an improved extraction method. *Food Control* 2014, *46*, 282-290.

[144] Lucker, E.; Horlacher, S.; Eigenbrodt, E., Brain in human nutrition and variant Creutzfeldt-Jakob disease risk (vCJD): detection of brain in retail liver sausages using

cholesterol and neuron specific enolase (NSE) as markers. *British Journal of Nutrition* 2001, *86*, S115-S119.

[145] Herde, K.; Bergmann, M.; Lang, C.; Leiser, R.; Wenisch, S., Glial fibrillary acidic protein and myelin basic protein as markers for the immunochemical detection of bovine central nervous tissue in heat-treated meat products. *Journal of Food Protection* 2005, *68*, 823-827.

[146] Lucker, E.; Eigenbrodt, E.; Wenisch, S.; Leiser, R.; Bulte, M., Identification of central nervous system tissue in retail meat products. *Journal of Food Protection* 2000, *63*, 258-263.

[147] Sultan, K. R.; Tersteeg, M. H. G.; Koolmees, P. A.; de Baaij, J. A.; Bergwerff, A. A.; Haagsman, H. P., Western blot detection of brain material in heated meat products using myelin basic protein and neuron-specific enolase as biomarkers. *Analytica Chimica Acta* 2004, *520*, 183-192.

[148] Lucker, E.; Eigenbrodt, E.; Wenisch, S.; Failing, K.; Leiser, R.; Bulte, M., Development of an integrated procedure for the detection of central nervous tissue in meat products using cholesterol and neuron-specific enolase as markers. *Journal of Food Protection* 1999, *62*, 268-276.

[149] Salman, M. D.; Jemmi, T.; Triantis, J.; Dewell, R. D., Assessment and modification of a Western blot assay for detection of central nervous system tissue in meat products in the United States. *Journal of Food Protection* 2005, *68*, 1706-1711.

[150] Kummer, V.; Maskova, J.; Rencova, E., Immunohistochemical detection of central nervous tissue in meat products. *Acta Veterinaria Brno* 2005, *74*, 449-454.

[151] Tersteeg, M. H. G.; Koolmees, P. A.; van Knapen, F., Immunohistochemical detection of brain tissue in heated meat products. *Meat Science* 2002, *61*, 67-72.

[152] Wenisch, S.; Lucker, E.; Eigenbrodt, E.; Leiser, R.; Bulte, M., Detection of central nervous tissue in meat products - An immunohistochemical approach. *Nutrition Research* 1999, *19*, 1165-1172.

[153] Wardlaw, G. M.; Kessel, M. W., Lipids. In *Perspectives in Nutrition*, 5 ed.; Meyers, L. M., Ed. McGraw-Hill: New York, 2002; pp 203-254.

[154] Biedermann, W.; Lucker, E.; Hensel, A., Detection of tissues of the central nervous system (CNS) as specified risk material (SRM) in meat products by means of gas chromatography mass spectrometry (GC-MS). *Berliner und Munchener Tierarztliche Wochenschrift* 2002, *115*, 131-133.

[155] Eng, L. F.; Ghirnikar, R. S.; Lee, Y. L., Glial fibrillary acidic protein: GFAP-thirty-one years (1969-2000). *Neurochemical Research* 2000, *25*, 1439-1451.

[156] Abdulmawjood, A.; Schonenbrucher, H.; Bulte, M., Collaborative trial for validation of a real-time reverse transcriptase-polymerase chain reaction assay for detection of

central nervous system tissues as bovine spongiform encephalopathy risk material: Part 1. *Journal of AOAC International* 2006, *89*, 1335-1340.

[157] Feinstein, D. L.; Weinmaster, G. A.; Milner, R. J., Isolation of cDNA clones encoding rat glial fibrillary acidic protein: expression in astrocytes and in Schwann cells. *Journal of Neuroscience Research* 1992, *32*, 1-14.

[158] Kale, M.; Kursun, O.; Pehlivanoglu, F., Detection of central nervous system tissues as bovine spongiform encephalopathy specified risk material in processed and raw meat products in Turkey. *Journal of Food Safety* 2007, *27*, 56-65.

[159] Sheppard, M. N.; Marangos, P. J.; Bloom, S. R.; Polak, J. M., Neuron specific enolase: a marker for the early development of nerves and endocrine cells in the human lung. *Life Sciences* 1984, *34*, 265-271.

[160] Weyandt, R. G., Detection of BSE-risk materials. *Fresenius' Journal of Analytical Chemistry* 2001, *371*, 574-575.

[161] Baumann, N.; Pham-Dinh, D., Biology of oligodendrocyte and myelin in the mammalian central nervous system. *Physiological Reviews* 2001, *81*, 871-927.

[162] Siegel, G. J.; Agranoff, B. W.; Fisher, S. K.; Albers, R. W.; Uhler, M. D., *Basic neurochemistry: molecular, cellular and medical aspects*. 6th ed.; Lippincott Williams & Wilkins: Philadelphia, 1999.

[163] Villmann, C.; Sandmeier, B.; Seeber, S.; Hannappel, E.; Pischetsrieder, M.; Becker, C. M., Myelin proteolipid protein (PLP) as a marker antigen of central nervous system contaminations for routine food control. *Journal of Agricultural and Food Chemistry* 2007, *55*, 7114-7123.

[164] Sandmeler, B.; Baeuerlein, R.; Villmann, C.; Duethorn, T.; Gareis, M.; Becker, C. M.; Pischetsrieder, M., Detection of central nervous system tissue in meat and meat products with a newly developed immunoassay selective for Myelin proteolipid protein. *Food Chemistry* 2007, *105*, 871-878.

[165] Bauerlein, R.; Sandmeier, B.; Villmann, C.; Hammon, A.; Gareis, M.; Becker, C. M.; Pischetsrieder, M., Development of a dot blot assay for the rapid detection of central nervous system tissue on meat and contact surfaces. *Journal of Agricultural and Food Chemistry* 2008, *56*, 44-49.

[166] Boggs, J. M., Myelin basic protein: a multifunctional protein. *Cellular and Molecular Life Sciences* 2006, *63*, 1945-1961.

[167] Gasteiger, E.; Gattiker, A.; Hoogland, C.; Ivanyi, I.; Appel, R. D.; Bairoch, A., ExPASy: the proteomics server for in-depth protein knowledge and analysis. *Nucleic Acids Research* 2003, *31*, 3784-3788.

[168] Rao, Q. C.; Hsieh, Y.-H. P., Effect of pH, temperature and storage time on the stability of bovine myelin basic protein. *Food Control* 2015, *50*, 166-172.

[169] Schonherr, J., Analysis of products of animal origin in feeds by determination of carnosine and related dipeptides by high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry* 2002, *50*, 1945-1950.

[170] Fontaine, J.; Horr, J.; Schirmer, B., Near-infrared reflectance spectroscopy enables the fast and accurate prediction of the essential amino acid contents in soy, rapeseed meal, sunflower meal, peas, fishmeal, meat meal products, and poultry meal. *Journal of Agricultural and Food Chemistry* 2001, *49*, 57-66.

[171] Fontaine, J.; Horr, J.; Schirmer, B., Amino acid contents in raw materials can be precisely analyzed in a global network of near-infrared spectrometers: Collaborative trials prove the positive effects of instrument standardization and repeatability files. *Journal of Agricultural and Food Chemistry* 2004, *52*, 701-708.

[172] Fontaine, J.; Schirmer, B.; Horr, J., Near-infrared reflectance spectroscopy (NIRS) enables the fast and accurate prediction of essential amino acid contents. 2. Results for wheat, barley, corn, triticale, wheat bran/middlings, rice bran, and sorghum. *Journal of Agricultural and Food Chemistry* 2002, *50*, 3902-3911.

[173] Gao, H. W.; Zhang, D. B.; Pan, A. H.; Liang, W. Q.; Liang, C. Z., Multiplex polymerase chain reaction method for detection of bovine materials in foodstuffs. *Journal of AOAC International* 2003, *86*, 764-767.

[174] Bellagamba, F.; Valfre, F.; Panseri, S.; Moretti, V. M., Polymerase chain reaction-based analysis to detect terrestrial animal protein in fish meal. *Journal of Food Protection* 2003, *66*, 682-685.

[175] Chen, F. C.; Hsieh, Y.-H. P.; Bridgman, R. C., Monoclonal antibodies against troponin I for the detection of rendered muscle tissues in animal feedstuffs. *Meat Science* 2002, *62*, 405-412.

[176] Chen, F. C.; Hsieh, Y.-H. P.; Bridgman, R. C., Monoclonal antibody-based sandwich enzyme-linked immunosorbent assay for sensitive detection of prohibited ruminant proteins in feedstuffs. *Journal of Food Protection* 2004, *67*, 544-549.

[177] Chen, F. C.; Hsieh, Y.-H. P., Porcine troponin I: a thermostable species marker protein. *Meat Science* 2002, *61*, 55-60.

[178] Rovira, P. J.; Scanga, J. A.; Grandin, T.; Hossner, K. L.; Yemm, R. S.; Belk, K. E.; Tatum, J. D.; Sofos, J. N.; Smith, G. C., Central nervous system tissue contamination of the circulatory system following humane cattle stunning procedures. *Food Protection Trends* 2007, *27*, 524–529.

[179] Rotterud, O. J.; Helps, C. R.; Hillman, T. J.; Fisher, A. V.; Harbour, D.; Anil, H.; Nesbakken, T., Hot boning of intact carcasses: a procedure to avoid central nervous system self-contamination in beef and beef products. *Journal of Food Protection* 2006, *69*, 405-411.

[180] [Anon] Glossary. http://www.nature.com/nrm/journal/v5/n8/glossary/nrm1438_glossary.html. (Access: June 6, 2015).

[181] Allore, R.; Ohanlon, D.; Price, R.; Neilson, K.; Willard, H. F.; Cox, D. R.; Marks, A.; Dunn, R. J., Gene encoding the beta subunit of S100 protein is on chromosome 21: implications for Down syndrome. Science 1988, 239, 1311-1313.

[182] Bennett, M. K.; Calakos, N.; Scheller, R. H., Syntaxin: a synaptic protein implicated in docking of synaptic vesicles at presynaptic active zones. Science 1992, 257, 255-259.