

Food for Thought for Food Animal Veterinarians

What practitioners should know about bovine spongiform encephalopathy to serve clients and the cattle industry

Jared D. Taylor, DVM, MPH

Bovine spongiform encephalopathy (BSE) has been one of the most widely discussed animal diseases of the past 20 years. Its prominence in the US dialogue increased greatly following diagnosis of the first case in the United States in December 2003. Yet, knowledge of the disease and its many impacts on agriculture and society is limited. Many excellent review sources¹⁻⁴ are available that detail the disease and its theorized agent, pathogenesis, and epidemiologic characteristics. The information provided here focuses on the responsibilities of veterinarians regarding the disease and their role as disease experts, agriculture advocates, and public health defenders.

It is imperative that veterinarians understand and be able to explain at least the basic premise of current testing and regulatory efforts to control BSE. Most experts agree that the US government was proactive in creating firewalls to prevent the entry and dissemination of BSE.^{3,6} These have been improved and elaborated on over time, with the most notable alteration being the implementation of additional public health safeguards and launching of a new surveillance program in response to the 2003 case. All protective measures (proposed and in place) can be divided into 3 semi-independent categories⁷: measures to prevent spread or dissemination of BSE within the cattle population, measures to protect public health, and surveillance systems to determine prevalence and guide future policy decisions.

It is important to understand that there are interrelated goals among the 3 categories. For example, when there is no disease in the cattle population, it can pose no risk to public health and the surveillance systems measure the success of the policies to prevent dissemination and guide decisions about future control measures. However, each of the 3 components has unique goals and methods.

Distribution of Infectivity and Specified Risk Materials

To understand the implementation of protective measures, the concept of distribution of infectivity

must be addressed. One of the first challenges in controlling BSE was to identify how the disease is transmitted. Historically, there was no consensus on the causative agent and no sensitive means of detecting pathognomonic markers in tissues other than the brain. However, it was suspected that other tissues may harbor the agent. Thus, a bioassay was used to determine the tissues capable of harboring and transmitting the disease. This meant exposing susceptible animals (typically mice) to various tissues from infected cattle and determining whether disease resulted. Sensitivity of this method is unknown because of differences that may exist among species (eg, mice vs cattle vs humans). To maximize sensitivity and reduce variability in incubation times, intracranial inoculation is the method generally used, which perhaps overestimates the potential for natural transmission. Transmission via the oral route has also been investigated extensively, but researchers often have used high exposure doses and small numbers of subjects.

Experimental transmission studies have revealed that earliest infectivity is for the distal portion of the ileum at approximately 6 months after exposure via the oral route. Other tissues become infective later in the incubation period, including tonsils and CNS material.⁸⁻¹⁰ It has been established that the brain and spinal cord have the highest infectivity. In fact, in clinical cases, nearly 85% of infectivity is restricted to these

Table 1—Infectivity for various tissues of an animal with bovine spongiform encephalopathy (BSE).¹¹

Tissue	g/animal	ID ₅₀ /g	ID ₅₀ /animal*	%†
Brain	500	10	5,000	60.1
Spinal cord	200	10	2,000	24.1
Trigeminal ganglia	30	10	200	2.4
Dorsal root ganglia	20	10	300	3.6
Ileum	800	800	260	3.1
Vertebral column	50	5,000	160	1.9
Spleen	3.2 × 10 ⁻² *	800	26	0.3
Eyes and remainder of head	3.2 × 10 ⁻² *	11,600	370	4.5
Total	18,500	NA	8,316	100

*Number of doses per infected animal that will result in infection in 50% of susceptible exposed animals. †Percentage of total infectivity represented by each tissue.
NA = Not applicable.

From the Center for Food Security and Public Health, College of Veterinary Medicine, Iowa State University, Ames, IA 50011. Dr. Taylor's present address is Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK 74078.

tissues (Table 1).¹¹ Bone marrow was found to be infective in 1 study,¹² but this finding has not been replicated. Infectivity has also been found in association with the trigeminal and dorsal root ganglia. As a result of these findings, the term **specified risk materials (SRMs)** has been created to designate tissues that could potentially be infective. Although exhaustive studies have not been performed to assess infectivity of all tissues, SRMs have been confirmed to pose the greatest risk, and as such, they receive extraordinary regulatory attention.

Because of the slow progression of the disease, age limits for cattle have been created to estimate when tissues should be considered potentially infective. The age chosen (most commonly 30 months) is in some ways arbitrary and pragmatic. Considerations that have contributed to this choice include the fact that the mean incubation period for BSE in cattle in the United Kingdom epidemic was estimated to be 60 months.^{13,14} Scrapie has often been used as a model for transmissible encephalopathies, and experiments with scrapie have revealed that infectivity is detectable at 50% of incubation. Thus, assuming BSE is similar to scrapie, it can be conservatively estimated to reach infectivity at 30 months (ie, 50% of 60 months).¹⁵ Other considerations include the physiologic ability to estimate this age (the second set of incisors should be erupted in cattle at 30 months of age). Finally, clinical disease has rarely been detected in cattle < 30 months old, and these were typically at the height of the epidemic in the United Kingdom (Figure 1).¹⁶ Age at which clinical signs become apparent is dose dependent,¹⁷ which means that higher amounts of exposure result in a shorter incubation period. Therefore, it could be assumed that even if US feeds contained infective materials, it would be at low amounts and would result in a relatively late onset, compared with that seen in cattle in the United Kingdom. This contention is supported by the age of the affected cattle thus far found in North America (the animal in Washington in 2003 was 6.5 years old, the animal in Texas in 2004 was 12 years old, and cattle found in Canada have been between 6 and 8 years of age).

On the basis of the aforementioned considerations, SRMs in the United States are currently defined as the brain, skull, eyes, trigeminal ganglia, spinal cord, vertebral column (excluding the vertebrae of the tail, transverse processes of the thoracic and lumbar vertebrae, and wings of the sacrum), and dorsal root ganglia of cattle 30 months of age and older and the tonsils and distal portion of the ileum of all cattle.¹⁸ It has been suggested that a more strict definition of SRMs be instituted (ie, lowering the threshold to a specified age < 30 months). Regardless of the specific age used in the definition, it is important to understand that the decision is a mixture of

science, pragmatism, and precaution. The guiding tenet is that removal of specific materials can virtually eliminate the risk of transmission. This approach has proven effective in rapidly reducing the number of cases of BSE in the United Kingdom (Figure 2).¹⁹ Methods for excluding SRMs from feeds formulated for cattle and the human food supply will thus play a prominent role in the discussion of measures to protect susceptible cattle and public health.

Measures to Prevent Introduction or Dissemination of BSE Within the Cattle Population

The first measures taken to protect the United States from BSE were to limit the risk of introduction. These were implemented soon after recognition of the disease, which was prior to the appreciation of a public health threat. In 1989, a ban was put in place to prevent entry into the United States of live animals and meat and bone meal (MBM) from any country that had

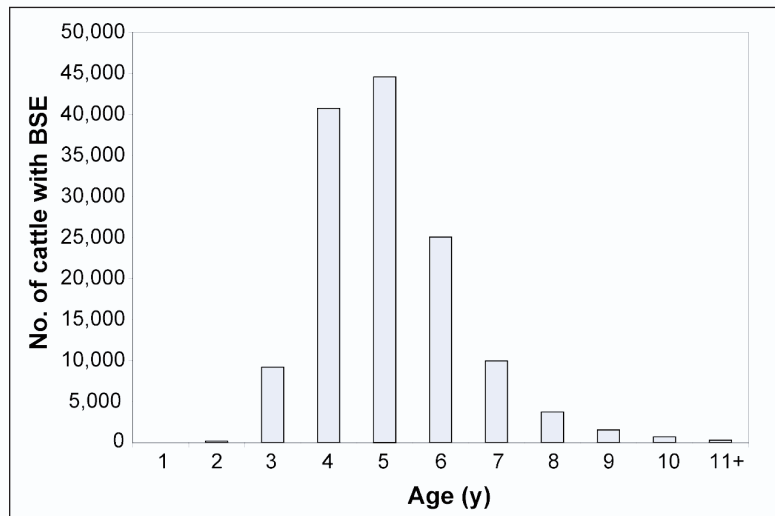


Figure 1—Age at onset of clinical signs of bovine spongiform encephalopathy (BSE) in cattle in the United Kingdom.¹⁶

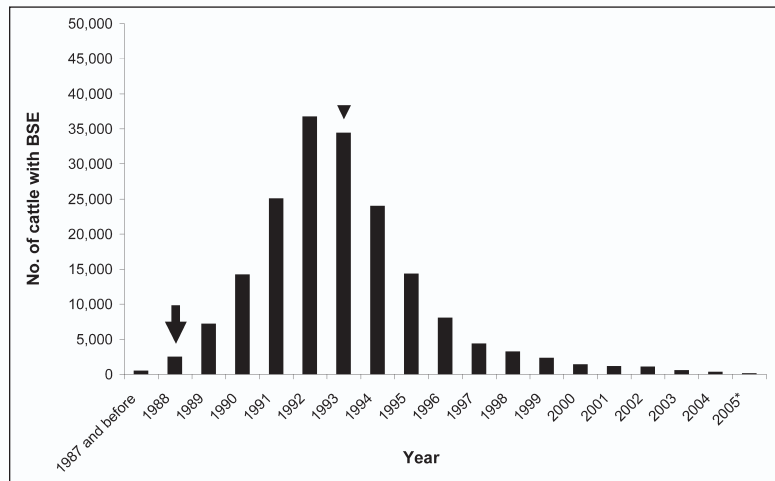


Figure 2—Number of cattle in the United Kingdom in which BSE was diagnosed during various years.¹⁹ A ban on inclusion of ruminant protein in feeds intended for use in cattle was instituted in 1988 (arrow), and the interval 1 incubation cycle (approx 60 months) after inception of that ban is indicated (arrowhead). *Represents data for only the first 3 months of 2005.

a confirmed case of BSE. In 1997, when it became apparent that BSE was in most, if not all, of Europe, the ban was further expanded to include all European countries. In 2000, the importation into the United States of all animal protein products (including products made from poultry, swine, and horses) from the European Union was prohibited. This additional restriction was a result of the concern for cross-contamination of those products by ruminant proteins.

Although there was no evidence that BSE was in the United States in the late 1990s, measures were implemented to ensure that it would not develop into an epidemic, even if it was found in the United States. In 1997, the FDA instituted a prohibition of most mammalian material in feeds formulated for ruminants. This included the most suspect material (MBM from ruminants) but still permitted the use of other proteins considered safe. Materials that remained acceptable included blood and milk products as well as protein derived from horses and swine. Ruminant-derived proteins could still be used in other feed products, but they were required to be maintained separately from those used in feeds formulated for ruminants and to include labeling that they were not to be fed to ruminants. Similarly, plate waste or table scraps were still allowed in feeds formulated for ruminants because these products had already undergone inspection for their use in the human food supply.

After the case of BSE in December 2003, additional precautions were proposed. These included prohibiting poultry litter in cattle feed because of the potential for spilled feed formulated for poultry to contain ruminant MBM, a ban on the use of blood products in feeds formulated for ruminants, and prohibition of feeding plate waste. Restrictions were also proposed for feed mills that produce feeds intended for cattle and feeds that could contain prohibited products (eg, foods intended for dogs and cats). It was proposed that these feed mills be required to use dedicated facilities to ensure there is no cross-contamination of prohibited materials into feed intended for cattle. As of August 2005, these proposals have not been enacted. The principal delay in this process involves the discussion of whether the new regulations should be expanded to include recommendations made by an **international review panel (IRP)**.²⁰

An IRP was commissioned by the USDA at the conclusion of the investigation of the December 2003 case of BSE. The purpose of the IRP was to consider the design and conduct of the investigation; determine what additional information should be gathered, if any; and provide suggestions for additional control measures that would be justified by the status of BSE in North America. This evaluation was to be based on the best available science and experiences of other countries. The IRP concluded that it was likely there was BSE material in the United States and that it had potentially been recycled. Thus, the IRP recommended an aggressive surveillance program to verify a low prevalence. If more than a minimal risk was found, the IRP would suggest a broader definition of SRMs be devised to include the brain, spinal cord, skull, and vertebral column (because of inability to exclude the dorsal root ganglion and cranial nerves from these tissues) from cattle 12 months of age and older.

Regardless of the ultimate age limit for defining SRMs, the IRP recommended eliminating SRMs from all feeds formulated for animals, including foods manufactured for dogs and cats. Additionally, they recommended the elimination of all mammalian and poultry protein from feeds formulated for ruminants (not just ruminant-derived MBM, as is currently excluded). These last 2 provisions were out of concern that feeds formulated for ruminants may be cross-contaminated by potentially infective material. Because of the extremely low amounts of material needed to transfer infectivity, it was the IRP's position that preventing the potential for cross-contamination would be more effective than testing for it.²¹ These recommendations are currently under consideration by the FDA. The agency has published a request for public comment on these proposals²² but has not published an interim or final rule.

The center of the debate regarding more stringent regulations is whether the costs associated with these measures are justified by the current risk. Some have argued that inspections have documented that compliance with current regulations is extremely good, meaning that little, if any, potentially infective material is entering feeds formulated for cattle. These opponents assert that when this fact is combined with the low prevalence in the United States, there is little or no risk of BSE entering feeds formulated for cattle. This would support the conclusion that current regulations are adequate. There is also opposition to the recommendation to exclude blood and blood products from feeds (most commonly used in colostrum supplements). Transmission has never been documented through ingestion of blood, and blood is not believed to harbor substantial amounts, if any, of the agent. Additionally, the IRP made no recommendation that blood products be banned in feeds formulated for ruminants.

Advocates for the proposed restrictions argue that current regulations make it possible, and perhaps even likely, that potentially infective material is being fed to US cattle. They assert that the true prevalence of BSE in the United States is unknown, and it would only take a limited number of infected cattle from which infective material could enter feed mills to amplify the disease. This could happen through cattle receiving feed that is intended for other species (which may currently legally contain ruminant MBM) and through feed intended for cattle that is cross-contaminated with feed intended for other species. Critics of the current policy also mention that the weakest link of feed protections would appear to be on farms. On-farm mixing is a widespread practice, and there is no effective monitoring of on-farm compliance; thus, opportunities exist for prohibited materials to find their way into ruminant feeds by accident or intent. The only way to eliminate this risk is by preventing the inclusion of SRMs in any feeds prepared for animals.

Proponents of the proposed regulations believe the prohibition of feeding blood products is justified on the basis of studies suggesting blood transfusions transmitted natural scrapie and experimental BSE infection in sheep²³ and possibly **variant Creutzfeld-Jakob disease (vCJD)** in humans.^{24,25} It has also been reported^{26,27} that stunning of animals with a captive bolt may cause emboli of brain tissue in the blood stream;

therefore, this could serve to contaminate blood with infective material even though the blood was not initially infective. When blood has been tested for infectivity in experimental challenge experiments, the animals have been euthanized rather than sent to slaughter. It is important to mention that results of experiments in cattle have documented emboli of brain tissues after air injection or pithing.²⁶ Other results documenting emboli of brain tissue were reported²⁷ in experiments conducted in sheep; thus, the relevance of these concerns is unknown. Finally, feeds containing blood products are almost always fed to neonatal calves, which would likely represent the most susceptible population. Again, even a rare infected animal could serve to spread and amplify the disease.

When discussing these topics with the public, veterinarians must realize the visceral disdain that many people have for the prospect of feeding rendered products or blood material to cattle. This reaction may or may not be relevant in the policy-making process. Nevertheless, the public's perceptions or opinions should not be disregarded or minimized in any way if there is to be a truly meaningful dialogue. When communicating with the public, veterinarians would be well served to consider how decisions may be influenced by emotions and opinions in addition to the cold hard facts. In addition, veterinarians must not be afraid to voice their opinions to the public or policy makers.

Measures to Protect Public Health

The first step in protecting the public from BSE or any food-borne disease relies on producers (with help from their veterinarians) sending only live, healthy animals to slaughter. The next measure is mandatory antemortem inspection of all cattle slaughtered for the retail meat market. This measure has been in place since 1906 and is designed to identify any animals with signs of potential systemic disease, including CNS disorders, fever, or invasive cancer, and exclude them from the food chain. When an animal has such signs, it is condemned and cannot enter the food supply. When such a condition is suspected but pathognomonic signs are not evident, the animal is documented as a suspect and undergoes stringent postmortem examination by a veterinarian. Obviously, this system was not designed to protect the food supply from BSE, but it is still an effective screening measure. Prior to 2004, an animal that was nonambulatory was cause for that animal to be classified as a suspect, but it was not sufficient cause alone for condemnation. Following confirmation of the initial case of BSE in the United States (identified in December 2003), new regulations were enacted that mandate condemnation of all nonambulatory livestock.¹⁸

The second line of defense for ensuring public health is postmortem inspection. However, because there are no gross lesions related to BSE, it is impossible for postmortem inspection alone to exclude BSE-infected cattle from the food supply.

The next line of defense relates to process controls that limit the potential for BSE-infective materials to enter the food supply. Even before the initial case in December 2003, provisions existed that limited consumers' exposure to potentially infective tissues. Spinal

cord material is not considered a component of meat; thus, it was never allowed to be included as a product derived by use of **advanced meat recovery (AMR)** systems, which use high-pressure techniques to remove meat from around bones. However, an audit performed in 2002 found that some product derived by use of AMR systems and labeled as meat contained spinal cord material.²⁸ Thus, routine sampling procedures were instituted in 2003 to confirm the lack of spinal cord material in AMR products. Tonsils, another potentially infective material, have never been permitted to enter the food supply.

Most measures specifically designed to protect the food supply were created in response to the initial case of BSE identified in December 2003. These new measures include the following:

- The carcass of any slaughter cattle being tested for BSE will be held until BSE-negative status is confirmed.
- All downer or nonambulatory disabled cattle are banned from entering the human food supply.
- The aforementioned SRMs are prohibited from entering the human food chain.
- Most of the vertebral column of cattle ≥ 30 months old is now designated as an SRM; thus, it can no longer be subjected to AMR systems.
- Mechanically separated beef can no longer enter the human food supply. Mechanical separation is a technique used prior to the development of AMR systems to recover meat from bones. It commonly results in inclusion of bone material and spinal cord in the end product and thus cannot be termed as meat. However, it was previously permitted as human food labeled as mechanically separated beef.
- The use of air-injection stunning of cattle at slaughter has been prohibited to reduce the potential for emboli of brain tissue entering the tissue of carcasses.

Additionally, a national animal identification plan (which was already in development) will become a higher priority and be implemented more rapidly. This will facilitate trace back of any affected animals and permit identification of members of an exposure cohort. A more rapid and complete investigation will be possible, with minimal need for depopulation, testing, and disposal. Such a situation will be beneficial to the US cattle population and for public health.

Few of these measures have drawn criticism. The provision that carcasses be held until negative test results are received was instituted in recognition of the disruption caused by recall of product already released to the retail market that may have been derived from the initial animal with BSE. The new requirement creates a logistic challenge for some meat processors, who are now forced to hold these carcasses in storage for a longer period; however, this is less of a concern now that rapid tests are being used in the screening program.

The provision for removing all downer or nonambulatory cattle from the human food supply has generated the most controversy. Many have stated that this eliminated otherwise healthy cattle that were nonambulatory or unable to stand only because of a broken limb, including young animals unlikely to be clinically

affected by BSE. Proponents of the restriction point out that animals clinically affected by BSE are more likely to suffer injuries; thus, it may be difficult to confirm that the injury or condition is unrelated to BSE. The Secretary of Agriculture has suggested that this regulation may be revised to permit nonambulatory cattle < 30 months old to enter the food supply, but no official rule change has been proposed as of August 2005.

The most effective means of protecting public health appears to be quite simple: identify infected cattle before or at the time of slaughter and prevent them from entering the food chain. Unfortunately, this goal is not currently achievable. Because of the limitations of available testing methods, testing would not be sufficient to identify all cattle incubating the disease. Thus, testing animals for BSE prior to allowing them to enter the food supply is less effective than removing SRMs from the food supply, regardless of the test status for the animal of origin.²⁹ Moreover, an infective dose for humans has yet to be determined, so it is impossible to know how little or how much needs to be contained in the products to pose a risk to humans.⁹ Finally, because the tests are currently conducted on brain matter, it is not clear what relationship such test results would have to potential infectivity of edible products. In consideration of these issues, testing of all cattle slaughtered for human consumption is not considered a reasonable measure by most experts²¹ and has been adopted by only 1 country (Japan). Most experts do not view testing as a food safety measure. Rather, they view it as a surveillance system for monitoring success of the controls implemented to prevent the introduction and dissemination of BSE.

Testing and Surveillance Programs

Two principle types of tests exist for BSE: those designed to detect infectivity and those intended to detect molecular or microscopic characteristics associated with the disease. As mentioned previously, detection of infectivity is performed through bioassays. Although this has been the criterion-reference standard for identifying the tissues that are of greatest concern for disease transmission, it is a long and expensive task not appropriately suited for large-scale testing. Therefore, all testing or surveillance programs rely on detecting characteristics of the disease.

The defining characteristics of BSE are the accumulation of a plaque-forming protein and eventual vacuoles in the brain.³⁰ Histologic examination can be diagnostic when spongiform changes are detected. However, it is insensitive because vacuoles develop late in the disease process. This leaves detection of the plaque-forming protein as the most sensitive and viable method for detecting the disease. This protein, termed a prion, is an aberrant version of a glycoprotein typically found throughout the body but that is concentrated in neurologic tissue.³¹ The abnormal protein is typically denoted as the **scrapie prion protein (PrP^{Sc})**, and the normal protein is often termed the **cellular prion protein (PrP^C)**. Various theories relating to the cause of BSE have been proposed, but a complete consensus has yet to be reached. Currently, PrP^{Sc} is the only reliable molecular marker for the disease and is

considered by most investigators to be the etiologic agent.^{32,34} Nevertheless, investigators have detected infectivity without detectable prion protein,³⁵ and some forms of PrP^{Sc} are noninfective.^{36,37}

Because the prion protein is a modified form of a native protein, there appears to be no immune response against it.^{38,39} Therefore, tests based on detection of antibodies or cell-mediated immune responses are not useful. Most tests seek to detect the PrP^{Sc} protein and are typically conducted on brain tissue; thus, there currently is no test that can be conducted on live animals to detect BSE. Methods of detecting PrP^{Sc} include **immunohistochemical (IHC)** testing, ELISAs, and western blotting.³² These techniques are all extremely sensitive when used to test cattle that have clinical signs of BSE.

The IHC test is considered by the USDA as the criterion-referenced standard. Attributes of IHC tests include comparatively high sensitivity and extremely high specificity. This is augmented by the ability for pathologists to confirm that the tissue being examined is brain tissue (more specifically, from the obex) and that the detected PrP^{Sc} is located and distributed in a manner consistent with BSE infection.⁴ However, it has been criticized for the fact that it examines a small amount of tissue from a single location and is subjective. In this regard, western blotting is preferred by some. Immunohistochemical testing reportedly⁴⁰ is effective in detecting the disease 4 to 6 months before onset of clinical signs. Research is ongoing to determine the stage of incubation at which PrP^{Sc} can be detected by use of western blotting or an ELISA. Although analysis of dilution experiments conducted in 1 study⁴¹ suggests the ability of these tests to detect minute amounts of PrP^{Sc}, the Spongiform Encephalopathy Advisory Committee speculated⁴² that these tests may yield only positive results on samples obtained approximately 3 months before the onset of clinical signs.

Combined with the extended incubation period, the inability to detect cattle during early incubation suggests that there is little value in testing young cattle. This premise is reflected in the surveillance programs enacted by various nations. The current active surveillance program for the European Union mandates testing of all slaughter cattle > 30 months old and all emergency slaughter cattle > 24 months old. In the United Kingdom, virtually no cattle > 30 months old are permitted to enter the food supply; instead, they are euthanized and incinerated. A random sample of the destroyed cattle > 30 months old is tested, as are all casualty and fallen stock > 24 months old.⁴³

The United States has also relied on testing high-risk cattle > 30 months old. Only Japan routinely tests healthy cattle < 30 months old, and in March 2005, an advisory panel of experts in the Japanese Cabinet Office Food Safety Commission acknowledged that testing is not justified in cattle < 20 months old.

In addition to age, other criteria can be applied to refine testing programs. Experience in the United Kingdom has revealed that passive surveillance in which clinical suspects are submitted for testing on the recommendation of a veterinarian is much more efficient than active surveillance in which cattle are tested because of age, injury, or as a random selection at slaughter. Passive

surveillance has resulted in 179,124 cases detected from 223,777 submissions (1 case/1.25 submissions). Active surveillance has resulted in 1,537 cases detected from 1,405,097 submissions (1 case/914 submissions).⁴⁴ This emphasizes the importance of veterinarians participating in surveillance programs and recommending testing of suspect animals examined on farms.

Additionally, the effectiveness of active surveillance can be improved by focusing on high-risk cattle sent for slaughter or rendering. In the United States, these cattle have been referred to as the 4 D's (diseased, down, dying, and dead). In the United Kingdom and other countries in Europe, they are referred to as fallen stock, emergency slaughter animals, and antemortem slaughter suspects. In the United Kingdom, testing of this group of cattle results in approximately 1 in 500 with positive results, whereas only 1 in 16,444 healthy cattle > 30 months old has positive results.⁴⁴ Similar trends have been observed in Switzerland,⁴⁵ Denmark,^b and the European Union as a whole (Table 2).⁴¹ In fact, detectable cases of BSE were 29.4 times as common in targeted high-risk cattle as in apparently clinically normal cattle.⁴⁶ This would suggest that if there are low amounts of BSE, it is most likely to be detected through testing of older cattle that have clinical signs consistent with BSE. Restricting testing to this population would be the most economically efficient means of identifying BSE infection in a country. Once it is confirmed, it may be justifiable to pursue active surveillance of a larger subset of the population to more accurately determine the prevalence.⁴⁷

The United States has had a targeted surveillance program for BSE since May 1990. Since that time, BSE has been a notifiable disease. The Food Safety Inspection Service along with the **Animal and Plant Health Inspection Service (APHIS)** have coordinated testing of high-risk cattle, including nonambulatory (downer) cattle, cattle that die on farms, cattle with signs of neurologic disease, and rabies-negative submissions.⁴⁸ The USDA tested 19,990 cattle during fiscal year 2002 and 20,000 cattle during 2003. Both of these figures are substantially higher than the standards set by the World Organization for Animal Health (**Office International des Épizooties [OIE]**), the standard setting organization for animal health for 167 member nations. In accordance with the OIE international standard, a BSE-free country such as the United States would only be required to test 433 cattle/y.²²

As part of the increased measures taken following the initial case in December 2003, the USDA APHIS initiated a more aggressive surveillance program. Before June 2004, all testing was conducted by use of IHC tests at the **National Veterinary Services Laboratory (NVSL)** of USDA APHIS in Ames, Iowa. To achieve the increase

in surveillance, the USDA licensed 5 rapid tests and 12 laboratories to perform screening tests. Any reactors on the screening tests are sent to the NVSL for confirmation by IHC testing. In the beginning of the new program, USDA policy provided for announcement of an inconclusive result after a reactor was identified by use of a rapid test. Results for 2 cattle were declared as inconclusive early during the surveillance program but were negative as determined by results of IHC tests conducted at the NVSL.

The USDA subsequently changed their policy to reflect the recommendations of the manufacturer of the rapid test. Therefore, when an initial test result is positive, the sample is immediately retested in duplicate by use of a rapid test. When at least one of the repeated tests yields positive results (ie, 2 of the 3 screening tests conducted have yielded positive results), the sample is deemed an inconclusive and sent to the NVSL. When the repeated duplicative screening tests both yield negative results, the initial result is considered a false-positive and no further investigation is conducted.⁴⁹ Repeating the screening test reduces the likelihood that improper tissue handling will yield a false-positive test result. Since the policy change was instituted, 1 animal with inconclusive results was identified on November 18, 2004; this animal was subsequently determined to have negative results on the basis of results of IHC testing.

The issue of a criterion-referenced standard for testing received new attention in June 2005 when the Office of the Inspector General for the USDA recommended reexamination of the 3 cattle that had yielded inconclusive results for the rapid test. These cattle had subsequently yielded negative results for the IHC test and thus had been declared false reactors. However, the Office of Inspector General suggested that the tissues be tested by use of a western blot method, which yielded a weak positive result for the reactor animal tested in November 2004. Tissues from this animal were forwarded to the OIE-recognized reference lab in Weybridge, England, for final judgment. Testing conducted at that laboratory via IHC and western blot methods determined that the animal was infected with BSE. After this confirmation, the USDA announced that in the future, all inconclusive results will be tested by use of both IHC and western blot methods for final determination of infection status.

The goal for the original APHIS testing program was to have 95% confidence that the program would detect BSE at a prevalence of 1 infected animal/1 million cattle. To achieve this degree of confidence regarding the US population of approximately 45 million adult cattle would necessitate testing nearly 3 million cattle. However, the amount of testing needed could be substantially reduced by accepting some assumptions. First, it was assumed that all cases of BSE in the United States would be in high-risk cattle or target populations. As stated previously, it is quite reasonable to assume a much higher prevalence in the cattle referred to as 4-D's, but not all cases would necessarily be limited to this population. Secondly, it was assumed that there would be approximately 195,000 nonambulatory cattle/y (determined on the basis of a survey of members of the American Association of Bovine Practitioners) and a

Table 2—Number of cattle tested for various high-risk populations in the European Union to yield 1 positive test result for BSE.⁴⁰

Population	2002	2003
Clinical suspects	2,646	2,890
Fallen stock and emergency slaughter cattle	1,085	1,656
Healthy cattle at slaughter	32,258	33,333

high-risk population of 446,000 cattle. It also was assumed that the tests were highly sensitive and specific; this could be considered a reasonable assumption for both the IHC and rapid tests when applied to cattle that have clinical signs of BSE. Lastly, it was assumed that the samples received would be representative of the entire high-risk population. Accepting these assumptions results in the need to test 12,500 cattle/y.

The USDA APHIS greatly increased the goal of the revised testing program implemented in 2004. The goal of the new program was to have 99% confidence that the testing would detect BSE in 1 animal/10 million adult cattle. Approximately 268,500 high-risk cattle would need to be tested to achieve this goal. Thus, beginning on June 1, 2004, APHIS initiated an intensive testing program to obtain samples from as many cattle in the target population as possible, with the intention of testing more than 250,000 cattle during the ensuing 12 to 18 months.⁵⁰ This goal was reached in March 2005, but testing is currently slated to continue in the same manner until the middle of 2005.

Before 2004, samples were collected for the testing program from cattle sent to slaughter operations that were deemed by Food Safety and Inspection Service personnel to be suitable candidates for testing. This approach is not sufficient to provide the number of cattle that must be tested under the revised program. Thus, USDA APHIS has been cooperating with producers, renderers, slaughter facility operators, and others to collect samples from high-risk cattle.

The USDA provides training to practitioners in the collection and submission of samples from appropriate cattle. Once trained, veterinarians are eligible for compensation (\$100) for each sample submitted. Owners also receive \$100 to offset the cost of disposal of the carcass. Veterinarians who have not been trained to collect or submit samples should contact their area veterinarian in charge (ie, the APHIS representative for each state) or state veterinarian to arrange for collection and submission of samples. It is imperative that veterinarians accept this responsibility and participate appropriately.

Veterinarians should encourage clients to submit samples for testing from any cattle that are in the target population. This includes older cattle that die on farms as a result of unknown causes, those with broken legs, or cattle that appear to have paralysis associated with parturition.

Following completion and analysis of the results of this undertaking, the APHIS will create another surveillance program. The intention is to use results from the current testing scheme to establish the amount of ongoing testing required to verify the effectiveness of control measures. It is important to understand that neither the original nor the current revised program made any assumption of prevalence, and they cannot directly be used to estimate prevalence. Assuming no positive results are found during testing for the current program, it can be stated with 99% confidence that there are < 5 infected cattle (clinically affected or within 6 months of onset of clinical disease) in the target population of approximately 446,000 cattle. If all of the aforementioned assumptions are accepted and no cattle have positive test results, it would mean there were < 5 infected

cattle in the total population of 45 million animals; this is the basis for the often stated claim of finding 1 infected animal/10 million cattle. However, the APHIS acknowledges that it is not appropriate to assume that results from the current testing program are representative of the entire cattle population; thus, such an extrapolation is tenuous at best. Only after completion of the program will the APHIS use the results to estimate prevalence in the US cattle population.⁵¹

The current surveillance plan was submitted to the Office of the Inspector General for the USDA for an audit review to determine whether the expanded program will accomplish its stated goal. One of the greatest criticisms made by the Office of the Inspector General was that the noncompulsory nature of sample submission violated the principles of random sampling.⁵⁰ Random sampling assumes that each animal in the target population has an equal chance of being selected for testing. This assumption is not valid when owners, veterinarians, renderers, and personnel at slaughter plants are relied on to submit samples from eligible cattle because some individuals may be more likely than others to submit tissues. This may result in over- or underrepresentation of animals on the basis of class, age, condition, or geographic location. The APHIS acknowledges the limitations created by this situation but stated they are largely a result of the lack of regulatory or legal requirements to report dead or down cattle. Because the APHIS does not know the exact distribution of cattle in the target population, it is impossible to initiate a program that would test a subgroup of cattle known to be representative of the entire population.

Veterinarians should recognize that full cooperation by the veterinary community is the best way to minimize distortion that may result from voluntary participation. Equal and active participation by veterinarians would approximate random sampling and make the results more meaningful. Similarly, it must be appreciated that the international community is closely observing the US response to BSE, including the surveillance programs. If it becomes apparent that inadequate or inappropriate cattle constitute a portion of the testing program, it will become much more difficult to convince trading partners to accept the validity of the surveillance programs. The so-called shoot, shovel, and shut-up approach to dealing with high-risk cattle is a detriment to US agriculture and public health.

Conclusions

The United States is unlikely to have BSE in epidemic proportions,⁵² and the 2 cases diagnosed thus far demonstrate that isolated cases will not be the demise of the beef industry. However, failure to be honest and open with the public about risks and uncertainties can have a negative impact on consumer confidence. The overassurance of officials in the United Kingdom that no public health threat existed because of BSE during the early stages of their epidemic ultimately served to do great damage to public trust.^{53,54}

It is essential that food producers and consumers understand what is known about BSE, what is not yet understood, and what is being done to protect US citi-

zens and cattle. This information needs to be conveyed in a sensitive and noncondescending manner. Veterinarians must recognize that as trained professionals, they may have a different perception of risk than do members of the public. There are many characteristics of BSE that have led to fear and apprehension disproportionate with its risk. These include the severity of vCJD, the mystery and uncertainty surrounding BSE, the long interval between exposure and clinical onset of the disease, and the lack of control consumers have in preventing exposure.³⁵ These concerns must be recognized and received with empathy. Only then will clients appreciate the insights veterinarians may provide on the details of risks and precautions.

Numerous measures are in place in the United States to control the introduction and spread of BSE. Although they can be divided into 3 discreet categories (protecting cattle, protecting humans, and surveillance), they are all intimately related and overlapping. Veterinarians have an obligation to be aware of these measures and their responsibility in sustaining them. These include diligence in monitoring to detect cattle with clinical signs compatible with BSE, participation in surveillance programs, and education of producers and consumers. It can also include active participation in the formulation or criticism of policy regarding the disease. As experts in animal disease and food safety, veterinarians are uniquely qualified to accomplish all of these tasks. Failure to meet these responsibilities will harm clients, the public, and the veterinary profession.

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