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Bovine Spongiform Encephalopathy (BSE)
Ongoing Surveillance Plan

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VS
Veterinary Services
Safeguarding Animal Health

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INTRODUCTION

The U.S. Department of Agriculture (USDA) has taken aggressive measures to prevent the introduction and potential spread of bovine spongiform encephalopathy (BSE), and has conducted surveillance since 1990 to monitor whether the disease was present. Surveillance was expanded in scope and intensity following the confirmation of BSE in an imported cow in December 2003. This expanded surveillance effort was designed to estimate the level of disease present in the United States and provide input for designing a long-term surveillance plan.

The present plan is intended to inform and educate USDA's partners and stakeholders on approaches to be employed in ongoing BSE surveillance. The plan retains the USDA's ability to detect BSE at 1 infected animal per 1,000,000 adult cattle in the population with a high degree of confidence, maintains surveillance at levels that exceed international standards, emphasizes sample collection from cattle subpopulations where BSE is most likely to be detected, and retains sample collections from all important surveillance sources.

The plan follows surveillance system design standards and guidelines established by the USDA's Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS), National Surveillance Unit (NSU). These guidelines are intended to assist planners and managers in considering specific objectives, design strategies, reporting systems, implementation methods, and long-term system maintenance. The guidelines ensure that the objectives of the surveillance system are predefined, and that the collection, organization, and analysis of appropriate data are considered before implementation. Further, the guidelines provide a mechanism for review and evaluation to assure that the surveillance is providing the appropriate type and quality of information.

Disease description

Bovine spongiform encephalopathy, commonly known as "Mad Cow Disease", is a transmissible neurodegenerative disease of adult cattle that emerged in Great Britain in 1985,[38] and has subsequently been identified in cattle of most European countries, Canada, the United States, and Japan. BSE belongs to the group of transmissible spongiform encephalopathies (TSEs), together with scrapie of sheep, chronic wasting disease of free-ranging and captive deer and elk, and Creutzfeldt-Jakob disease (CJD) of humans. TSEs have long latency periods, are untreatable, and currently cannot be prevented by vaccination since there is absence of a host immune response to infection.

Most scientific evidence suggests that prions are the causative agent of TSEs;[32] however, the nature of the prion remains undetermined. A unique characteristic of the prion is its resistance to inactivation by most conventional physical or chemical decontamination methods.[37] The prion consists mostly of protein, largely comprised by a proteinase-resistant, disease-associated isoform (PrP^{res}) of host-encoded prion protein (PrP^{C}).[29,32] The pathogenesis of the TSEs requires the formation of PrP^{res} from PrP^{C}.[16] Interaction

with PrP^{res} leads to post-translational conformational modification of PrP^{C},[24,26,33] resulting in its conversion to PrP^{res}.[14,19] The subsequent pathological accumulation of PrP^{res} in certain tissues defines the TSEs.[13,34] The pathogenesis of BSE appears to involve a much more restricted tissue distribution of PrP^{res} accumulation than other animal TSEs, having reduced involvement of the lymphoreticular system.

The origin of the BSE agent is unresolved. Theories have considered derivation from a TSE agent of another mammalian species, such as scrapie,[40] or spontaneous genetic mutation of the bovid prion protein gene.[31] The emergence of BSE coincided with reduced use of hydrocarbon solvents in the production of meat-and-bone meal (MBM) through carcass rendering.[40]

Transmission of BSE is thought to primarily occur through ingestion of feedstuff, especially ruminant-derived MBM, contaminated with the BSE agent.[41,43] Calves born to infected cows have increased risk to develop BSE,[44] especially if born around the time of disease onset in the dam.[22] However, the risk is probably influenced by conserved management practices where both the dam and calf have been fed concentrated feedstuff containing MBM early in life.[12] Horizontal transmission of BSE between cattle is not believed to occur. Most cattle become infected within the first 6 months of life.[12] The mean incubation period for BSE is around 60 months, with clinical onset of disease occurring on average at 4-5 years of age.[11] The age range of affected animals is very wide, although BSE is rarely confirmed in animals less than 30 months of age.[4,48]

BSE is invariably fatal. Clinical signs have an insidious onset and are largely nonspecific. Signs that may be associated with BSE include apprehension, ataxia, emaciation, hypersensitivity to touch or sound, head shyness, panic-stricken response, kicking in the milking parlor, reluctance to enter the milking parlor, abnormal ear movement or carriage, increased alertness behavior, reduced milk yield, bruxism, and change in temperament.[35,43] The duration of clinical signs averages 1 to 2 months prior to death or slaughter, but may range from weeks to a year.[43]

Although live animal tests are under development, at present, none are available to reliably detect BSE. Diagnosis is achieved postmortem through examination of central nervous system (CNS) tissue, and is contingent on identification of characteristic histopathologic lesions, detection of PrP^{res}, or electron microscopic visualization of scrapie-associated fibrils.[6] Because PrP^{res} is the only currently known disease-specific macromolecule, all commercially available diagnostic assays rely on its immunological detection.[10] These assays have limited diagnostic sensitivity in that PrP^{res} accumulation may not be detectable until late during the incubation period, within months prior to onset of clinical disease.[27] Infected animals that are early in the incubation period can only be identified through demonstration of tissue infectivity using bioassay. The only lesions associated with BSE are found microscopically within CNS tissue.[38,39] Lesions develop late in the disease process, roughly coinciding with the onset of clinical signs. These consist of non-inflammatory vacuolar degeneration, or spongiform change, of grey matter and neuronal cell bodies. Astrocytosis and cerebral amyloidosis, features of other TSEs, are unusual with BSE.

Breed-dependent differential susceptibility or incubation period has not been observed with BSE,[42] and there is little variability in the bovine PrP gene.[23] The consistent neuroanatomical lesion profile in the brains of cattle affected with BSE,[36,39] and uniform glycoform ratios of PrP^{res} as determined by immunoblotting,[21,28] suggest the existence of a single strain of the BSE agent. However, a recently described atypical form of BSE, termed bovine amyloidotic spongiform encephalopathy (BASE), has modified glycoform patterns similar to sporadic CJD in humans, and may represent an alternative strain of BSE agent.[18]

Although TSEs are usually confined to an individual species, concern has arisen for the potential of inter-species transmission of BSE. The BSE agent is widely recognized as the cause of variant CJD in humans, based on epidemiological and mouse inoculation studies,[15,46] and biochemical PrP^{res} characteristics.[21] In addition, natural exposure to BSE agent has led to similar encephalopathic disease in captive wild ungulates and cats,[25] and in domestic cats.[47]

Measures established by the United States to prevent new cases of BSE have included restrictions on the importation of ruminants and ruminant-derived products from countries affected with BSE or that present undue risk of BSE (9CFR Parts 93, 94, 95, 96, and 98) and prohibition of feeding mammalian-derived proteins, with exceptions, to ruminants (21CFR589.2000). Mitigating measures have been considered to effectively reduce the likelihood of BSE introduction and amplification in U.S. cattle.[20] Food-safety precautions implemented by the United States to protect the consumer have included the exclusion of non-ambulatory cattle from slaughter for human consumption, prohibitions on the use of specified risk material as human food, and prohibitions on the use of mechanically separated beef in human food (69 FR 1826, January 12, 2004; 69 FR 42255, July 14, 2004); prohibition of the use of air-injection stunning devices to immobilize cattle during slaughter (69 FR 1885-1891); and prohibitions on the use of certain tissues in advanced meat recovery systems and additional process controls on these systems (69 FR 1874-1885).

Purpose, rationale, and objectives of surveillance

Animal and public health concerns have led to the establishment of active surveillance programs among other regulatory measures to monitor and prevent disease. Active surveillance for BSE was initiated in the United States in 1990. In response to identification of a BSE-affected imported dairy cow in December 2003, the U.S. Enhanced BSE Surveillance Program was implemented in June 2004. Through these efforts, 2 cases of BSE were identified through March 2006. Both cases were in beef cattle over ten years old (born before the feed ban of 1997), one located in Texas and one in Alabama.

Based on data collected in the United States over the last seven years, including over a half million samples from the Enhanced Surveillance program, the USDA has developed an estimate of prevalence of BSE among U.S. cattle that was extremely low, projected at

less than 1 case per million animals in the standing adult cattle population at the 95% confidence level.[1] In addition, the USDA demonstrated that surveillance efforts to date far exceed the World Organization for Animal Health (Office Internationales des Epizooties [OIE]) "type A" surveillance requirements. Prevalence is expected to decline as long as mitigation efforts that maintain low risk for introduction and spread of the BSE agent among U.S. cattle are equivalent to or better than those evaluated by the Harvard Risk Assessment.[20] The present plan details the objectives and methods considered pertinent and necessary for Ongoing BSE Surveillance.

The principal purposes of Ongoing BSE Surveillance are:

1. To continue to assess and monitor change in the BSE status of U.S. cattle.
2. To provide mechanisms for detection of BSE prevalence if it were to increase above 1 infected animal per million adults.

Expected outcomes

The results of Ongoing BSE Surveillance will be used for decision-making and policy development regarding design and implementation of future BSE surveillance programs and control efforts. Results will also be used to facilitate contingency plans for national BSE control and response programs and to evaluate the effectiveness of mitigations and control measures that have been implemented to reduce the risk of introduction and spread of BSE among U.S. cattle. The planned response to confirmed cases of BSE are described elsewhere.[3,7]

Additionally, the implementation of a strong BSE surveillance strategy will help provide reassurance to consumers and international trading partners regarding our ability to detect a problem if one should arise. The results of the surveillance program will be an important component of documenting the BSE status of U.S. cattle. The USDA has designed this surveillance program to meet or exceed the internationally accepted surveillance practices recommended by the OIE. Compliance with OIE guidelines is an important component of assuring trading partners of the quality of our surveillance efforts.[2] USDA expects that this robust surveillance program will continue to provide the foundation for market confidence in the safety of U.S. cattle.

Stakeholders and responsible parties

Users of surveillance system information include policy makers within USDA, the U.S. livestock industry, consumer groups, trading partners, State Animal Health Offices, and data providers (sample collectors and veterinary diagnostic laboratories). In addition to information users, beneficiaries of the surveillance information include the public, the U.S. cattle industry, consumer groups, and industries engaged in export markets for cattle-derived products.

Data collection will be performed by a variety of parties. Sample data will be collected by accredited veterinarians, Food Safety Inspection Service (FSIS) veterinarians and inspectors, veterinary diagnostic laboratory or public health laboratory personnel, and

qualified VS personnel (including animal health technicians and those involved with data entry). VS Area Offices will direct efforts for resolution of problematic data.

Samples will be tested for BSE through cooperation of NVSL and contracted veterinary diagnostic laboratories. Positive samples will be confirmed by NVSL. American Association of Veterinary Laboratory Diagnosticians (AAVLD) accredited laboratories participating in the National Animal Health Laboratory Network (NAHLN) may be contracted by NVSL to perform BSE screening tests, provided quality assurance standards are met (as described by the most current version of NVSL protocols GPPISOP3501 and 3303).

Training of sample collectors will be completed by Area Veterinarian in Charge (AVIC) office personnel with oversight by VS regional offices. Data entry training will be completed by these parties as well as the BSE Help Desk.[a]

Assessment of data quality, data analysis, and interpretation will be completed by the NSU, VS TSE program manager, and VS TSE epidemiologists. Reporting and dissemination of surveillance results will be primarily the responsibility of the NSU. In addition to standard Agricultural Marketing Service audit procedures, the NSU will conduct a review of the surveillance system's effectiveness. NVSL will be responsible for quality assurance of laboratory results.

POPULATION DESCRIPTION AND SAMPLING METHODS

Current OIE guidelines emphasize the development of ongoing surveillance programs that focus on obtaining quality samples from high risk subpopulations rather than on a target number of animals.[2] As a result, if surveillance can be efficiently targeted to the highest risk subpopulations, meaningful surveillance can occur with a fairly low number of animals (for an example of how the OIE numbers are calculated, see the Summary of BSE Enhanced Surveillance in the United States).[9] For this reason, an important focus of the Ongoing Surveillance will be obtaining samples from cattle that are "clinical suspects." This subpopulation of cattle, particularly cattle over 30 months of age, has been found to exhibit the highest prevalence of BSE. Inferences derived from surveillance findings will be generalized to the adult U.S. cattle population (i.e. target or inference population), which consists of approximately 42 million adult cattle.[5]

Study (Targeted) Population

Ongoing Surveillance will target subpopulations from the Enhanced Surveillance population with the highest probability of BSE detection. Because BSE is exceedingly uncommon among U.S. cattle, the chosen targeted population will create intentional bias in the sample frame which favors detection of disease. Cattle that are dead with unknown clinical history will be limited in the surveillance sampling strategy because they provide substantially less information than animals accompanied by clinical history. As used in this Ongoing Surveillance plan, "dead with unknown clinical history" refers to cattle that

[a] Veterinary Services BSE Help Desk: (866) 370-6611, bse.help@aphis.usda.gov

are already dead prior to being presented for BSE sample collection and that have no known clinical history other than "dead of unknown cause." Efforts for collecting clinical history data will be augmented for Ongoing Surveillance. At least one clinical sign must be identified for each sampled animal. Apparently healthy animals will not be targeted for surveillance. For those cattle without obvious CNS signs, sample collection on-farm will be preferred over other collection sites because the subtle and nonspecific nature of behavioral changes associated with BSE are best identified by those who handle cattle on a daily basis,[2] and personnel on-farm are most suitable for providing this information.

The targeted population for Ongoing Surveillance consists of cattle of any breed that fit one of the following clinical presentation criteria:

1. Cattle of any age with CNS signs

   This category includes cattle exhibiting signs consistent with a central nervous system disorder (including rabies-negative cases from public health laboratories, and FSIS condemns for "CNS signs" or "rabies").

   Additionally, this category includes cattle highly suspicious for BSE as indicated by VS Memo 580.16, which includes: 1) cattle affected by illnesses that are refractory to treatment (including anorexia, loss of condition in spite of good appetite, pneumonia, decreased milk yield) and are displaying progressive behavioral changes that are not of an acute nature (including apprehension, nervousness, excitability, aggression, head shyness, hypermetria, kicking when milked, difficulty in rising, excessive nose scratching, or hesitation at gates/barriers); 2) cattle displaying progressive neurological signs that cannot be attributed to infectious illness and are not responsive to treatment.

2. Cattle ≥ 30 months of age[b] that are condemned during antemortem inspection or are excluded from slaughter due to poor health status (nonambulatory, unhealthy, or dead)

   This category includes:

   a) Cattle that are condemned by FSIS at antemortem inspection for any reason (other than "CNS signs" or "rabies," which are covered above under "Cattle of any age with CNS signs").

   b) Cattle without a history of CNS signs for which sample collection occurs on-farm, at veterinary clinics, or at livestock sale or auction barns and that are dead, nonambulatory, or have clinical signs that may be associated with BSE. For those cattle that are dead prior to arrival of sampling

[b] Age of 30 months or older is evidenced by the eruption of at least one of the second set of permanent incisors.

personnel on-farm, additional clinical history must be available other than "dead of unknown cause."

c) Cattle presented to veterinary diagnostic laboratories for necropsy or for ancillary diagnostics without a history of CNS signs but which had clinical signs that may be associated with BSE.

d) Cattle from renderers or 3D/4D facilities (up to a maximum of 5,000) that are dead, nonambulatory, or sick. Collection of clinical history is preferable for these samples but is not required.

The Enhanced Surveillance data provide information on the number of clinical suspects and the number of nonambulatory, unhealthy, or dead animals over 30 months classified by collection site.[9] We expect that collection sites yielding the highest proportion of clinical suspects relative to the number of animals sampled will continue to do so. Accordingly, because renderers and 3D/4D facilities produced the most samples but proportionately the fewest clinical suspects in the Enhanced Surveillance program, we will limit samples from these facilities to 5,000 animals.

Sample Points and Numbers to Meet OIE Surveillance Standards

APHIS is committed to maintaining BSE surveillance that at least meets OIE guidelines. The OIE BSE surveillance guidelines recommend a target number of surveillance points for Type A surveillance based on the size of a country's cattle population. These points are accrued over 7 consecutive years, and are weighted according to the surveillance stream and age of the animal sampled. For a large cattle population, using the design prevalence of 1 case per 100,000 adult cattle and 95% confidence, 300,000 total points over 7 years, or 42,857 points per year are required for Type A surveillance.[2]

The four surveillance streams identified in the OIE Code are clinical suspects; casualty slaughter; fallen stock; and healthy slaughter. OIE guidelines recommend sampling from at least three of the four surveillance streams. BSE surveillance efforts in the US have always focused on the three surveillance streams where BSE is more likely to be found – clinical suspects, casualty slaughter, and fallen stock. During the 7 consecutive years prior to March 17, 2006, the United States collected 735,213 BSE samples from these surveillance streams and accumulated 2,973,804 OIE points.[9]

If the Ongoing Surveillance plan maintains similar sample numbers from these surveillance streams, approximately 10,500 cattle per year would be sufficient to meet the OIE minimum number of sample points for Type A surveillance.

2,973,804 points ÷ 735,213 samples = 4.1 points per sample

and

42,857 points required per year ÷ 4.1 points per sample = 10,453 samples per year

The Ongoing Surveillance plan targets relatively few of the lowest value samples (i.e., only 5,000 renderer and 3D-4D origin samples as compared to over ½ million during Enhanced Surveillance). Thus, the point value per sample will be higher than 4.1, and 10,453 samples from Ongoing Surveillance will easily exceed the OIE requirements.

Sample Points and Sample Size for BSE Prevalence Estimates

The OIE minimum number of samples as outlined would be sufficient for a design prevalence of 1 case per 100,000 adult cattle. In the interest of maintaining confidence in previous BSE prevalence estimates, a more sensitive design prevalence of 1 case per 1,000,000 adult cattle will be adopted for Ongoing Surveillance.

The point tables described by OIE surveillance guidelines were designed using the BSurvE model to represent the most conservative scenario of the characteristics of the cattle populations of all its member States. These conservative demographic characteristics describe a population that culls cattle very rapidly (mean age of approximately 4 years) and results in point values that are that are much lower than the BSurvE model would calculate for cattle in most countries. The United States has relevant demographic data pertaining to the adult cattle population that indicate 25% of the adult cattle population of approximately 42 million are dairy production type and 75% are beef cattle.[30] While the U.S. dairy population undergoes rapid culling similar to the conservative characteristics used to develop the OIE table, beef cattle generally remain in the herd to a much older age until they no longer produce calves.

The BSurvE model can be used to determine sample point values based on a particular population's demographics. Because actual U.S. data are available regarding population characteristics, and because this population differs significantly from that used for the conservative OIE estimates, it is appropriate to base the sample size estimates on the points calculated through BSurvE given U.S. demographics (these points are hereafter referred to as "analytical points"). The higher average age at which beef cattle are culled influences the BSurvE output and results in substantially higher point values. Hence, sample values calculated with BSurvE from actual U.S. data result in higher point values than the conservative OIE estimates . Each analytical point calculated by BSurvE corresponds to a single non-targeted sample.[45]

According to OIE, BSurvE and Cannon and Roe[17]calculations, the required number of non-targeted samples needed to detect a prevalence of 1 case per million adult cattle with 95% confidence (given a population size of 42 million adult cattle) is 3,000,000, 2,995,730, and 2,891,389, respectively. Conservatively using the value of 3 million, we calculate that we will need to accumulate 428,571 analytical points (with negative results) per year across a period of 7 years to meet this objective.

$$3{,}000{,}000 \text{ analytical points} \div 7 \text{ years} = 428{,}571 \text{ analytical points per year}$$

The prevalence analysis conducted on U.S. surveillance data collected from March, 1999 through March 2006 reports 6,745,010 points resulting from 735,213 samples. The average sample was worth 9.5 analytical points.[1]

6,745,010 analytic points ÷ 735,213 samples = 9.5 analytic points per sample

If USDA maintained an equivalent mix of surveillance streams during Ongoing Surveillance, then approximately 45,113 samples per year would be required to meet this objective.

428,571 analytical points per year ÷ 9.5 analytical points per sample = 45,113 samples per year

However, greater than one half million of the samples from Enhanced Surveillance were collected from fallen stock – the surveillance stream that produced the lowest point values. Sampling efforts can be focused on higher value surveillance streams – clinical suspects and casualty slaughter – with a limited number of samples obtained from the fallen stock surveillance stream. This will increase the average point value per sample. Therefore, we estimate that 40,000 samples collected from these 3 surveillance streams – with a focus on clinical suspects and casualty slaughter - will exceed the number of points necessary to maintain confidence that prevalence is less than 1 infected animal per million adult cattle. Further, since the data are analyzed over 7 consecutive years, the estimate of sample size may be adjusted each year as appropriate to assure a robust prevalence estimate.

Study Area Under Surveillance

The collection sites for this sampling plan have been selected to include animals from all sections of the United States and comprise nationally representative avenues through which cattle exit production. In combination, these data sources provide the opportunity for cattle residing in any part of the country or segment of industry to be sampled:

- Slaughter facilities are located throughout the United States and service every constituent of the production industry. Additionally, western States that practice open range grazing and do not have access to renderers, or may not observe animal deaths, will still ship cull cattle to FSIS-inspected facilities in other States.
- Rendering facilities or 3D/4D facilities are located throughout the United States. Some of these facilities draw from the same populations as the slaughter facilities, as they are the disposal facility for offal and condemned animals from the slaughter facilities. Focusing on these facilities, and on rendering facilities in geographic locations with only limited disposal alternatives will help ensure a broad geographical representation. The inclusion of these facilities ensures access to the fallen stock surveillance stream.
- On-farm sample collection allows that samples will be collected wherever cattle reside. Efforts will be made across the nation to encourage the

participation of veterinary professionals and paraprofessionals in on-farm sample collection.
- There are no areas in the nation that cannot submit fresh whole cattle brain to a public health or veterinary diagnostic laboratory.

A qualitative, not quantitative, analysis of sample origin and collection site will be employed for Ongoing Surveillance sampling.[c] With increased emphasis on the importance of obtaining samples from high risk subpopulations, establishing and meeting a specific numeric target for the total number of cattle sampled becomes relatively less important. The results of Enhanced Surveillance allowed us to identify the collection sites that are more likely to yield clinical suspects. Nevertheless, it is important to obtain coverage of all potential streams at all collection sites in order to maximize the likelihood that we will obtain samples from clinical suspects.

OIE Guidelines and Surveillance Streams

Samples will be assigned to the surveillance streams described in Article 3.8.4.2 of the OIE Terrestrial Animal Health code. The stream to which a sample is assigned will be based on clinical signs that are provided, sample source, and condemnation code data. Note that samples within the "Routine Slaughter" stream (from Article 3.8.4.2) will not be collected during Ongoing Surveillance. The OIE surveillance streams and the criteria by which samples will be assigned to them are listed below.

1. **Clinical suspect** – cattle displaying behavioral or clinical signs consistent with BSE.

   Article 3.8.4.2 of the OIE Terrestrial Animal Health code describes this group as follows: "Cattle affected by illnesses that are refractory to treatment, and displaying progressive behavioral changes such as excitability, persistent kicking when milked, changes in herd hierarchical status, hesitation at doors, gates and barriers, as well as those displaying progressive neurological signs without signs of infectious illness are candidates for examination. These behavioral changes, being very subtle, are best identified by those who handle animals on a daily basis."

   Samples will be assigned to this surveillance stream if they are from cattle defined as highly suspicious as outlined in VS Memorandum 580.16, were tested negative for rabies at a public health or veterinary diagnostic laboratory, had CNS signs or were condemned by FSIS for CNS signs or rabies, or if the likelihood ratio for clinical signs associated with BSE is above an appropriate cutoff value (methods for these determinations are described elsewhere).[9] Most of these samples are

[c] A quantitative analysis of geographical representation is difficult to interpret since cattle movement in the United States is poorly defined, and a sample's site of collection site may not correspond to where the animal was born or resided most of its life (i.e., the area where BSE transmission would have occurred). Statistical comparison of geographic regions in the Enhanced Surveillance analysis and the paucity of positive cases provide no evidence that BSE prevalence varies by region within the United States.

anticipated to be derived from samples collected on-farm. A substantial number will also be contributed by FSIS, veterinary diagnostic laboratories, and public health laboratories.

2. **Casualty slaughter** –cattle over 30 months of age that are non-ambulatory, recumbent, unable to rise or to walk without assistance, sent for emergency slaughter, or condemned at antemortem inspection.

Samples will be assigned to this surveillance stream if the likelihood ratio for clinical signs being associated with BSE is below an appropriate cutoff value, and if the sample meets the OIE criteria for this stream. Most of these samples are anticipated to be derived from FSIS-inspected slaughter plants. However, a considerable number will be contributed by other data sources such as on-farm and veterinary diagnostic laboratories.

3. **Fallen stock** – cattle over 30 months of age that are found dead on farm, or during transport to or at an abattoir.

Samples will be assigned to this surveillance stream if the likelihood ratio for clinical signs being associated with BSE is below an appropriate cutoff value, and if the sample meets the OIE criteria for this stream. These samples are anticipated to contribute a negligible portion of total points.

Table 2. OIE point values for each surveillance stream by cattle age

	Clinical suspect	Casualty slaughter	Fallen stock	Routine slaughter (Apparently Healthy)
Age ≥ 1 year and < 2 years	N/A	0.4	0.2	0.01
Age ≥ 2 years and < 4 years	260	0.4	0.2	0.1
Age ≥ 4 years and < 7 years	750	1.6	0.9	0.2
Age ≥ 7 years and < 9 years	220	0.7	0.4	0.1
Age ≥ 9 years	45	0.2	0.1	0.0

Sample collection sites

The following sites have been selected based on observations from Enhanced Surveillance including relative quality of data collected, average point value per sample, and total sample numbers. Additionally, these collection sites comprise nationally representative avenues through which cattle may exit the adult cattle population and be available for sampling. Cattle meeting the targeted criteria will be sampled from the following sites:

1. **On-Farm**
These samples may be collected by accredited veterinarians, Federal or State employees (including animal health technicians), or VS-approved dead stock haulers. Under VS Area Office oversight, sample collectors with other

qualifications may be enlisted when resources preclude the participation of aforementioned sample collectors in a given area. Although these samples may have a higher cost relative to other data sources, they are anticipated to have higher value to surveillance since the accuracy, quantity, and perceived validity of clinical history is superior relative to other data sources.

2. Veterinary Diagnostic Laboratories

Cattle submitted for necropsy, or fresh whole brainstem submitted for ancillary diagnostics to veterinary diagnostic laboratories, including those not involved in BSE testing, will be sampled by laboratory personnel. Such samples are usually accompanied by significant clinical history and thus are of high value to surveillance.

3. Public Health Laboratories

Samples from cattle that are rabies suspects and test negative for rabies will be submitted for BSE testing by laboratory personnel. All samples derived from this data source can be characterized as clinically suspicious for BSE, and thus are of high value to surveillance.

4. Slaughter (FSIS)

Cattle 30 months or older condemned at antemortem inspection, and cattle of any age condemned for "CNS signs" or "rabies," will be sampled by FSIS employees or designated off-site sample collection facilities.

5. Facilities contracted to collect samples from cattle condemned by FSIS at antemortem inspection

Samples derived from animals condemned by FSIS personnel at antemortem inspection may be collected by personnel of a contracted rendering or 3D/4D facility, or other APHIS approved facility. Under these circumstances, communication of condemnation codes, clinical signs, and condemnation tag numbers (Z-tags) to the contracted facility is imperative.

6. Rendering or 3D/4D facilities

In order to represent the "fallen stock" surveillance stream and a wide variety of data sources, 5,000 samples will be collected over a 12-month period from targeted cattle presented to rendering or 3D/4D facilities. A quota is selectively applied to this collection site type since these samples tend to lack adequate clinical history, making them a less reliable source for clinical suspect animals.

Sampling progress will be monitored on a monthly basis. If the results differ considerably from expectations, the sampling strategy may be adjusted. For example, should we find that our expectations of sites most likely to yield clinical suspects are not met, we may refocus sampling efforts to other collection sites. Similarly should we find there are substantially large numbers of non-clinical-suspect cattle being sampled, we may reduce the intensity of our sampling in these categories. Such restrictions may involve reducing or stopping the collection of samples on-farm, at veterinary clinics, or

auction barns or reducing or stopping the collection of cattle condemned by FSIS. However, samples will be collected without restriction throughout the sampling period from cattle with CNS signs, rabies suspects, those considered highly suspicious for BSE, or cattle condemned by FSIS for CNS disorders or rabies regardless of the avenue through which they present to surveillance, and regardless of the degree with which sampling goals have been met.

Sample points derived from Enhanced Surveillance have provided a substantial cushion for potential deficiency during Ongoing Surveillance. Since international standards allow points to be included in surveillance analysis over a 7-year time period, there is considerable time to adjust Ongoing Surveillance to meet surveillance needs with the most cost-efficient methods of sampling.

COLLECTION METHODS

Data Collection Methods

Data collection methods will utilize the structures and processes established in the Enhanced Surveillance program. Sample data should be collected using the forms employed by Enhanced Surveillance: USDA BSE Surveillance Submission Form and USDA BSE Surveillance Data Collection Form. Forms are completed by the sample collector either by hand (paper forms) or electronically, through the NAHLN Web site (nahln.aphis.usda.gov/nahln/jsp/login.jsp). If the collection site cannot complete data entry electronically, paper copies must be forwarded to a designated USDA:VS office or the BSE Helpdesk for data entry. Training of data entry is overseen by VS area and regional offices, and the BSE Help Desk.

A hard copy of the BSE Surveillance Submission Form must be submitted to the diagnostic laboratory. If samples are not accompanied by the appropriate submission forms with all necessary information, it is the responsibility of the diagnostic laboratories to contact the sample collection site. Diagnostic laboratories may report collection sites that are repeatedly problematic to the appropriate AVIC for correctional efforts.

The USDA BSE Surveillance Submission Form must be completed for each set of samples from a particular collection site and date. The USDA BSE Surveillance Data Collection Form must be completed for each animal sampled. All types of ID present on the animal must be provided, including silver tag number, owner ear tag number, vaccination tag number, condemnation tag number, back tag number, bangle tag number, ear tattoo, brands, or microchip. In the case of samples from cattle condemned at antemortem inspection, the "Z" tag (FSIS condemnation tag number) must be recorded. A "Primary Reason for Submission," is used to assure the sample collector that the animal being sampled is appropriate for surveillance (i.e. is a member of the targeted population). It is imperative that the clinical signs section be completed as thoroughly and accurately as possible because data from this section of the form are used for allocation of samples into appropriate surveillance streams during data analysis.

Data relevant to the results of laboratory testing are entered through the NALHN interface by diagnostic laboratory personnel. Results can include any of the following:

- Not Detected – negative by ELISA or IHC
- Not detected, not obex* – negative by ELISA; although the sample appeared to be brainstem, the laboratory technician could not identify the sample as obex tissue
- Not tested* – sample not tested because sample could not be recognized as brainstem by the laboratory technician
- Initial reactor – positive on first screening (ELISA) test
- Inconclusive – following a positive on the first screening test, at least one additional test is positive when the screening test is repeated in duplicate
- IHC Inconclusive – sample with equivocal immunohistochemistry results
- Positive – samples positive either by IHC or immunoblotting

*For these selections, the reason for the result must be further specified as one of the following:

- Advanced tissue decomposition
- Wrong anatomic location
- Tissue disrupted preventing anatomic orientation

Sample Collection Methods

Samples may be collected by authorized Federal or State personnel, accredited veterinarians, APHIS-contracted employees, or diagnostic laboratory personnel.

Animal identification items (drawings or digital pictures of brands, removed tattooed hide, ear tags, etc.) should be collected from each animal sampled, bagged, labeled with the sample number, attached to a copy of the USDA BSE Surveillance Submission Form, and saved by the sample collector until negative results are received.

Brainstem samples may be collected through the foramen magnum, using a brain spoon or other extraction techniques (such as water extraction or compressed air), after disarticulation of the atlanto-occipital joint. Alternatively, a brainstem sample may be collected by dismantling the calvarium (e.g. when retrieving the whole brain for rabies diagnosis). An appropriate brainstem sample includes obex with little contamination or postmortem decomposition. Samples with post-mortem or post-collection decomposition such that they cannot be recognized as brainstem will not be tested by the diagnostic laboratory. Sample collectors should submit samples that have questionable testability and allow laboratory technicians to decide if tissue integrity precludes testing. Diagnostic laboratories will be compensated for efforts related to determining sample testability.

Samples may be held prior to submission to the diagnostic laboratory, provided they are refrigerated (e.g., for sending multiple samples in one shipment). These should be

submitted as soon as possible, but may be held for no longer than 7 days. Samples should not be frozen.

Fresh brainstem samples are individually packaged in plastic tubes labeled with a unique BSE sample identification bar code supplied by the USDA. Samples are enclosed with cold packs in insulated packages and are shipped by overnight contract delivery service (e.g. Federal Express), same-day courier service, or by hand delivery to a NALHN laboratory that offers BSE test service the following business day. Once shipped, delivery of samples to diagnostic laboratories should be completed within 24 hours so as to preserve sample integrity. If samples are received by a diagnostic laboratory on a non-operating weekday, samples will be held under refrigeration by the diagnostic laboratory and subsequently tested on the next operating weekday. If test results are urgently needed, the AVIC office may dictate that samples be redirected to an operating diagnostic laboratory. For samples with "inconclusive" test results, all remaining tissue must be immediately forwarded to NVSL (per the most current version of NVSL protocol GPPISOP0029). The timeline of procedures and reporting that occur in response to a positive case are described elsewhere.[3,7]

The diagnostic laboratory is responsible for entering test results into the NAHLN database and for notifying the sample submitter of test results electronically, by phone, or in writing. This should be completed within 24-48 hours of test completion. Additional parties, including the AVIC and State veterinary office, may also be notified of results given that an arrangement has been agreed upon between the diagnostic laboratory and additional party. APHIS Animal Identification Coordinators (AIC) may assist with sample delivery verification and troubleshooting. Training of sample collectors is completed by AVIC offices and VS regional offices, and, at their discretion, state veterinary offices. It is the responsibility of the AVIC offices to monitor sample collectors in the relevant area for habitual poor sample collection technique.

Animal Disposal

Carcasses from negative animals are disposed of in compliance with Federal, State, and local laws. Carcasses and offal from "inconclusive" or positive animals may be disposed of by one of the following: rendering for non-animal feed use by dedicated facilities, burial in a landfill, burial on-farm, alkaline digestion, or incineration. Rendering facilities may refrigerate or freeze carcasses, or may proceed with rendering and hold batches of final products, pending test results. Should a positive animal occur with the latter method, an indemnity would be supplied for the disposed batches of products.

Clinical Case Definition

Clinical case definitions are not applicable to the present surveillance methods. Because clinical signs have such poor specificity and sensitivity for BSE diagnosis, and because the diagnostic assays used for BSE diagnosis are considered to have near perfect analytic sensitivity and specificity (i.e. cattle with detectable disease are reliably differentiated from cattle without detectable disease), BSE diagnosis is solely based on laboratory

criteria. The case definition used for surveillance is the laboratory case definition for BSE as defined by the most current version of NVSL document GPISOP0034.

Laboratory Criteria for Diagnosis

The diagnostic strategy implemented for BSE surveillance is described elsewhere (most current versions of NVSL protocols GPPISOP0027 and GPPISOP0034).

ANALYSIS, REPORTING, AND PRESENTATION

The NSU is the party primarily responsible for data analysis and reporting. Reports produced by the NSU for the Deputy Administrator of VS and his designates will include monthly reports used for program monitoring and oversight, and an annual summary report analogous to that produced at the conclusion of the Enhanced Surveillance program. The annual summary report is intended to tally surveillance points derived from BSE surveillance efforts over the last 7 years using the OIE Code. Additionally, this report will provide an estimate of BSE prevalence, and/or demonstrate freedom from disease in U.S. cattle. The information provided by the annual report may be further tabulated for public consumption at the request of the Deputy Administrator; however monthly reports are anticipated to remain for internal USDA use only. Measures that will be portrayed by the monthly reports, in tabular and graphical format, include monthly and cumulative numbers of targeted samples, non-targeted samples, "not detected, not obex" samples, and total samples stratified by collection site type, submission reason, and geographical area. Monthly reports will be used to assess progress toward national sampling goals with respect to point totals.

Data analysis will be performed throughout the Ongoing Surveillance to assess sampling progress. Data quality will be monitored periodically using an error-checking routine to identify information that is outside expected values or for key data that are missing. Samples that do not meet the targeted sampling criteria will be identified, and feedback will be provided through appropriate channels to sample collectors that submit non-targeted samples. Many mechanisms are already in place in the existing BSE database that prevent common data entry errors and that require recording of important sample data.

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