CHAPTER 2

Central Nervous System Tissue in Meat Products: An Evaluation of Risk, Prevention Strategies, and Testing Procedures

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Contents

Abstract

Since the outbreak of bovine spongiform encephalopathy (BSE) in the United Kingdom in 1986 and its subsequent link to the human neurological disorder variant Creutzfeldt–Jakob disease (vCJD),

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presence of tissues from the central nervous system (CNS) in meat products has been considered a public health concern and, thus, has been banned from entering the human food chain in many countries. Despite this, potential can exist during harvesting to contaminate or cross-contaminate edible meat products with CNS tissue that is designated as a specified risk material (SRM) in many countries. Methods used to detect CNS tissue in meat products vary greatly in their sensitivity, specificity, cost, labor and expertise needed, ease of completion, and type of results given (qualitative vs quantitative) and, within these constraints, appropriate testing methods must be selected to monitor or verify that meat products system controls are effective in removing CNS tissue from the human food chain. The extent to which monitoring procedures are needed should be based on the public health risk of CNS tissue in meat products as determined by each sovereign nation and/or third-party international organizations such as the World Organization for Animal Health (OIE). Risk associated with consumption of CNS tissue should be estimated by sovereign nations by establishing prevalence of BSE within their borders. Using this information, science-based decisions may guide international policy and trade. Using available scientific information, appropriate testing methods for monitoring or verification, and prevalence information, nations can estimate and reduce, to the extent deemed necessary, the public health risk of vCJD.

I. INTRODUCTION

Bovine spongiform encephalopathy (BSE) was first identified in the United Kingdom in 1986 and became a reportable disease in 1987 (Wells *et al.*, 1987). Since the BSE outbreak in the United Kingdom, the disease has spread to 24 other countries including the United States, Canada, Japan, and most countries of the European Union (EU) (OIE, 2007). BSE is a fatal neurodegenerative disorder that affects the central nervous system (CNS) of adult cattle (DeArmond and Prusiner, 2003). While CNS tissue is not the only specified risk material (SRM) associated with BSE, it has historically been discussed with the most trepidation because of the high tissue infectivity titers it displays (personal communication with Danny Matthews of the United Kingdom Department for Environment Food and Rural Affairs and Gerald Wells of Fulmer Consulting Ltd.). Consumption of CNS tissue (and other SRM) that is infected with BSE is thought to cause the human neurological disease, variant Creutzfeldt–Jakob disease (vCJD), and consumption of BSE-infected brains, spinal cord, tonsils,

distal ileum, dorsal root ganglia, trigeminal ganglia, and eyes has resulted in transmission of BSE to other cattle (Hill *et al.*, 1997; Wells *et al.*, 1998). Currently, regulations are in place requiring the removal of SRM from the food and feed chain in countries affected with BSE (Table 1).

During slaughter, potential exists for the contamination and/or cross-contamination of meat with CNS tissue by animal stunning, improper removal of SRM, carcass splitting, carcass washing, and exsanguination. Due to risk associated with cross-contamination of meat during harvest of BSE-infected cattle, testing methods have been developed and implemented to identify presence of CNS tissue in meat products. This chapter investigates the potential risk of a public health threat due to consumption of BSE-infected meat products based on prevalence information, potential routes of CNS tissue contamination of beef carcasses, and current testing methods employed to identify CNS tissue; and, it discusses governmental, industrial, and scientific methods that have been identified to reduce or eliminate CNS tissue cross-contamination onto meat and meat products.

TABLE 1 Age at which tissues are designated as SRMs in Australia, Japan, the United Kingdom, and the United States

Tissue	Australia[a]	Japan[b]	United Kingdom[c]	United States[d]
Tonsils	Not an SRM	All ages	All ages	All ages
Intestine (duodenum to rectum)	Not an SRM	All ages	All ages	All ages
Skull	Not an SRM	All ages	>12	>30
Brain	Not an SRM	All ages	>12	>30
Eyes	Not an SRM	All ages	>12	>30
Spinal cord	Not an SRM	All ages	>12	>30
Trigeminal ganglia	Not an SRM	All ages	Not an SRM	>30
Dorsal root ganglia	Not an SRM	All ages	>24	>30
Vertebral column	Not an SRM	All ages	>24	>30
Mesentery	Not an SRM	Not an SRM	All ages	Not an SRM

[a] DAFF (2007).
[b] MHLW (2005).
[c] Food Standards Agency (2007).
[d] USDA-FSIS (2004).

II. PREVALENCE AS AN EVALUATOR OF BSE FOOD SAFETY RISKS

In order to determine the food safety risk from consumption of BSE-infected meat products, many factors must be taken into consideration. First and foremost, the infective dose of prions in humans must be quantified. To date, no scientific research has determined the infectious dose of BSE prions in humans, and, therefore, it is impossible to exactly quantify the risk of human consumption of beef products that may have small amounts of CNS tissue present. However, through use of known tissue infectivity for transmission to cattle, surveillance at all animal production sectors and at harvest, and slaughter control processes, risk can be estimated and greatly reduced. Therefore, each country, and each facility within those countries, must evaluate the likelihood of a BSE-infected animal entering their slaughter process and, subsequently, their food chain, undetected, and must identify the control measures needed to prevent or reduce (to the extent needed) that risk. Countries that conduct a surveillance program and determine BSE to be a low food safety risk would not, in theory, need to implement as many controls as countries that have a high prevalence of the disease. Countries that have a high prevalence of the disease and, thus, a higher food safety risk would, in theory, implement more controls to ensure that SRM does not enter the human food chain.

Currently, the key factor in trade of beef and beef products between nations is whether BSE has been found in the indigenous cattle population of the exporting country. More important, however, than the actual number of BSE cases are the surveillance techniques implemented by each country and the control measures in place to prevent, to the greatest degree possible, a food safety threat. The World Animal Health Organization (OIE) outlines two different types of surveillance for BSE in the indigenous cattle population in their Terrestrial Animal Health Code (OIE, 2006b). The goal of each category of surveillance is to determine (using a 95% confidence interval) the BSE prevalence in a country by testing cattle subpopulations. Each subpopulation is assigned a point value (lower-risk cattle being worth fewer points and higher-risk cattle being worth more points) and, in order to meet OIE standards, each country must accumulate enough points to satisfy the requirements of the OIE surveillance program with which they attempt to comply.

The first type of OIE surveillance program (denoted as ''Type A'' surveillance by OIE) is conducted by countries to determine prevalence of BSE and allows for detection of BSE if there is one BSE case (with a 95% confidence interval) per 100,000 adult cattle (OIE, 2006b). Countries implementing Type A surveillance must accumulate points based on the points system outlined in the Terrestrial Animal Health Code (OIE, 2006b).

In this system, a young animal tested at slaughter showing no symptoms is worth 0.1 point on the OIE point scale, whereas an animal that is between 4 and 7 years old showing clinical signs of BSE is worth 750 points (OIE, 2006b). The United States and Japan have implemented surveillance programs that meet or exceed OIE Type A surveillance. In 2006, Japan tested over 6,000,000 cattle for BSE including every animal at harvest, regardless of age, and found 10 animals to be positive for BSE (OIE, 2007). Through the duration of their enhanced BSE surveillance program, the United States reported test results for over 735,000 adult cattle and found 2 positive animals (USDA, 2006b), and reported a total OIE point accumulation of over 2,900,000—nearly 10 times the OIE recommended level of testing to determine prevalence of BSE (USDA, 2006a). Through Type A surveillance, countries establish the prevalence of BSE in their country. On completion of Type A surveillance (i.e., when the prevalence of BSE is well established within a country), each individual country may continue surveillance on a smaller scale using OIE Type B surveillance (OIE, 2006b).

Countries designated as being at negligible risk for having BSE in their indigenous cattle herd (see below), and countries that have completed a Type A surveillance program, may implement a Type B surveillance to monitor their indigenous cattle population for BSE. Type B surveillance is designed similarly to Type A surveillance in that it uses the same point system and scale for at-risk animals (OIE, 2006b). Type B surveillance is designed, however, to detect BSE if there is 1 BSE-positive animal in every 50,000 adult animals (with a 95% confidence interval) (OIE, 2006b). Australia is a country that uses Type B surveillance. In 2005, Australia tested 501 animals, enough to meet the requirements of OIE Type B surveillance (Australia National Health Information System, 2007).

It is important for all countries to conduct Type A surveillance and determine the prevalence of BSE within their borders. Furthermore, nations must continue to diligently monitor for BSE in their cattle population to prevent the inadvertent spread of the disease. Even though the factors that contribute to the spread of BSE are well known, the origin of the disease is still unknown (ILC, 2006). It is possible that BSE occurred sporadically and, therefore, all nations must actively monitor their cattle population and continue to implement risk mitigation factors such as ruminant-to-ruminant feed bans (Brown *et al.*, 2006).

In addition to creating standards for surveillance of BSE, in 2006 the OIE stipulated three categories of BSE risk in a cattle population and, thus, in a country, based on results of the surveillance plans described above (OIE, 2006a). To date, OIE has not given any country a designation based on the new criteria. However, many countries, including the United States, have presented OIE with the results of their Type A surveillance and have petitioned for OIE to determine their status, and many countries

will likely be designated during the voting session in May 2007 (personal communication, Dr. Chuck Lambert, the Under Secretary for Marketing and Regulatory Programs, USDA). Determination of status by a third-party international organization may allow for increased understanding of the risk posed by importing beef and beef products.

The first category of BSE risk as stipulated by OIE is countries with ''negligible BSE risk'' (OIE, 2006a). These are countries that meet the OIE surveillance guidelines and have not detected indigenous BSE (OIE, 2006a). Australia is an example of a country that, theoretically, would be designated as having negligible BSE risk and, as such, has not implemented stringent domestic SRM removal and disposal laws (Table 1). In countries with negligible BSE risk, prevention and surveillance are of paramount importance. Stringent live-animal importation and feeding laws must be observed to prevent the introduction of the disease into the population.

The second category of BSE risk includes countries with a ''controlled BSE risk'' (OIE, 2006a). Countries in this category have detected BSE in their indigenous cattle herd and have implemented necessary control measures to ensure food safety (OIE, 2006a). The United States, the United Kingdom, and Japan are examples of countries that could be designated as controlled BSE risk countries; tissues designated as SRM in these countries are listed in Table 1. Each of these countries that, hypothetically, would be designated within this category has had drastically different experiences with controlling BSE. Because BSE was discovered in the United Kingdom, control measures were not immediately available and prevalence of the disease reached very high levels. Once control measures were in place and the proper amount of time elapsed for them to take effect, cases of BSE in the United Kingdom declined (OIE, 2007). The United States, on learning of BSE and the experiences of the United Kingdom, implemented preventive measures to minimize transmission of the disease in its cattle herd and has had only two cases of indigenous BSE (USDA, 2006a). In contrast, Japan did not implement BSE control measures until the disease was found in its cattle herd and Japan is still experiencing new cases of BSE, including 10 cases in 2006 (OIE, 2007). In order to be considered for the controlled BSE risk category, countries must meet the requirements of Type A surveillance and complete a risk assessment (OIE, 2006b).

The third category of BSE risk includes countries that have an ''undetermined BSE risk'' (OIE, 2006a). Countries in this category do not perform BSE surveillance, do not report any BSE surveillance results to the OIE, or do not meet the requirements of either of the other two categories (OIE, 2006a). Any country that does not meet the requirements of the ''negligible BSE risk'' or ''controlled BSE risk'' categories would

hypothetically be designated as an ''undetermined BSE risk'' country by OIE.

Testing methods and procedures for BSE are outside the scope of this chapter; however, without internationally accepted and practiced methods of BSE testing for prevalence determination, all countries are not parallel and the risk of consuming meat products from countries that do not conform to international standards cannot be estimated. This problem is further exacerbated by countries that consider themselves free of BSE but do not test for the disease based on international standards, orat all. Once prevalence is established through an accepted surveillance program, countries can be categorized and risk can be more closely estimated. Once risk can be estimated, control measures can be implemented and that risk can be reduced at measurable amounts until an acceptable level of safety for all parties concerned is achieved. Each individual sovereign country has the right and responsibility to protect their population from public health threats. Currently, the food safety threat to public health of BSE is unknown because the infectious dose is unknown. Nevertheless, countries can make judgments based on available scientific knowledge concerning tissue infectivity, prevalence information provided by each country to third-party international agencies such as OIE, and countermeasures (to prevent BSE and vCJD amplification) in place in each country from the farm to the consumer. If countries determine that BSE is a hazard of private or public health concern, there are specific countermeasures that can be used to prevent the presence of potentially infective tissue in meat products.

III. CARCASS CONTAMINATION WITH POTENTIALLY INFECTIOUS TISSUES

Currently, there is no test (other than histology, possibly) that is capable of identifying the presence of SRM on a beef carcass. As such, this chapter will hereafter concentrate on the contamination of carcasses by CNS tissue and the diagnostic testing for such contamination. The authors do, however, acknowledge that other potentially infective tissues, including SRM, exist. Therefore, if, during their risk analysis, a country or facility determines that CNS tissue (that could potentially harbor BSE and, thus, vCJD-causing prions) in meat products is a food safety risk, routes of contamination with CNS tissue from the carcass to itself and to other carcasses must be identified and reduced to the lowest possible amount. Additionally, the effect of abattoir personnel coming into contact with CNS tissue and spreading it throughout the production plant must be considered. The following is a discussion of potential routes of carcass contamination with CNS tissue.

A. Stunning

In almost all countries, cattle are rendered unconscious by a device that delivers blunt force trauma to the forehead of the animal. There are several types of stunning devices that have been used in the past, including penetrative and nonpenetrative captive bolt stunners powered by gun powder, pneumatic captive bolt stunning devices, and air-injection penetrative captive bolt stunners. Some pneumatic captive bolt stunning devices inject air intracranially and, combined with the invasive action of the captive bolt, have been shown to dislodge brain and spinal cord material such that CNS tissue may enter the bloodstream and be transported into the lungs or heart of animals. Because of this, the use of air-injection captive bolt stunning devices is prohibited in the United States (USDA-FSIS, 2004) and in many other countries. In some countries, the practice of inserting a rod through the captive bolt stunning aperture and destroying the brain stem and the spinal cord in order to completely stop nerve firing and subsequent animal jerking (especially leg kicking), known as pithing, is used to augment worker safety.

Garland *et al.* (1996) reported grossly visible brain tissue varying in size from several millimeters to 14 cm in the lungs of 2.5–5.0% of cattle at slaughter when a pneumatic air-injection captive bolt stunner was used. Similarly, Schmidt *et al.* (1999) evaluated presence of blood clots in hearts of cattle in 15 packing plants in the United States. In plants where air-injection stunning devices were used, 33% ($n = 1050$) of hearts evaluated contained large clots in the right ventricle. Additionally, in plants that used pneumatic (non-air-injecting) and captive bolt stunning devices, 12% and 1% of hearts, respectively, contained clots in the right ventricle. Schmidt *et al.* (1999) also reported presence of large (10 to 13-cm long) pieces of spinal cord in the hearts of two animals that were stunned using pneumatic air-injection stunning devices. They noted that, in cow/bull harvest facilities (as opposed to steer and heifer facilities) due to the animal's old age, the captive bolt remained inside the skull longer and, thus, more air was injected into the cranial cavity. Therefore, severe disruption of the brain and spinal cord ensued which was then transferred in large pieces via venous blood to the heart (Schmidt *et al.*, 1999).

Anil *et al.* (1999) stunned 60 animals with one of four different types of stunning devices, including a penetrative captive bolt stunner used with and without air-injection, a nonpenetrative captive bolt stunner, and a pneumatic air-injection stunning device. Blood samples were collected for 60 s following stunning, and the buffy coat of the blood was assayed using an enzyme-linked immunosorbent assay (ELISA) for presence of Syntaxin 1-B and Annexin V (Anil *et al.*, 1999). They reported that CNS tissue was present in the jugular venous blood of 4 of 15 animals stunned using a pneumatic air-injection captive bolt stunner and 1 of 16 animals stunned

by a captive bolt stunner followed by pithing. No CNS tissue was found in venous jugular blood of animals stunned by use of a penetrative captive bolt stunner without pithing or nonpenetrative captive bolt stunning (Anil *et al.*, 1999).

Prendergast *et al.* (2003) collected Syntaxin 1-B and glial fibrillary acidic protein (GFAP) swab samples from 21 different locations in a beef packing abattoir, including slaughtered animals, slaughter equipment, the abattoir environment, and personnel. Samples collected after stunning indicated that the aperture created by the use of a penetrating captive bolt stunner allowed for CNS tissue to drain from the animal and onto the floor, equipment, and personnel of the abattoir. Furthermore, Prendergast *et al.* (2003) reported that CNS tissue remained on the captive bolt stunner, providing a vehicle for cross-contamination with CNS tissue.

Rovira *et al.* (2007) evaluated two different methods of stunning cattle. The authors rendered 10 animals insensible in a controlled laboratory setting with a non-air-injection penetrative captive bolt stunning device. Following stunning, five of the animals were immediately exsanguinated, while the other five were administered an electric shock by a hands-free heart defibrillator in an attempt to stop blood circulation. Blood samples were collected from jugular catheters before stunning and at 90-s intervals for 6 min. Scientists did not find any CNS tissue in the whole or buffy coat of any of the blood samples, regardless of the stunning method. To further investigate potential CNS tissue spread during stunning, the researchers collected blood samples from 360 animals, immediately after sticking, at 12 commercial beef packing facilities that used non-air-injection pneumatic captive bolt stunning devices. Only 1 of the 360 samples collected was positive for CNS tissue. Finally, Rovira *et al.* (2007) evaluated blood samples from 30 cattle collected during Kosher slaughter (because animals harvested in this manner are not rendered unconscious before exsanguination) and found no CNS tissue contamination. These researchers concluded that (1) because the heart functions normally after stunning, the interval between stunning and sticking is the period of highest risk for dissemination of CNS tissue; and (2) non-air-injection penetrating captive bolt stunning devices are safe, if used properly, and do not create or perpetuate CNS tissue cross-contamination hazards (Rovira *et al.*, 2007).

In summary, stunning of animals creates the potential for CNS tissue contamination, slaughter equipment, and abattoir personnel, depending on the type of stunning and whether pithing is used. Further investigation is needed to determine methods that may be implemented to control this contamination. Air-injection stunning devices used to render animals unconscious may dislodge brain and spinal cord tissue and could allow for dissemination of CNS tissue through the bloodstream. The threat of facility personnel coming into contact with CNS tissue draining from the

stunning aperture is of particular concern as human movement throughout facilities and outside of the facility could potentially spread CNS tissue to areas thought to be free of CNS tissue. In countries or facilities which determine that BSE is a food safety risk, efforts should be undertaken to promote methods of stunning that do not penetrate the skull of the animal, to limit the draining of CNS tissue from the stunning aperture (perhaps by ''corking'') and to minimize employee contact with CNS tissue. Furthermore, countries that use pithing and/or air-injection stunning devices should be encouraged to cease those practices because they could contribute to the spread of CNS tissue to meat products.

B. Carcass splitting

In abattoirs around the world, beef carcasses are normally split laterally down the center of the vertebral column, usually by use of a circular band saw, to separate them into ''sides.'' Carcass splitting disrupts, severs, and spreads the spinal cord tissue along the vertebral column of a carcass. Additionally, carcass splitting saws accumulate spinal cord tissue inside the saw housings during the splitting process and spread that CNS tissue to the split surfaces of subsequent carcasses.

Helps *et al.* (2002) compared the CNS tissue contamination from use of a common commercial carcass splitting saw (Jarvis Buster VI) to that from use of an experimental oval-shaped saw designed to remove a portion of the vertebral column without disrupting the spinal cord. Samples were collected from five areas of the carcass, from saw operators' aprons, and from aerosol screens placed near the site of splitting. Results indicated that use of the experimental oval-shaped saw to remove the spinal cord and surrounding vertebral column resulted in significantly less CNS tissue contamination of the carcass and the saw operators' apron than use of the commonly used carcass splitting saw. No CNS tissue was found in any of the aerosol screens near the carcass splitting environment (Helps *et al.*, 2002).

Helps *et al.* (2004) slaughtered two female cattle, followed by one male, and then four female cattle and collected swab samples from the split vertebral-column surfaces. Real-time polymerase chain reaction protocols were followed to determine the extent to which tissue from the male carcass accumulated in the splitting saw and was disseminated to subsequent female carcasses. Under simulated abattoir conditions (i.e., washing the saw for 5 s between carcasses and washing the carcasses before collecting samples), these researchers reported that 0.01% of the tissue recovered from the split vertebral-column surface of the final female carcass in the sequence was from the male carcass and that 10% of the tissue remaining in the housing of the saw was from the male carcass. It was concluded from that study that ''should a BSE-positive carcass be

identified, significant contamination of carcasses further down the line cannot be ruled out'' (Helps *et al.*, 2004).

Bowling *et al.* (2006) evaluated cross-contamination of carcasses with CNS tissue via splitting saws at commercial beef packing facilities and identified the split surface of the aitch (pelvic) bone as a viable testing site at which to measure cross-contamination with CNS tissue. They reported that (1) the carcass splitting saw completely severs the aitch bone before beginning to split the vertebral column and, therefore, CNS tissue on the aitch bone would likely result from cross-contamination derived from the carcass splitting saw; (2) this was confirmed by collecting swab samples from the split surface of the aitch bone and from the *cutaneous omobrachialis* muscle of the same carcass and having the DNA of the samples compared; and (3) in each of five cases, the DNA from the excised muscle samples did not match the DNA from the material collected from the split aitch bone surface of the same animal (Bowling *et al.*, 2006).

In a subsequent trial, Bowling *et al.* (2006) collected samples from the aitch bone after carcass splitting and after carcass washing in five commercial beef packing plants. Samples were analyzed for the presence of GFAP, using the procedures of Reddy *et al.* (2006). They reported that 5.6% of 320 samples collected after carcass splitting were positive for GFAP, while 2.5% of samples collected after carcass washing were positive for GFAP (Bowling *et al.*, 2006), indicating that carcass splitting saws cross-contaminate CNS tissue and that final carcass washing cabinets do not completely remove that contamination. In another trial, Bowling *et al.* (2006) evaluated four different saw-washing procedures performed with three different water temperatures circulating within the saw. Results indicated that tissue accumulation inside the saw housings and on the saw blade was not different among different water washing temperatures. The authors noted that, under normal slaughter conditions, hot (60 °C) water was circulated within the carcass splitting saw to reduce microbiological cross-contamination and was not likely to be changed to reduce CNS tissue contamination. Finally, Bowling *et al.* (2006) evaluated two different carcass splitting-saw models and determined that both harbor CNS tissue in the saw housings and on the saw blade, and that neither one demonstrated any comparative advantages over the other with respect to preventing cross-contamination with CNS tissue.

In addition to investigating the effects of carcass splitting and alternative methods of spinal cord removal on CNS tissue dissemination, researchers have also investigated methods for removing meat products from the carcass without splitting the carcass. Rotterud *et al.* (2005) investigated hot boning of carcasses, the process of removing prerigor meat from the carcass as a procedure to prevent CNS tissue contamination due to splitting. The researchers laterally split carcasses with a circular saw to simulate conventional carcass splitting (although carcass splitting is

more conventionally accomplished with a band saw) and horizontally split intact carcasses between the 10th and 11th vertebrae; intact carcasses were never split laterally, thus limiting dissemination of CNS tissue. Results from carcass sampling sites indicated that GFAP ranged from ''not detectable'' to 932.0 ng/mg total protein on conventionally split carcasses and from ''not detectable'' to 5.8 ng/mg total protein on intact carcasses. Results from minced (ground) beef samples indicated that there was no difference in GFAP concentrations between splitting methods. However, GFAP concentration on surfaces of tables on which conventionally split carcasses had been fabricated was nearly 100 times higher than the GFAP concentration on surfaces of tables on which intact carcasses had been fabricated. These researchers concluded that (1) boning of intact carcasses split horizontally rather than laterally resulted in significantly lower amounts of CNS tissue on carcasses and fabrication tables, (2) a cost-benefit analysis must be completed to determine the risk of minute amounts of CNS tissue in meat products, and (3) the cost of shifting to hot boning of intact carcasses may be high (Rotterud *et al.*, 2005).

In summary, scientific evidence indicates that carcass splitting could potentially contribute to CNS tissue cross-contamination if not controlled. In addition to spreading CNS tissue from the carcass being split, carcass splitting saws may harbor and disseminate CNS tissue to subsequent carcasses. Alternative methods for carcass splitting, spinal cord removal before carcass splitting, and carcass boning/fabrication have been shown to be more effective than traditional carcass splitting at reducing the spread of CNS tissue. However, factors such as cost, time the process takes, existing facility design, effectiveness, and the likelihood of a public health risk must be taken into account when use of these alternative methods is considered. Additionally, the risk of minute amounts of CNS tissue in meat products must be quantified in order to determine if drastic changes to beef slaughter and fabrication practices are needed.

C. Removal of CNS tissue

SRM which include CNS tissue are tissues from animals that are known to carry, transfer, or perpetuate infectivity of the BSE causative agent, prions. Because BSE is an adult-onset disease, identification of tissues as SRM is dependent on the age of the animal at slaughter, and definitions of SRM vary by country, based on differences in interpretation of scientific evidence and the amount of risk allowed (Table 1). Care must be taken when removing SRM (including CNS tissue) to prevent contamination of products due to improper or incomplete removal of SRM and to prevent cross-contamination of SRM to meat products via personnel or equipment.

To quantify potential CNS tissue cross-contamination during the slaughter and fabrication processes, Prendergast *et al.* (2003) collected samples

from (1) the hands and aprons of facility workers immediately after head removal and during transfer of heads from the chain to an SRM bin, (2) the wash water draining from the skull after washing, (3) the knives and aprons of employees on the fabrication floor, and (4) tables and conveyor belts on the fabrication floor. While both hands and aprons of workers that removed heads and workers who placed the heads into SRM bins were contaminated with SRM, the hands were significantly more contaminated. Wash water draining out of the skull after head washing was also contaminated with CNS tissue. On the fabrication floor, CNS tissue contamination occurred on the aprons and knives of workers, on samples collected from the striploin on the fabrication tables after 2 hours of operation, and in the carcass separation saws and on the carcass conveyor belts after 6 hours of operation (Prendergast *et al.*, 2003).

One strategy that has been implemented to prevent SRM tissue cross-contamination during SRM removal involves use of dedicated knives and splitting saws where SRM could be potentially handled. This strategy could be used during carcass splitting (and where saws are used on the fabrication floor), during head removal, and to remove the spinal cord. A ''dedicated'' splitting saw can be used for all carcasses over the age limit at which spinal cord is designated as an SRM. Many facilities have implemented a standard operating procedure for ''dedicated'' knife SRM removal whereby edible tissues are removed by a knife with a handle of a specified color, while SRM is removed with a knife having a handle of a different color. When the latter procedure is followed, care must be taken not to allow cross-contamination among the two kinds of knives.

Proper removal of SRM (and, thus, CNS tissue) from beef carcasses is of paramount importance for processors that export, as different trading partners consider BSE differently, as a food safety risk; international standards should be followed to determine acceptable practices in trade. In international trade, presence of CNS tissue in meat products (from countries that have had indigenous cases of BSE) is unacceptable and, thus, must be completely prevented.

IV. METHODS OF DETECTION OF CNS TISSUE IN MEAT PRODUCTS

Many methods have been investigated and are in use today for detection of CNS tissue in meat products, but not necessarily for all SRMs. In order to identify which test is the most efficacious, the factors of subjectivity versus objectivity, labor, the need for quantitative versus qualitative results, cost, training of qualified personnel, sensitivity, and specificity must be considered. In addition, effects of processing (including grinding, heating, and the addition of other ingredients that may interfere with the assay) must be

considered. Methods of testing for presence of CNS tissue in meat products include tissue dissection and visual inspection, histological staining, immunohistochemistry (IHC), immunochemical assays such as ELISA and Western blot analysis, quantification of cholesterol, reverse transcription polymerase chain reaction (RT-PCR), and gas chromatography-mass spectrometry (GC-MS). The following is a description of these methods and specific CNS tissue markers used by each method.

A. Histological staining and IHC

Wenisch *et al.* (1999) investigated histological staining and immunohistochemistry (IHC) as a means to detect CNS tissue in sausage products. They added bovine brain tissue to normal sausage formulations at concentrations of 0.0%, 7.4%, and 33.3% and heated the mixtures at 80–100 °C for 1 hour. Results indicated that, due to homogenization, histological identification of CNS tissue was not possible, regardless of staining method or amount of brain in the mixture. Immunostaining with mouse antibody for human neuron-specific enolase (NSE) revealed the presence of brain tissue, and varying degrees of staining could be determined based on brain tissue concentration in the sausage mixture. These researchers concluded that the histological procedure was an unreliable method for detecting presence of CNS tissue in processed meat products due to homogenization and high-pressure heating, while IHC with NSE is an effective method for detecting CNS tissue in such products (Wenisch *et al.*, 1999).

Kelley *et al.* (2000) evaluated hematoxylin and eosin (HE) histochemistry, IHC, and polarization microscopy for determining presence of CNS tissue in ground meat products produced by advanced meat recovery (AMR) systems. They collected ground beef samples from establishments that used vertebrae (as a raw material) in their AMR systems and 64 control samples of ground beef from AMR systems in establishments that hand-deboned the vertebral column before generating their products. Of the 196 samples collected from establishments that used vertebrae in their AMR systems, 19 were not subjected to desinewing, a process of pressing the product through fine screens to reduce fragment size to 2–3 nm. Of those 19 samples, CNS tissue was detectable by HE histological staining in only 2 samples, while no CNS tissue was found by HE histological staining in any of the other 177 samples subjected to desinewing, or in any of the 64 control samples. The researchers next investigated use of neurofilament and GFAP antibodies for immunohistochemical staining and found CNS tissue in 7 of 17 samples with both methods. Results indicated that peripheral nervous tissue (rather than CNS tissue) was present and detected by both GFAP and neurofilament staining. The researchers also investigated synaptophysin, a transmembrane glycoprotein localized in the CNS and not found in peripheral nervous tissue.

They found it to be a useful marker for CNS tissue and, more specifically, a useful marker for differentiating between CNS tissue and tissues of the peripheral nervous system in AMR products (Kelley *et al.*, 2000).

Tersteeg *et al.* (2002) evaluated the immunostaining ability of four antibodies in minced and intact meat products with CNS tissue contamination at 0%, 5%, 10%, and 20% levels and heat treatments of 0, 70, and 115 °C. The antibodies evaluated were anti-neurofilament (anti-NF), anti-myelin basic protein (anti-MBP), anti-NSE, and anti-GFAP. Results from this study indicated that anti-MBP was the most effective staining method due to its ability to properly stain target tissue even after heating and mincing treatments. Anti-NF was able to detect CNS tissue in all raw meat products; however, when heat treatments were applied, anti-NF staining diminished. Anti-GFAP staining was effective at creating a strong staining reaction with minced and intact products; however, background staining diminished the researchers' ability to identify CNS tissue, and heating of samples produced a background staining that made interpretation impossible. Anti-NSE stained effectively in raw and minced unheated meat products; but when heat treatments were applied, the anti-NSE was undetectable in all products. Furthermore, at the 1% CNS tissue level, staining with anti-NSE was ineffective. These researchers concluded that anti-MBP was the most effective IHC staining antibody due to its ability to effectively stain CNS tissue in raw, minced, and heated meat products (Tersteeg *et al.*, 2002).

In summary, histological staining has been shown to be an ineffective method of CNS tissue determination in processed meat products subjected to homogenization even at CNS tissue concentrations of up to 33.3%. Additionally, histology is a qualitative method that requires highly trained personnel and is subject to misleading results due to the small proportion of the product that can be viewed microscopically. Similarly, IHC methods require highly trained personnel, expensive equipment, a long period of time to complete, and are limited by the amount of sample that is viewed for detection. As such, histological staining and IHC methods are poor CNS tissue screening tests and are more applicable in confirmatory/reference assay roles. Due to the seemingly poor sensitivity of these assays (reported to be 10 times lower than the immunochemical methods described by Hossner *et al.*, 2006), other methods may be more effective.

B. Immunochemical assays and quantification of cholesterol

There are many immunochemical methods that have been implemented to detect CNS tissue in meat products. Additionally, different CNS tissue markers have been used to investigate, qualitate, and quantitate CNS tissue presence in meat products. Immunochemical methods offer

highly sensitive and specific CNS tissue detection capabilities and are not subjective.

Lucker *et al.* (1998) evaluated histological and immunochemical methods of detection of CNS tissue in meat products and reported that because the cholesterol content of tissues of the CNS is 2000 mg/100 g compared to ~100 mg/100 g in other tissues, cholesterol could be used as a screening marker for CNS tissue presence in meat products. Their data suggested that cholesterol content increased by 26 mg/100 g of fresh substance for each percentage of brain tissue added to a reference product produced in their laboratory, and that normal cholesterol content of emulsion-type cooked sausages and liver sausages was 115 mg/100 g and 181 mg/100 g of product, respectively. The researchers indicated that any amount of cholesterol over those reference cutoff values should be further evaluated with a more specific CNS tissue marker (Lucker *et al.*, 1998). In an assessment of various histological staining methods specific to CNS tissue, the researchers were unable to identify CNS tissue in any of the samples. As a result, the researchers then investigated immunohistochemical staining of products containing 0%, 7.4%, and 33.3% of brain tissue by use of monoclonal anti-NSE antibodies and were able to detect CNS tissue due to the increased staining intensity associated with increased brain content of the samples. Finally, Lucker *et al.* (1998) evaluated immunochemical detection of NSE tissue by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting. NSE is a dimeric protein composed of three immunologically distinct subunits ($\gamma\gamma$ and $\alpha\gamma$) which are present in CNS tissue in greater concentrations than in non-CNS tissues (Kato *et al.*, 1982). Results indicated that brain was detected at a 1% concentration using the Western blotting method in emulsion-type sausages, and at a <4% concentration in liver-type sausages. The researchers concluded that the cholesterol assay offered an inexpensive and rapid screening method for CNS tissue in meat products, particularly for processed products such as sausages, and that NSE immunochemistry is a highly specific marker for CNS tissue presence in meat products (Lucker *et al.*, 1998).

Lucker *et al.* (1999) evaluated 402 retail sausage samples from four different categories of sausages including (1) cooked emulsion-type sausages, (2) cooked blood sausages, (3) cooked sausages of the fat or gel type, and (4) heat-treated meat products such as hamburgers or meatballs. The upper limit of acceptable cholesterol content in each product was first determined using methods previously reported (Lucker *et al.*, 1998), and then products were divided into two groups based on cholesterol levels. The first group consisted of all products except liver sausage, and they ranged in cholesterol levels from 119 to 132 mg/100 g product. For liver sausage, the upper limit of cholesterol was 201 mg/100 g due to the higher endogenous levels of cholesterol in liver tissue. Using the

upper limits for cholesterol concentration to detect CNS tissue described by Lucker *et al*. (1998), scientists determined that 16 of 402 retail sausage samples could contain CNS tissue and that those samples required further testing using immunochemical detection of NSE (by the methods of Lucker *et al*., 1998). Positive NSE immunochemical results were obtained in 7 of the 16 samples that had cholesterol levels above the acceptable limit. These researchers concluded that CNS tissue was present in German retail sausage products and reiterated the effectiveness of their previously reported testing method (Lucker *et al*., 1999).

Lucker *et al*. (2001) evaluated 126 liver sausages for presence of CNS tissue using an immunoassay that detects presence of NSE. They detected CNS tissue in 5 of 126 samples assayed using NSE immunochemistry, and concluded that NSE immunochemistry was a highly specific and moderately sensitive method for detection of CNS tissue in non-heat-treated meat products (Lucker *et al*., 2001).

GFAP is an antigen that is largely restricted to occurrence in CNS. It is a specific marker for differentiated astrocytes and is the cytoskeletal protein providing structural support to CNS tissue cells (Eng and Ghirnikar, 1994). Schmidt *et al*. (1999) first reported a method for using GFAP in a colorimetric ELISA to detect CNS tissue in meat products. These researchers quantified presence of GFAP in bovine brain, cerebral cortex, spinal cord, sciatic nerve, diaphragm, blood clots, skeletal muscle, and ground beef. They reported large amounts of GFAP in tissues from the CNS, small amounts of GFAP in the sciatic nerve, and were unable to detect any GFAP in samples from skeletal muscle, ground beef, or blood with the exception of a sample from a neck muscle, which the researchers concluded was likely cross-contaminated during the slaughter process. A limit for detection of GFAP using the colorimetric ELISA was reported to be 1.0 ng, and intra-assay variation ranged from 3.25% to 4.0% (Schmidt *et al*., 1999).

Schmidt *et al*. (2001b) admitted having made mistakes in calculating the levels of GFAP in tissues in their previous study (Schmidt *et al*., 1999) and reported that GFAP is present at ~2 ng/mg in spinal cord, 600 ng/mg in brain, 12 ng/mg in sciatic nerve, and 2 ng/mg in cervical ganglia. They also reported development of a more sensitive fluorescent ELISA (F-ELISA) for detecting GFAP in meat products that increased sensitivity of the assay, lowering the detection limit to 0.2-ng GFAP. In another study, these scientists reported that GFAP was detectable after the addition of normal sausage ingredients and after being heated up to 80 °C; only after heating sausage samples to 115 °C for 100 min did GFAP become undetectable (Schmidt *et al*., 2001a). Additionally, Schmidt *et al*. (2001a) reported that the F-ELISA was more sensitive than was the Syntaxin 1-B ELISA. Schmidt *et al*. (2001b) compared the F-ELISA to a commercial ELISA (R-Biopharm, Inc., Darmstadt, Germany) test kit based on their

colorimetric ELISA (R-ELISA) and reported that the F-ELISA was more sensitive than was the R-ELISA, but that their results were deemed inconclusive due to the poor homogenization of spinal cord/ground beef mixtures analyzed.

Agazzi *et al.* (2002) compared the commercially available Brainostic (ScheBo-Biotech, Marietta, Georgia, United States) NSE Western blot test kit to the R-ELISA using heat-treated sausages (0, 80, and 120 °C) that were spiked with CNS tissue concentrations of 0%, 0.5%, 1.0%, and 2.0%. After sausage samples were sent to 29 laboratories for determination of CNS tissue presence using both the Brainostic™ test kit and the R-ELISA, results indicated that sensitivity of both tests in nonheated and moderately heated samples was 0.5% CNS tissue (raw weight basis). In strongly heated samples, the R-ELISA was more specific than the Brainostic™ test kit, which exhibited a low level of false-negative results. As such, the detection limit for the Brainostic™ test kit in strongly heated products was determined to be 2%, whereas the detection limit for the R-ELISA remained at 0.5% (raw weight) (Agazzi *et al.*, 2002).

Hajmeer *et al.* (2003) compared the commercial Brainostic™ test kit to the R-ELISA by mixing spinal cord with a ground product from carcasses of three grades (utility, select, and choice) to yield samples containing CNS tissue concentrations of 0.0%, 0.0125%, 0.025%, 0.05%, 0.1%, 0.2%, 0.4%, 0.8%, and 1.6% for products from each grade of beef. For ground beef of each grade, five samples were assayed using each diagnostic test for presence of CNS tissue. Results from the Brainostic™ diagnostic test kit indicated that the limit of detection of NSE was 0.25%, regardless of quality-grade origin of the beef, and the limit of detection of CNS tissue for the R-ELISA was 0.025%, regardless of quality grade of beef. They concluded that the Brainostic™ diagnostic kit was 10 times less sensitive than the R-ELISA, took 30 hours to complete compared to 2 hours for the R-ELISA, and costs ~4 times more than the R-ELISA to perform (Hajmeer *et al.*, 2003). Findings of this latter study agreed with the cautions of Agazzi *et al.* (2004) with regard to the R-ELISA, indicating that the test is an effective commercial tool for qualitative analysis of CNS tissue presence in meat products, but that caution should be used when attempting to quantify the amount of CNS tissue present in samples because there is a mixture of brain and spinal cord in the R-ELISA standards (Hajmeer *et al.*, 2003).

Agazzi *et al.* (2004) prepared products, spiked with bovine brain tissue at concentrations of 0.0%, 0.5%, 1.0%, and 2.0% that were subjected to three differing heat treatments (none, 80 °C for 20 min, and 120 °C for 20 min), coded them, and sent samples of each product to each of 19 laboratories for analysis by the R-ELISA method. They reported that all samples with 0% CNS tissue were correctly identified as negative for presence of CNS tissue and that, at the 0.5% level of CNS tissue

concentration, the number of false negatives exceeded 5%. Therefore, 1.0% CNS tissue was determined to be the limit of detection of the R-ELISA, irrespective of heat treatment applied; R-ELISA is an effective qualitative detection tool for CNS tissue above those levels (Agazzi *et al.*, 2004).

Hossner *et al.* (2006) compared the GFAP F-ELISA developed by Schmidt *et al.* (2001a), the R-ELISA, and immunohistochemical (IHC) methods used by USDA-FSIS (USDA-FSIS, 1998) to detect CNS tissue in meat products by spiking ground beef samples with brain, spinal cord, or dorsal root ganglia, and then homogenizing samples to obtain CNS tissue concentrations varying from 0.05% to 0.5%. In addition to laboratory analysis of spiked samples, these researchers collected samples from the AMR systems of five commercial beef packing facilities. Results indicated that the F-ELISA was able to detect 0.3-ng GFAP while the R-ELISA was only able to detect 1.2-ng GFAP per well; and, in addition to being more sensitive, the F-ELISA was also more specific than the R-ELISA. Results indicated that the intra-assay coefficients of variation were 0.61–13.20% and 3.0–36.0% for the F-ELISA and the R-ELISA, respectively, and that the interassay variation was 6–26% and 18–32% for the F-ELISA and the R-ELISA, respectively. None of the methods tested could routinely detect dorsal root ganglia at any of the concentrations tested. These researchers reported that (1) the F-ELISA was able to detect brain and spinal cord at 0.05% raw weight, whereas the R-ELISA was only able to detect spinal cord at 0.3% and was unable to detect brain tissue at any level; (2) the IHC method was able to detect 0.3% spinal cord and 0.5% brain (raw weight) with 100% of samples testing positive; (3) when lower concentrations of brain and spinal cord were used, the IHC method was able to detect CNS tissue in only 70–90% of the samples; and (4) of the AMR samples evaluated by all three methods, 17.2%, 3.2%, and 2.1% of samples assayed by the F-ELISA, the R-ELISA, and IHC methods, respectively, were positive for CNS tissue contamination (Hossner *et al.*, 2006). Hossner *et al.* (2006) concluded that (1) the GFAP F-ELISA is the most effective method to detect CNS tissue in meat products, but not dorsal root ganglia (Hossner *et al.*, 2006); (2) the R-ELISA should be used with caution because results were highly variable, the assay was not as sensitive as the F-ELISA, the assay was highly sensitive to room temperature, and the assay could not detect brain tissue at any of the concentrations tested; and (3) the IHC method was less sensitive and more time consuming than was the F-ELISA and required trained personnel and specialized equipment to perform.

In summary, immunochemical testing is a highly sensitive, specific, and objective method of determining CNS tissue presence. Furthermore, immunochemical methods are relatively rapid, easy to complete with little training, and equipment costs are low. All of these traits lead to the conclusion that immunochemical assays are well suited for screening of

meat products for presence of CNS tissue. Drawbacks to use of immunochemical methods for detection of CNS tissue include (1) tissue-specific results that are not capable of differentiating species or age of animals; (2) unquantifiable loss of protein and, thus, sensitivity due to heating or further processing; and (3) a lack of ability to quantify results, especially in heat-treated or processed products (Biedermann *et al.*, 2004).

C. Gas chromatography-mass spectrometry

Another method that has been evaluated for its ability to detect CNS tissue in meat products is GC-MS. Niederer and Bollhalder (2001) reported that fatty acids could be detected at a sensitivity of 0.01% by solid phase extraction, acidic methanolysis to methylated esters (FAME), and evaluation via GC-MS. Biedermann *et al.* (2002) evaluated the brain-specific fatty acids, docosahexaenoic acid (DHA; C 22:6), lignoceric acid (C 24:0), nervonic acid (C 24:1), and cerebronic acid (C 24oh) as potential markers for CNS tissue in meat products. After creating standardized meat products with known CNS tissue concentrations, fatty acid concentrations were determined and, by modifying the protein extraction methods of Niederer and Bollhalder (2001), they were able to achieve a tenfold increase in sensitivity which resulted in a detection limit of 0.01% CNS tissue (Biedermann *et al.*, 2002). By modifying the GC-MS methods of Niederer and Bollhalder (2001), Biedermann *et al.* (2002) were able to correctly identify 60 samples with varying CNS tissue amounts as positive or negative. Use of GC-MS detection of CNS tissue in meat products could serve as a valid reference method for immunochemical or immunohistochemical determination of CNS tissue in meat products (Biedermann *et al.*, 2002).

Lucker *et al.* (2004) evaluated the ability of GC-MS using several brain-specific fatty acids to identify and quantify CNS tissue in meat products. The researchers determined specific fatty acid content of brains from cattle, calves, sheep, pigs, turkeys, as well as muscle and adipose tissue. They determined that species and age characterizations could be made based on the concentration of specific fatty acids present in a sample. Sensitivity of GC-MS CNS tissue detection was reported to be 0.01% raw weight, but the practical sensitivity was 0.1–0.5% raw weight CNS due to the fatty acid baseline content in muscle and adipose tissue.

Detection of CNS tissue by GC-MS is a highly sensitive assay. The ability of GC-MS to differentiate fatty acids between species and ages of animals is very strong, and it is a useful analytical tool with respect to addressing regulatory issues concerning SRM removal. However, detection of CNS tissue by GC-MS is likely impractical on a large scale, everyday basis due to its high cost, the length of time required to conduct the assay, and the technical expertise required of technicians (Biedermann *et al.*, 2004). Furthermore, GC-MS detection of CNS tissue, thus far, has

only accounted for fatty acids that are found in the brain and not those found in other CNS tissues (i.e., spinal cord). A method that is incapable of detecting tissues from the spinal cord could result in false-negative results and inaccurately low numbers of positives when CNS tissue is quantified in meat products. Research is needed to identify fatty acids present in spinal cord and brain in order for GC-MS to serve as a reference assay for CNS tissue in meat products.

D. Polymerase chain reaction

Detection of GFAP mRNA in minced meat and meat products by an RT-PCR was described by Seyboldt *et al.* (2003). These researchers collected samples of liver, kidney, spleen, lung, lymph node, heart, skeletal muscle, and spinal cord of adult cattle and, using RT-PCR methods, detected GFAP mRNA in heart, skeletal muscle, and spinal cord samples (Seyboldt *et al.*, 2003). The unexpected presence of GFAP in heart and skeletal muscle samples was explained by the scientists as likely peripheral nervous system tissue; however, the possibility exists that, due to stunning and other slaughter processes, CNS tissue could have cross-contaminated tissues from other parts of the animal and/or carcass. They further reported that CNS tissue could be detected at concentrations of 0.5% in a brain/minced meat homogenate stored for up to 35 days and heated up to 70 °C; nevertheless, sensitivity of the RT-PCR assay is likely below 0.5% CNS tissue on a wet weight basis (Seyboldt *et al.*, 2003). These researchers explored the use of restriction fragment length polymorphisms (RFLP) as a means to identify the animal species from which the CNS tissue originated that was found in meat products. Using brain tissue from bovine, equine, ovine, porcine, turkey, chicken, and deer sources, these researchers found that species identification was possible, except that deer and bovine species could not be differentiated (Seyboldt *et al.*, 2003). More research is needed to determine the sensitivity of the aforementioned assay. By using an RT-PCR assay as a reference, a species-specific assay could be implemented. However, due to equipment costs, the amount of technical expertise required to run the assay, and the time needed, RT-PCR is not likely to become a broad-based screening method in commercial applications.

CNS tissue in meat products can be detected in a variety of ways and with varying degrees of sensitivity and specificity. For industrial application, immunochemical detection of NSE or GFAP as a screening method, followed by a confirmatory species- and/or age-specific assay (if needed), seems most appropriate and efficient. Each assay described herein has specific limitations that preclude the declaration of one single assay as the most appropriate for every type of testing. Researchers and industry scientists must evaluate specific objectives of their sampling and testing

program against capabilities of each assay and determine which assay or combination of assays is most prudent for their work. Methods that require highly trained personnel, long periods of time to complete, expensive equipment, and those that are subjective, and/or that do not evaluate all CNS tissues (i.e., brain, DRG, and spinal cord) are likely to be used only as reference assays and not as screening methods. Further research is needed to increase the sensitivity of rapid, inexpensive assays to a level that would allow companies to identify, distinguish, and quantify peripheral nervous system tissue and dorsal root ganglia in meat products in production plants.

V. CONCLUSION AND FUTURE TRENDS

Meat products containing SRM (and possibly CNS tissue) tissue were banned from human consumption because of knowledge that the infective agent of both BSE and vCJD, the prion, if present, can be contained within such tissues, as well as others. Since BSE and vCJD were linked, fewer than 200 people worldwide have died of vCJD, suggesting intuitively that the infective dose of BSE prions in humans is relatively high compared to the infective dose needed to transmit the disease in cattle. During cattle slaughter, there are many potential sites or sources of CNS tissue contamination, and numerous efforts have been investigated and implemented to control them. For countries that consider BSE a food safety concern that is reasonably likely to occur, further research is needed to identify an effective method of carcass splitting that does not disrupt the spinal cord or, alternatively, that removes the spinal cord and DRG before carcass splitting. Currently, in the EU and Japan, a system is used that vacuums the spinal cord longitudinally from the spinal foramen before splitting. However, this system is incapable of removing the spinal cord at the high production speeds used in the United States and some other countries. Considerations such as cost, time needed to perform, and potential damage of high-value edible meat items near the vertebral column must be taken into consideration when developing a new carcass splitting technology. In addition, research is needed to create rapid testing methods that are highly sensitive and specific to CNS tissue for commercial use in plants. Currently, many tests exist that are able to detect minute amounts of tissues from the brain and spinal cord in meat products. Development of a test able to detect dorsal root ganglia at low concentrations, or other SRM (e.g., distal ileum and tonsils), would be beneficial.

Before the first case of BSE occurred in the United States, beef exports were worth approximately $3 billion/year. In the years since BSE was detected in the United States, beef exports plummeted to extremely low levels, resulting in a $165–$190 reduction in the value of each beef animal

harvested; comparable losses in carcass value were experienced in countries throughout the world when BSE was detected in those countries. There is consensus that BSE-infected tissues, when consumed by humans at high enough doses, can cause susceptible humans to contract the neurological disorder, vCJD. In the absence of knowledge regarding the infective dose of BSE prions to humans, CNS and other tissues were banned for human consumption. It is now believed that the infective dose must be very high. In addition to this information, prevalence information collected by many countries indicates that BSE prevalence may vary tremendously, and that worldwide prevalence is declining due to implementation of successful control measures. Furthermore, there is scientific evidence indicating that CNS tissue presence in meat products usually occurs at extremely low levels—in those plants, at least, rates of cross-contamination are low. In the future, as knowledge about pathogenicity of BSE becomes clearer, cattle slaughter practices can be more effectively directed toward more complete removal of CNS and other SRM tissues from edible products. As testing methods become more sensitive and specific, it is conceivable that the outright ban on all CNS tissue will be removed and replaced with standards for acceptable limits of CNS tissue content based on risk as determined by individual countries or, perhaps, by an international body such as OIE or Codex Alimentarius. No country or process is 100% effective at removing CNS tissue from meat products. However, because of a low or decreasing prevalence of BSE, the use of best practices for SRM removal, good manufacturing practices (e.g., ''dedicated'' tools and equipment), and standard operating procedures (SOP) for detection and removal of CNS tissue from meat products, the risk of humans contracting vCJD from eating meat products is extremely low. Therefore, a more prudent policy of risk mitigation as it pertains to humans contracting vCJD from eating meat products contaminated with CNS tissue and, in exceptionally rare cases with BSE prions, would be to allow international bodies, countries, and individual facilities to address risk based on hazard analysis and critical control points (HACCP) principles of risk analysis and reduction. Agreement on, and implementation of, international testing policies for prevalence of BSE in the indigenous cattle herd of countries and on methods for determination of CNS tissue presence (even in the smallest of amounts) would allow for trade to occur based on knowledge of risk, as opposed to zero tolerance policies.

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