

(VCI/CVE/KBT- I)

VETERINARY COUNCIL OF INDIA

(Statutory body of Government of India established under Indian Veterinary Council Act, 1984)



Continuing Veterinary Education (CVE) Programmes

Module
On
**BOVINE SPONGIFORM ENCEPHALOPATHY
(BSE, Mad Cow Disease)**

A-Wing, 2nd Floor, August Kranti Bhawan,
Bhikaji Cama Place, New Delhi – 110 066

Training Module developed with inputs from:

1. Dr. J.L. Vegad, Retired Head, Department of Pathology, College of Veterinary Science and Animal Husbandry, Jabalpur-482002, Madhya Pradesh.
2. Dr. R.S. Chauhan, Joint Director, Centre for Animal Disease Research and Diagnosis, Indian Veterinary Research Institute, Izatnagar-243122, Uttar Pradesh.
3. Dr. Rajendra Singh, Principal Scientist, Centre for Animal Disease Research and Diagnosis, Indian Veterinary Research Institute, Izatnagar-243122, Uttar Pradesh.

Published and printed by: The Secretary, Veterinary Council of India, A-Wing, 2nd Floor, August Kranti Bhavan, Bhikaji Cama Place, New Delhi-110066.

Printed at:

Preface

Consequent upon the decision of the Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture, Government of India to implement the Continuing Veterinary Education (CVE) programmes, an activity of Professional Efficiency Development Scheme, through Veterinary Council of India as its nodal agency in the country, the Council has been implementing these programmes through conducting skill based trainings on identified topics since December, 2007. The primary objective of these trainings is to upgrade the knowledge and skills of the registered Veterinary practitioners aimed at improving quality of Veterinary services.

Though Bovine spongiform encephalopathy (BSE) has not been reported in the country, its outbreak in the United Kingdom has resulted in huge economic loss as well as concern to human health. The disease first reported in 1986 in UK, has significant importance as a potential transboundary disease requiring preparedness. The disease is a recent addition to the group of diseases known as 'transmissible spongiform encephalopathies (TSEs) and is transmissible orally and parenterally through Specific risk materials (SRM) including the brain, spinal cord, dorsal root ganglia, trigeminal ganglia and eyes. OIE has advocated surveillance of BSE to establish the disease status in the member countries. As such, there is a necessity to bring in awareness about the disease amongst the registered veterinary practitioners.

This Module developed and finalized by the experts in the subject emphasizes on the status of the disease, etiology, epidemiology, transmission, pathogenesis, clinical symptoms, diagnosis, treatment, surveillance and its laboratory techniques. It also includes the various formats for recording history of bovines, microscopic and histopathological interpretation of bovine brain.

The contents of this Module are also available on the website www.vci-india.in.

CONTENTS

	Topics	Page No.
1.	Introduction	1
2.	Etiology	2
3.	Epidemiology	3
4.	Transmission	4
5.	Incubation period	4
6.	Pathogenesis	5
7.	Clinical symptoms	5
8.	Lessions	5
9.	Diagnosis	6
10.	Prevention and Control	7
11.	Treatment	7
12.	Surveillance of BSE and its Laboratory Diagnostic Techniques	8
13.	Appendix	23
14.	Further Readings	25

INTRODUCTION

- Bovine spongiform encephalopathy (BSE) is a neurodegenerative disease of adult bovine, which is invariably fatal. The disease is caused by oral exposure to scrapie like agent in the ruminant derived protein of meat-bone-meal supplements. The onset of the disease is insidious and clinical course is variable. The disease is slowly progressive in nature with long incubation period (average 4-5 years). The clinical signs in adult bovine (from 2 years of age to mostly 4-5 years of age) as manifested by disturbances in behaviour, locomotion and sensitivity are distinctively suggestive of the disease. Because of the peculiar and uncontrolled behaviour of the affected cow the disease is commonly known as **Mad Cow Disease**. Grossly no lesions are observed in brain, however, microscopically hallmark lesions are characterized by vacuolation of neurons and neuropil in certain brain nuclei along with astrogliosis and loss of neurons.
- BSE is a recent addition to the group of diseases known as '**transmissible spongiform encephalopathies (TSEs)**'. The animal TSE's include: scrapie of sheep and rarely of goats, transmissible mink encephalopathy (TME) of mink, chronic wasting disease (CWD) of deer, feline spongiform encephalopathy (FSE) of cat and the exotic ungulate encephalopathy of greater kudu, nyala and oryx animals. In these species, BSE agent is believed to be the common source of encephalopathies.
- Human TSE's include: Kuru (known for over 200 years ago in Papua New Guinea due to ritual cannibalism, but does not exist now), Creutzfeldt- Jacob disease (CJD) with three phenotypic variants viz., sporadic CJD (accounts for 85% of the cases in 50-70 years age group), familial CJD (10-15% of the cases due to gene mutation) and iatrogenic CJD (<5% of the cases due to medical and surgical intervention), Gertsman-straeussler – Scheinker-Syndrome (GSS, due to germ line mutation in gene) and fatal familial insomnia (FFI). These disorders are caused due to abnormal folding of normal cellular protein called prion protein (PrP^C). The infective prion protein, named as scrapie prion protein (PrP^{Sc}), is manifested as sporadic, hereditary and/or infectious in nature. Some other atypical neurodegenerative diseases in humans' viz., Alzheimer's disease, Parkinson's disease and Amyotrophic Lateral Sclerosis (ALS) show similar manifestations.
- The above diseases including BSE are characterized microscopically by the development of vacuoles or empty spaces (vacuolation) both in the neurons and the neuropil (i.e., complex tissue network of the cytoplasmic processes of neurons and neuroglia in the grey matter) along with astrogliosis and neuronal loss. So, microscopically BSE is identified by the presence of these three hallmark lesions.
- The BSE was first detected in 1986 in United Kingdom in dairy herds and had caused human fatalities due to consumption of BSE infected meat. The condition in humans was similar to Creutzfeldt-Jacob disease (CJD), except for different age group (about 27 years). This new variant of CJD (nvCJD) indicated that the infectious agent crossed from bovine to human beings probably due to more homology of prion protein of these species. The affected individuals showed clinical signs of tingling or burning feeling, unsteadiness, involuntary jerking and depression followed by death. This event caused greater concern all over the world due to its zoonotic implication.

Etiology

- TSEs are caused by a unique protein called prion. Dr Stanley Prusiner, Professor of Biochemistry, University of California, San Francisco received the Nobel Prize in 1997 for the discovery of Prion.
- This protein has two isoforms: infectious isoform called scrapie prion protein (PrP^{Sc}) and normal cellular prion protein (PrP^C). Although both forms are similar in their biochemical compositions yet differ in their physical and chemical properties including conformation.

- PrP^c is a small glycoprotein of 254 amino acids (33 – 35 KDa) found anchored on cell surface in many mammalian cells (genitalia, heart, lung, lymphocyte, follicular dendrite cells) but its expression is highest in neurons and astrocytes.
- The PrP^c plays role in recognition/communication between cells, memory and maintenance of circadian rhythms and sleep patterns. Since PrP^c knockout mice models showed no adverse effect, its precise role is still unknown. However, PrP^c is a necessary substrate for modified prion formation (PrP^{sc} PrP27-30 kDa fraction).
- There are many hypotheses to describe the conversion of PrP^c to PrP^{sc}. Probably, change in conformation of PrP^c to PrP^{sc} takes place during post-translational processing which results into development of different kinds of strains. Unlike viruses, it has no nucleic acid. However, the process by which PrP^c is converted to PrP^{sc} which in turn gets accumulated to an abnormal level in the cytoplasm of neurons to cause their vacuolation, degeneration and associated reactive changes (astrogliosis) is not fully understood.
- PrP^c is rich in alpha helices, whereas the PrP^{sc} has much fewer alpha helices and many more beta sheets and thus very sturdy for degradation.
- It is believed that there are different strains of the BSE agent due to different conformations of PrP^{sc}.
- The agent is highly resistant to many standard decontamination procedures, including treatment with formalin, chloroform and ether; also to heat, ultraviolet and ionizing radiations, ultra-sonication, proteases and nucleases.

Infectious Dose

- Oral exposure of bovine to a pool of 1 g of BSE-infected brain has shown that bovine can be infected experimentally.
- Comparison of responses between bovine and mice to intra-cerebral inoculation with BSE-infected bovine brain indicates that mice are 1000 times less sensitive to infection than bovine.

Route of Infection

- The oral route in mice is 60,000 times less efficient than the intra-cerebral route. Challenge of bovine by intra-cerebral and oral routes using BSE-infected brain pool indicated 100,000-fold difference in the efficiency of infection by the oral route, compared to that of the intra-cerebral challenge.
- Incubation periods for BSE are much more variable for oral exposure with a fixed dose than for intra-cerebral inoculation.

Epidemiology

- The outbreaks in the UK are believed to have resulted from the feeding of scrapie-containing sheep meat-and-bone meal (MBM). The outbreak was increased by feeding bovine MBM to young calves. The epidemiological investigation revealed that the infective agent of scrapie escaped inactivation due to change in the rendering process from dry to steam and hydrocarbon extraction. This caused crossing over of the species barrier from sheep to bovine. Further the agent from affected cow jumped to human beings due to consumption of infected cow meat and closer homology of infected prion protein to normal prion protein between the two species.
- Between November 1986 and November 2002, 1, 81,376 cases of BSE were confirmed in the UK and 4.4 million bovine slaughtered during the eradication programme. Epidemic peaked in January 1993 with nearly 1,000 new cases reported weekly. Almost two-thirds of the dairy herds in the UK had at least one case of BSE, while only one in six beef herds had reported cases.

- In July 1988, ruminant protein in ruminant feed was banned in U.K. The ban significantly reduced the incidence of new clinical cases in five years, which is the incubation period of the disease.
- However, approximately 36,000 new cases have been diagnosed since the ban, which indicates the ban was not totally effective. As a result, a ban from feeding any mammalian protein to any farm animal was implemented in the U.K. in 1996. The number of new cases has continued to decline since then at a steady rate.
- Most recently, in August 2008, a case of atypical BSE has been reported from US in a 2-year-old heifer. Like the classical BSE, atypical form is not associated with ingestion of BSE-contaminated feedstuffs, but is transmitted genetically. This has further complicated the BSE scenario.
- Since 1989, when the first BSE case was reported outside the UK, a total of 1,88,556 cases have been reported from a number of different countries- Canada (10), Austria (5), Belgium (125), Czech Republic (9), Denmark (15), Finland (1), France (900), Germany (312), Greece (1), Hong Kong (2), Israel (1), Italy(117), Japan (26), Liechtenstein (2), Luxembourg (2), Netherlands (75), Oman (2), Portugal (875), Republic of Ireland (1353), Slovakia (15), Slovenia (15), Spain (412), Sweden (1), Switzerland (453), United Kingdom (9183823), United States (3).

BSE status in India

- So far BSE has not been reported in India.

Transmission

- The epidemiological evidence strongly indicates that disease was caused initially, during the early 1980s, by the feeding of rations containing MBM supplements to bovine, contaminated with the scrapie agent (a prion).
- The epidemic then became amplified by the subsequent recycling through MBM of infected bovine within the bovine population.
- Not only BSE was spread to other countries in Europe via MBM and live bovine, but it was most probably the origin in humans of a new variant of Creutzfeldt-Jacob disease (nvCJD), first described in U.K. in 1996.
- While BSE is not contagious by direct contact, it is transmissible orally and parenterally.
- Specific risk materials (SRM) include the brain, spinal cord, dorsal root ganglia, trigeminal ganglia and eyes.

Horizontal Transmission

- There is no evidence of horizontal transmission of BSE between bovine.

Maternal Transmission

- There is no convincing evidence that maternal transmission plays a part in the transmission of BSE whereas both horizontal (70% to 80%) and maternal (20% to 30%) transmission are considered to occur in sheep scrapie.

Transmission through other biological materials

- Muscle tissue (meat) may carry the infectious agent.
- Veterinary and human biologicals (vaccines, hormones) prepared from bovine materials may carry the BSE agent.
- Although, embryos, semen, ova, blood or blood components, and milk and milk products are unlikely to carry BSE agent and transmit the disease to recipients, they cannot be considered as zero risk materials.

Incubation Period

The incubation period is unusually long of about 4-5 years. All cases in British bovine (mainly Friesian/Holstein cows) have occurred in adult animals, with a peak incidence in those between 3-5 years of age.

Pathogenesis

The infection occurs through ingestion of ruminant-derived MBM. The infectious prion, being mostly resistant to proteolytic enzymes, escapes digestion in the gastrointestinal tract and enters through M cells lining the mucosa of Peyer's patches. After amplification in the Peyer's patches, it travels to the brain through nerve endings. In scrapie, there is a much greater involvement of the lymphoreticular tissue than BSE.

Mechanisms in the pathogenesis of the disease involve conversion of PrP^c in the CNS, slowly and progressively, to PrP^{Sc} which is capable of inducing other normal PrP^c molecules to undergo conformational change to the PrP^{Sc} form, resulting in the generation of extremely large numbers of abnormal molecules. When this happens, the proteins, which are normally in the liquid form, begin to solidify within the brain cells. The abnormal prion accumulates in the cells of the nervous system, causing nervous symptoms and finally death. When the infected cells die, the defective prions are released into normal tissue and go on to infect more cells. Ultimately, large clusters of cells die, leaving the brain filled with microscopic holes (vacuolations). This is a prolonged process, and symptoms of the disease may not appear for years.

Clinical symptoms

- The clinical manifestation in prion diseases are varying owing to species specific PrP^{Sc} in different conformation.
- Clinical signs include behavioural, gait, and postural abnormalities which usually begin with apprehension, anxiety, and fear.
- The affected bovine show a variety of nervous signs, mainly changes in behaviour, abnormalities in posture and gait, and extreme sensitivity to sound, touch and light (hyperaesthesia).
- Affected bovine lose weight after the onset of clinical signs until they die, or require euthanasia. This period may range from 2 weeks to 6 months.

Lesions

Postmortem Lesions

No gross postmortem changes are observed in the brain.

Histopathological findings

The characteristic changes comprise discrete ovoid and spherical vacuoles or microcavities in the neurons and neuropil of certain nuclei (collection of neurons) of the brainstem. In addition, reactive astrogliosis and neuronal degeneration are also associated with vacuolar changes. The changes described in the brains of bovine affected with BSE are clearly pathological, and distinguishable from single large intracytoplasmic vacuoles recognized in healthy bovine.

Diagnosis

- At present, there is no reliable test to detect the disease in live animals. Diagnosis is based on clinical signs, and confirmed by characteristic pathological changes found in the brain of affected animals and also by several other tests.

- Different diagnostic tests for detection of BSE in brain samples are: Histopathological examination, immunohistochemistry, Western immunoblotting, ELISA, and electron microscopic examination.
 1. **Histopathological examination:** Confirmation of BSE is done by conventional light microscopy of H&E stained tissue sections. This is the main laboratory method used to confirm a diagnosis of clinical case of BSE. The changes in the brain (grey matter) include spongiosis (vacuolation), gliosis, and neuronal loss without inflammatory lesions.
 2. **Immunohistochemistry:** This test is used to detect the disease agent. Because of its sensitivity, the test is used to identify specific PrP^{sc} peptide sequences in formalin-fixed as well as frozen brain tissues. This test can be adopted to detect the causative agent in autolysed brain samples, where histological structures are not distinct.
 3. **Western immunoblotting:** This is also used to detect the disease agent by distinguishing PrP^{sc} from PrP^c biochemically based on their insolubility and protease resistance. Unlike PrP^c, limited proteolysis of PrP^{sc} by proteinase K results in production of a smaller protease resistant residue of approximately 142 amino acids (PrP 27 - 30 kDa, PrP^{sc}) that is detected by this test. The salient features of the test are as follows :
 - The test is conducted in unfixed, fresh or frozen brain samples.
 - The prionics western blot technique shows a high sensitivity than the fibril detection test.
 - The western blot test can also detect the causative agent in the histopathologically negative samples. This test is used to screen a large number of brain samples obtained from slaughter houses.
 4. **Electron microscopic examination:** The test is used to detect SAF in fresh frozen brain samples. Fresh cervical spinal cord or caudal medulla (3 g) may be taken frozen as soon as possible after death.
 5. **Identification and isolation of the agent:** Bioassay of brain tissue of terminally affected bovine by parenteral inoculation of mice is the only method currently available for detection of infectivity. However, this is impractical because of minimum incubation period approaching 300 days. But in case of hamsters it takes only 70 days.
 6. **Rapid Tests:** A number of rapid tests are currently under validation. These include: an indirect ELISA (cELISA), a colorimetric sandwich ELISA (sELISA), a chemiluminescent sandwich ELISA (sELISA II), and an automated conformation-dependent immunoassay (aCID). At present 4 similar postmortem tests are under development as well as one live test based on FT-IR (Fourier Transform Infrared Spectroscopy).

Pre-clinical test

- No valid preclinical test is available till date.

Prevention and Control

- Since there is no treatment, control measures should include immediate destruction of affected animals and ban on the feeding of ruminant tissues to livestock.
- Carcasses and all parts from affected bovine must be destroyed.
- Other measures include pathological surveillance to occurrence of clinical neurological disease.

- There must be a total ban on the importation of live ruminants, and ruminant products, such as MBM from specified risk materials (brain, eyes, dura mater, pituitary, skull, spinal cord, dorsal root ganglion, vertebral column, spleen, intestine, tonsils and lungs), biological materials (blood/plasma, hormone, etc.) and embryos/ova/semen from countries endemic for BSE and scrapie.
- The surgical equipment used for post-mortem of suspected case should be decontaminated or autoclaved at 134°C for 1 hr or discarded.
- Raising of prion resistant domestic animals that do not replicate prion protein.
- Controls on recycling of mammalian protein' and effective tracing/identification of bovine is advisable.
- Surveillance of BSE by selecting the part of bovine population and analysis of all potential risk factors.
- Screening of brains of bovine over 24 - 30 months of age, native borne animals with BSE compatible clinical signs or moribund bovine without signs of illness and screening of selected sub-populations of bovine imported from countries or zones not free from BSE, bovine infected MBM or offspring of BSE affected bovine, fallen stock and emergency slaughter bovine other than routine slaughter bovine. Random sampling of brains from normal bovine not recommended for surveillance.
- Being OIE list B pathogen and highly resistant to general disinfectants, all care must be taken in handling the infected material. The infected material should be handled in BSL 2-3 cabinet.

Treatment

- Treatment is not available presently.

Biosafety Guidelines (Bio safety level 2 or 3)

- Necropsies and processing of brain tissues require bio-safety level 2 or 3 precautions.
- Use waterproof gown, cut-resistant gloves underneath two pairs of surgical gloves and surgical mask with a wrap around splash guard transparent visor while conducting the necropsy. Avoid contamination from aerosol during necropsy and tissue or fluid manipulation.
- Avoid puncture of skin. If it is there swab with 1N NaoH for 5 minutes and then wash with plenty of water.
- Place instruments in large stainless steel dish soaked in 2N NaoH for 1 hr or 1N NaoH for 2 hrs or autoclave at 134°C for 1 hr. Decontaminate the table and other surfaces with repeated wetting over 1 hr. with 2N NaoH.
- Formalin fixed tissues are post-fixed in 95-100% formic acid for 1 hr followed by fresh 10% buffered formalin for at least 48 hrs before histopathological processing to avoid contamination of laboratory equipments from infective prion.
- Examine formalin-fixed brain on a table covered with an absorbent pad with an impermeable backing.
- All absorbent cotton, covers, pads, disposable clothing, gloves, imbedding moulds, section waste and other handling materials are disposed in biosafety receptacles for incineration.
- Liquid waste is collected in a 4 lit waste bottle containing 600 ml 6N NaoH and diluted to a final volume of 4 lit to maintain the optimum concentration for disinfection of BSE agent.

- Use disposable specimen cups or slide mailers for reagents, disposable petri dishes for Immunohistochemical methods.
- During tissue preparation wear gloves, apron, and eye protection. Use disposable specimen cups for manual staining.

Inactivation of infective prion protein (PrP^{Sc}) by:

- 1N NaOH or 2N NaOH or 4Mol /lit Guanadine hydrochloride, sodium hypochloride (≥2% free Cl₂)
- Steam autoclaving at 132°C for 4.5 hrs or 1 hr.
- Dry the waste at 132°C for 4.5 hrs or be incinerated.
- Liquid waste treated with 1N NaOH followed by autoclaving at 132°C for 4.5 hrs.
- Boiling in SDS or prolonged protease digestion.
- High infected brain by autoclaving at 132°C for 4.5 hrs.

SURVEILLANCE OF BSE AND ITS LABORATORY DIAGNOSTIC TECHNIQUES

Salient features of BSE surveillance protocol prescribed by OIE

BSE surveillance in the targeted bovine population (indigenous, crossbred and high risk bovine-imported from BSE infected countries or offspring of BSE affected cows or animals fed with contaminated MBM of ruminant origin) should be carried out through:

1. Passive surveillance:

By creating awareness of BSE among various groups through:

- a) Carrying out regular publications (folders/letters/leaflets etc.) and workshops.
- b) Continued training programmes for field veterinarians about the detection of BSE affected bovine, if any, through physical tests for detection of disturbances in behaviour, locomotion and sensitivity (through CD) and collection, preservation and despatch of brains from animals exhibiting nervous signs compatible with BSE for laboratory confirmation.
- c) Organization of workshops to teachers and veterinarians on neurological diseases in ruminants with emphasis on identification and reporting of clinical BSE suspects, if any in the country.

By investigation of clinical BSE suspect, if any, in bovine population:

- a) Based on disturbance in behaviour, sensitivity and locomotion in bovine over 2 years of age as observed by the owner and field veterinarian must be examined for brain pathology.

2. Active surveillance through:

- a) Examination of brainstems preferably from clinical suspects (BSE compatible signs), emergency slaughter/recumbent and fallen (dead carcasses or culled on-site carcasses for rendering) bovines. Besides, random sampling of brainstems from the slaughtered bovine at abattoir may also be included though it should be avoided.
- b) Risk analysis of all other potential factors for BSE Viz., rendering units and their functioning, source and production of MBM or greaves of ruminant origin, occurrence of other TSEs in animals, importation of animals and animal by- products from countries with BSE and scrapie, etc. should be carried out.

Risk factors involved in the occurrence of Bovine Spongiform Encephalopathy in the country (OIE)

The first step in determining the BSE risk status of the cattle population of a country or zone is to conduct a risk assessment by identifying all potential factors for BSE occurrence and their historic perspective.

Release assessment

Release assessment consists of assessing the likelihood that a transmissible spongiform encephalopathy (TSE) agent has been introduced via the importation of the following commodities potentially contaminated with a TSE agent:

- a. meat-and-bone meal (MBM) or greaves;
- b. live animals;
- c. animal feed and feed ingredients;
- d. products of animal origin for human consumption.

Exposure assessment

It consists of assessing the exposure of the BSE agent to cattle, through a consideration of the following:

- a. Epidemiological situation concerning all animal TSE agents in the country or zone;
- b. Recycling and amplification of the BSE agent through consumption by cattle of MBM or greaves of ruminant origin, or other feed or feed ingredients contaminated with these;
- c. The origin and use of ruminant carcasses (including fallen stock), by-products and slaughterhouse waste, the parameters of the rendering processes and the methods of animal feed manufacture;
- d. Implementation and enforcement of feed bans, including measures to prevent cross-contamination of animal feed.

The potential for the release of BSE agent through Importation of MBM or greaves

MBM or greaves of ruminant origin plays the significant role in BSE transmission. But it has to be analysed that whether these feed materials been imported within the past 8 years and if so, where from and in what quantities of these feedstuffs imported.

Knowledge of the origin of meat-and-bone meal, greaves or feedstuffs containing either meat-and-bone meal or greaves, is necessary to assess the risk of release of BSE agent. MBM and greaves originating in countries of high BSE risk pose a higher release risk than that from low risk countries. MBM and greaves originating in countries of unknown BSE risk pose an unknown release risk. For this sufficient documentation regarding importation of MBM, greaves or feedstuffs, country of origin, annual volume of importation, the rendering processes used to produce MBM and the composition (on a species and class of stock basis) of the imported MBM, greaves or feedstuffs containing them should be maintained.

Importation of live animals potentially infected with a TSE

Countries which have imported ruminants from countries infected with animal TSEs are more likely to experience BSE. Cattle pose the only known risk and animals imported for breeding may pose a greater risk than animals imported for slaughter because of the hypothetical risk of maternal transmission and because they are kept to a greater age than animals imported for slaughter. But here also it is necessary to have knowledge about the country of origin and its BSE status, feeding and management of the animals in the country of origin, the slaughter, rendering and recycling practices of that country.

Importation of products of animal origin potentially infected with a TSE

Semen, embryos, hides and skins or milk are not considered to play a role in the transmission of BSE. But here the risk is influenced by the species of origin of the animal products and whether these products contain tissues known to contain BSE infectivity, country of origin and its animal TSE status, feeding and management of the animals in the country of origin and slaughter, rendering practices in that country.

The potential risk of BSE through consumption of MBM or greaves of ruminant origin

The consumption by bovines of meat-and-bone meal or greaves of ruminant origin plays the significant role in BSE transmission. Because the commercially-available products of animal origin used in animal feeds may contain meat-and-bone meal or greaves of ruminant origin.

Epidemiological situation concerning all animal TSE in the country or zone

BSE may have originated from scrapie of sheep. Countries with scrapie may be at greater risk than those which have demonstrated scrapie freedom. Theoretically, scrapie in small ruminants might mask the presence of BSE and no field methods are available to differentiate between different TSEs.

The origin of animal waste, the parameters of the rendering processes and the methods of animal feed production

- TSE of livestock has long incubation periods and insidious onset of signs, so cases may escape detection.
- Pre-clinical TSE cannot be detected by any method and may enter rendering, in particular if specified risk materials are not removed.
- Tissues most likely to contain high titres of TSE infectivity (brain, spinal cord, eyes) may not be harvested for human consumption and may be rendered.
- TSE of livestock may manifest in sudden death, chronic disease, or recumbency, and may be presented as fallen stock or materials condemned as unfit for human consumption.
- TSE agent survival in rendering is affected by the method of processing.
- TSE agent is present at much higher titres in central nervous system and reticulo-endothelial tissues (so-called 'Specified Risk Materials', or SRM).

If potentially infected animals or contaminated materials are rendered, there is a risk that the resulting MBM could retain TSE infectivity.

Hence documentation describing the collection and disposal of fallen stock and materials condemned as unfit for human consumption, the rendering process and parameters used to produce meat-and-bone meal and greaves, documentation describing methods of animal feed production, including details of ingredients used, documentation describing monitoring and enforcement of the above should be maintained and analysed.

The overall risk of BSE in the cattle population of a country or zone is proportional to the level of known or potential exposure to BSE infectivity and the potential for recycling and amplification of the infectivity through livestock feeding practices. For the risk assessment to conclude that the cattle population of a country or zone is free from BSE risk, it must have demonstrated that appropriate measures have been taken to manage any risks identified.

DIAGNOSIS

Neurological disorders may occur as a result of primary lesions in the brain or secondary to lesions elsewhere in the body like liver disease, enteric disease, poisoning and feed related diseases. Many etiological agents namely bacteria (listerial meningitis) viruses

(rabies), toxins, pesticides, nutritional and metabolic imbalances (mineral deficiency or toxicity) and genetic abnormalities may cause nervous disorders which are confused with BSE. Most of these agents produce distinct microscopic lesions in the different anatomical location of the brain that can be identified in an appropriate sample by a trained pathologist. Therefore, submission of entire brain, representative portions of the spinal cord suggesting cord lesions, and representative pieces of other organs is essential for laboratory diagnosis, if differential diagnosis is to be made. Otherwise, in case of BSE surveillance only brainstem is required for laboratory diagnosis.

- The clinical signs of BSE are confused with other neurological disorders; hence confirmatory diagnosis is achieved by histopathological, immunohistochemical (IHC), dot blot or Western immunoblot methods on brain samples.
- Amyloid plaques in brain sections are also of diagnostic significance; these can be demonstrated by PAS staining or by α -PrP antibodies. The SAF of PrP 27-30 can be demonstrated in brain tissue by electron microscopic.
- There are no authenticated clinical tests available to diagnose BSE. Nevertheless, molecular analysis of PrP gene in DNA isolated from blood leucocytes and detection of higher levels of protein 14-3-3 in CSF of BSE affected bovine are reported as sensitive methods.

Clinical signs

Most significant symptoms in animals with BSE are:

- ✓ **Disturbances in behavior:** Affected bovine will show *apprehension* to enter the milking parlor or may kick vigorously during milking. In dry cows, *pelvic limb in-coordination* and weakness is noticed.
- ✓ **Disturbance in locomotion:** Abnormal posture and movement when the animal is led by the owner. Swaying of the pelvic limbs and pelvic limb hypermetria are noticed when the animal is observed at pasture. With the advancing severity, there is generalized weakness, resulting in falling and recumbency. As the disease progresses, there is loss of bodily condition, decreasing body weight, and reduction in milk, besides nervous signs. The protracted clinical course, extending over a period of weeks (6-8) or months, would eventually require slaughter on welfare ground or animal dies.
- ✓ **Disturbance in sensitivity:** *Hyperesthesia* to touch or sound or light which can be detected by touching the tip of pen at the neck area, beating of any sound behind the animal or touching the broom stick at the hock region, the affected animals will show hyper reaction to these stimuli.
- ✓ If such animals are noticed in the population, they should be notified and diagnostic investigation of such clinical suspects carried out as per national policy.
- ✓ Carry out differential diagnosis from other nervous diseases eg., rabies, encephalic listeriosis, nutritional deficiencies/excess (vitamin B1), and metal (Lead) and non metal poisoning, metabolic disorders (ketosis, hypomagnesaemia, hypocalcaemia), etc.

Precautions to be taken in handling of suspected cases:

- The cases should be handled and necropsy conducted under appropriate containment facilities (comply with national bio-containment and bio-safety regulations).
- Personnel should wear protective clothing, gloves and safety glasses. Bio-safety level 2 procedures should be followed during handling of suspected materials.

- Disposal of formalin and formic acid used in fixing the tissues should be decontaminated as described under the heading 'inactivation of infected prion protein' above.

Collection of Brain Samples from Target Population mentioned below:

- Bovine over 30 months of age displaying behavioral or clinical signs consistent with BSE (called clinical suspect).
- Bovine over 30 months of age that are non ambulatory, recumbent, unable to rise or walk without assistant; bovine over 30 months of age sent for emergency slaughter or condemned at anti-mortem inspection (called casualty or emergency slaughter or downer bovine).
- Bovine over 30 months of age which are found dead or killed on farm, during transport or at an abattoir (called fallen stock).
- Bovine over 36 months of age at routine slaughter. In slaughter house material the possibility of getting a case of BSE is very remote.

In order to implement efficiently a surveillance strategy for BSE, there must be documentation of the age distribution of the adult cattle population and the number of cattle tested for BSE stratified by the age and sub population. This approach assigns point values to each sample based on the sub population as mentioned in the table. The number of points a sample is assigned is determined by the sub population from which the sample is collected and the age of the animal sampled. The total points accumulation is then periodically compared to the target numbers of points for the country, zone or compartment. The samples should be representative of the herd of the country, zone or compartment and include consideration of demographic factors such a production, type and geographic location, and the potential influence of culturally unique husbandry practices. The approach used and the assumptions made should be fully documented, and the documentation retained for 7 years.

Table: Surveillance point values for samples collected from bovines in the given subpopulation and age category.

Age (Yrs.)	Clinical suspect			Causality slaughter			Fallen stock			Routine slaughter		
	SP	NOA	Total SP	SP	NOA	Total SP	SP	NOA	Total SP	SP	NOA	Total SP
> 1 & < 2	NA			0.4			0.2			0.01		
>2 & < 4 (Young adult)	260			0.4			0.2			0.1		
> 4 & < 7 (Middle adult)	750			1.6			0.9			0.2		
> 7 & < 9 (Older adult)	220			0.7			0.4			0.1		
> 9 (Old)	45			0.2			0.1			0.0		
Total	-			-			-			-		

SP: Surveillance points per animal; NOA: No. of animals

Collection of brain for BSE surveillance

While collecting the brain, persons should wear protective clothing like thick gloves, goggles, plastic apron, and gum boots etc. to avoid infection from the viral, bacterial and prion infections and should handle the tissue under bio-safety level 2 procedures.

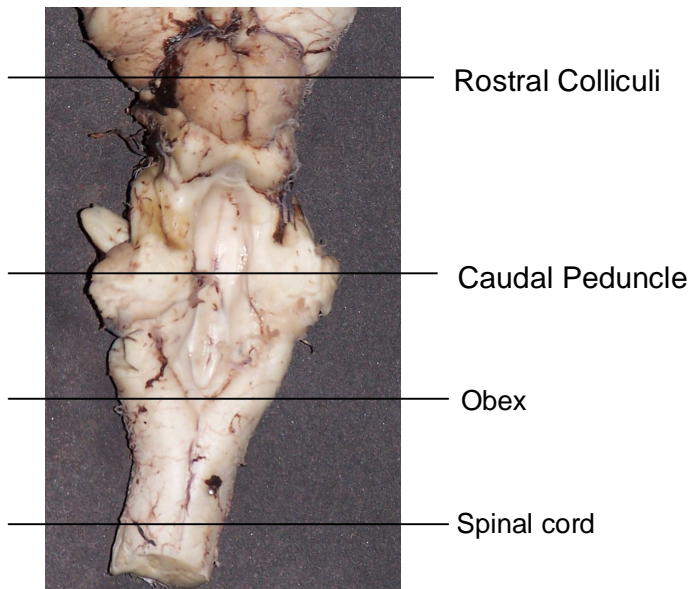
1. Stabilize the head in vice or against solid object.

2. Disarticulate the head through atlanto-occipital articulation approaching from the ventral aspect of the neck.
3. De-skin the head by cutting skin around head, ear, and horns.
4. Remove the muscles, lymph and salivary glands.
5. Make one transverse incision with saw that connects the supra-orbital foramen, just caudal to the orbits and two lateral incisions, from each supra-orbital foramen to the occipital condyle on both the sides to cut into the foramen magnum.
6. Remove the skull cap by severing with chisel and then remove meninges by cutting dura mater over the cerebellum and medulla oblongata.
7. Slightly tilt the skull on either side, then with support of spatula take out brain over plastic tray by tilting the skull backward and cutting the optic chiasma and cranial nerves. Clear the meninges remnants from the brain. Collect the brains in thick plastic bags under propylene/plastic containers and transport over ice to laboratory for further processing.

Processing of brain for sampling tissues for Histopathology, IHC and other tests:

1. From the freshly collected brain, take out small pieces (2-4 g) from the cranial spinal cord, medulla caudal to the obex, avoiding damage to the obex region, and parts of cerebellum and cerebral hemispheres in a plastic tube/cassette (covered with OCT compound) and send to the laboratory over ice; which is then stored frozen (-80°C) prior to testing to detect disease specific PrP by Western immunoblotting /electron microscopy. DNA isolation for genotyping of PrP^{Sc} can also be done in case the sample is found positive for BSE or inconclusive HP results obtained.
2. Fix up the rest of the brain in large container in 10% neutral buffered formalin. Usually 4.5 lit of formalin solution is required for fixation of one adult bovine brain. Fixation of brain requires 15 days with two change of formalin one week apart. The time required for proper fixation of brain is high because of its compactness and rich in lipid content as compared with other tissues wherein fixation is achieved within 6-12 h for 5mm thick block of tissue. Send the brains for further laboratory examination. **OR**

3. Remove the cerebral hemispheres and cerebellum after completing the step 1 to get brainstem and fix up only the brainstem in 10% neutral buffered formalin (10-15 times the volume of brain) which should be changed twice weekly. Or for faster fixation (at least 48 h), cut coronal sections (7mm thick) of the medulla at the obex, medulla at caudal cerebral peduncles and mid brain at rostral colliculi (Fig. 1). The fixed brainstem is used for histopathology and immuno-histochemistry methods.



Superior view of brainstem after removal of the cerebellum and cerebral hemispheres to show the specific sites for BSE surveillance

Dispatch of brain samples:

1. Place the brainstem in large plastic containers (leak proof) having 10% neutral buffered formalin and label it properly. Number of brainstems, properly tagged with, can be accommodated in one container for sending the samples. The unpreserved brain pieces collected at step 1 (See above) in plastic tubes should be sent over ice for Western blotting/ electron microscopy methods, etc.
2. Enclose detailed history sheet of bovine like whether indigenous, exotic, crossbred, dead carcass, emergency slaughtered carcass, abattoir slaughtered bovine, clinical BSE suspect bovine, other neurological signs, age, origin of specimen, owners name, with the sample as well as separately (BSE surveillance proforma).

Processing of fixed brainstem and unfixed frozen brain pieces:

1. Take out the formalin fixed brainstem and cut into coronal slices (2 mm) through medulla at the obex, through caudal cerebral peduncles and mid brain through rostral colliculi (Fig. 1).
2. Keep the coronal sections in formic acid (95-98%) for 60 min. to decontaminate prion infectivity, if any, and to increase the sensitivity of the immuno-staining and reducing the rate of false positives due to detection of endogenous cellular PrP. However, formic acid antigen treatment alone is not adequate for elimination of endogenous PrP^c.
3. Rinse the coronal sections 3 times with 10 volumes of water each time then rinse the sections in running distilled water or deionized water for 10 min. and then transfer the coronal sections to fresh 10% neutral buffered formalin and hold for 24 h to re-equilibrate.

Processing and paraffin embedding (routine method)

1. Remove the formalin from the coronal sections by placing the cassettes, numbered appropriately, in running water overnight.
2. Carry out dehydration in ascending grades of ethanol (50% ethanol, 70%, 80%, 90%, 95% and absolute ethanol 1 and absolute ethanol 2) for 1 hr. each. The volume of alcohol should be at-least 50 times more than the tissue placed for dehydration.
3. Clearing of tissues is done by placing the cassettes in ethanol and xylene mixture (1:1) for 1 hr., then the cassettes are transferred to xylene 1 and xylene 2 for 1 hr. each or these may be transferred to mixture of xylene and benzene (1:1) and then in benzene for 1 hr. each. On complete clearing, the tissue becomes transparent.
4. Transfer the cassettes in paraffin wax embedding bath or in oven fixed at 60-62°C temperature for impregnation two changes of 1 hr. duration each.
5. After impregnation of tissues make paraffin tissue blocks using conventional technique.
6. Trim the blocks at 10-15 μ on microtome to remove the extra wax and expose the whole surface of tissue.
7. Cut 3-5 μ thick sections and place the sections from the water bath onto charged slides (super frosted end slides, 75 x 25 mm with a 19 x 25 mm frosted end). To avoid the problem of detachment of the sections from the slide use poly-l-lysine (Sigma) coated slides.
8. Use the paraffin embedded tissue section slides for routine hematoxylin and eosin staining for detection of hallmark lesions of BSE as well as for immunohistochemistry to detect prion protein in bovine brain samples.

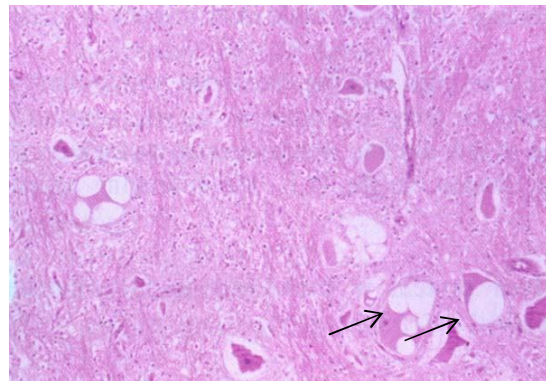
Staining procedure for H&E stain

1. Dewax the slide in xylene I and II for 10 min. or more each.
2. Remove the xylene by passing through descending grades of alcohol (ethanol). 100%, 95%, 90%, 80%, 70%, 50% of 3 - 5 min. each
3. Rinse the slide with distilled water.
4. Stain with Harris's hematoxylin for 15 min.
5. Rinse slides in tap water to remove extra pigment.
6. Differentiate the slides in acid alcohol (99CC of 70% alcohol and 1CC of conc. HCl) by 3-10 quick dips. Check the differentiation under the microscope – nuclei should be distinct and the background very light or colorless.
7. Rinse briefly in tap water.
8. Dip in ammonia water (50 ml water + 5 - 8 drops of ammonia) until sections are bright blue.
9. Wash in running tap waters for few min.
10. Pass the sections through ascending grade of alcohol (50%, 70%, 80%, 90%) 1 – 2 min. each.
11. Stain the section with Eosin for 15 sec. – 2 min. depending on the age of the Eosin and the depth of counter stain desired.
12. Pass the slide through 95% alcohol, absolute alcohol 1 & 2 for atleast 1-2 min.
13. Clear the slides in Xylene I & II for 10 min. or more each and than place the slides in Xylene III overnight.
14. Take out the slides from xylene Couplin jar and mount in DPX mountant.

Interpretation

- Examine the coronal sections and look for various nuclei (collection of neurons) for the presence of vacuolar changes, astrogliosis and loss of neurons.
- When the result of the histopathological examination is inconclusive or negative or the brain material taken at postmortem was unsuitable for the histopathological examination because of autolysis or damage, it is important to apply additional laboratory diagnostic methods such as immunoblotting, immunohistochemistry or the detection of scrapie associated fibrils.

Microscopic grading of the lesions in H&E sections may be done as per the method by Well *et al.*, 1989 for BSE and McGorum *et al.*, 2000 for scrapie. There are 3 hallmark lesions: vacuoles in perikaryon in neuropil and in the nuclei, degeneration of the neurons and astrogliosis in specific sites as mentioned above (vacuolar changes in neurons in red and oculomotor nuclei of the midbrain and occasional solitary vacuole are normally found in bovine). In case if BSE is established, then for monitoring purposes hind brain alone will be taken from foramen magnum without removal of calvarium; and medulla oblongata will be examined only.



Neuron showing vacuolation in the cytoplasm (Internet)

Immunohistochemistry for detection of PrP^{sc}

The IHC technique is more sensitive than routine histopathology as it can detect cases in the last month of incubation before the occurrence of vacuolar changes. This technique works well in autolyzed tissues in which morphological evaluation is no longer possible. The IHC is also as sensitive as Western blotting for detection of PrP^{sc}. IHC allows detection of PrP^{sc} accumulation, like the vacuolar pathology, exhibits a typical distribution pattern and appearance. In countries with low BSE incidence, IHC is, therefore, a method of choice for both confirmatory diagnosis and surveillance. The localisations of PrP protein on formalin fix sections can be achieved using the Streptavidin/Avidin-Biotin complex technique (Haritani & others 1994; Fatzer and others 1998) or by the recommended test kit.

A. Test Protocol as per the instructions mentioned in VMRD BSE antigen test kit (Bovine Spongiform Encephalopathy Antigen Test Kit, Immunohistochemistry, VMRD, Inc., PO Box 502, Pullman, WA 99163 USA, Email: vmrd@vmrd.com)

1. Bake the paraffin cut section slides at 56°C for 2 hrs. to remove excess paraffin.
2. Dewax the slide in xylene I and II for 10 min. each.
3. Rehydrate the sections by passing through descending grades of alcohol (ethanol). 100% (2 times 4 min. each), 95% (1 time, 3 min.), 80% (1 time, 3 min.).
4. Place the slides in a container for 10 min. in 200ml of freshly prepared 3% H₂O₂ in methanol (20ml of 30% H₂O₂ in 180ml methanol).
5. Rinse the slides into distilled water 10 times or may be held in water for a few hours at this stage.
6. Incubate slides in 95% formic acid for 5 min. and then rinse and neutralize by putting slides through 3 changes (3 min., 2 min., 2 min.) of 0.1M Tris-HCl, pH 7.6.
7. Transfer slides to a heat proof container containing modified citrated buffer (1x target retrieval solution, pH 6.1, kept at 4°C until used) for antigen retrieval.
8. Transfer the container to a microwave oven and incubate at 121°C for 20 min.
9. Allow slides to cool to room temperature.
10. Transfer slide rack to TBST (Tris Buffered Saline with Tween 20) for 10 min. Avoid air bubbles under the cover plate. Drain out the buffer.
11. Apply 100µl proteinase K for exactly 1.5 min. supplied with the kit.
12. Promptly rinse the slides with TBST buffer and drain out the buffer.
13. Antibody staining: Apply 100µl of 1X Monoclonal Antibody (Freshly diluted 1:1000 to a final concentration of 1µg/ml in antibody diluent supplied with the kit. Incubate for 10 min.
14. Rinse slides 3 times with TBST buffer and then drain out the buffer from the slide.
15. Apply 100µl of Anti-Mouse IgG-Biotin supplied with the kit and incubate for 10 min.
16. Rinse slide 3 times with TBST buffer and then drain out the buffer from the slides.
17. Apply 100µl of Streptavidin- HRP supplied with the kit and incubate for 10 min.
18. Apply 100µl of AEC Substrate Chromogen supplied with the kit and incubate for 5 min., then add a second 100µl of AEC and incubate a second 5 min., lastly add a third 100µl of AEC and allow to incubate for 10 min. Each slide requires a total of 300µl AEC applied over 20 min.
19. Rinse slides 2 times with TBSE buffer as in step 14.
20. Counter stain in Haematoxylin for 10 min.

21. Rinse gently in distilled water.
22. Dip slides 10 times into 37mM ammonium hydroxide water solution.
23. Rinse slides in tap water for 2-5 min. Leave slides in water until ready to proceed to mounting and cover slip.
24. Cover slip using aqueous base mounting medium. Xylene and alcohol dehydration or xylene based mountain will remove the immuno stain.

Interpretation

Strong positive signals are detected in neurons cytoplasm and in the neuropil area in positive reaction.

Test Preparations

Prepare 1X Target Retrieval Solution: 50 ml of 10X Target Retrieval Solutino (A) is diluted for use with 450ml of double-distilled or deionized water. After dilution, pH should be between 6.0-6.2. if not, it may be adjusted down with 2N HCl or up with 2N NaOH. Store 1X Target Retrieval solution at 2-7°C (35-45°F) between uses.

Prepare 1X Monoclonal Antibody: Monoclonal Antibody F99/97.6.1 (C) should be diluted on the day of use according to instructions on the label using Antibody Diluent (D). After dilution, antibody remains refrigerated (2-7°C; 35-45°F) or on ice until use. Storage of diluted antibody is not advised.

Prepare 0.1M Tris-Hcl Buffer: Dissolve 12.1g of Tris Base (MW 121.1g) in 800ml double distilled water. Adjust pH to 7.6 with concentrated HCl and dilute to 1 liter. This may be stored at room temperature.

Prepare TBST Buffer (50mM Tris-HCl, 300mM NaCl, 0.1% Tween 20): Dissolve 6.06 g Tris base (MW 121.1g) and 17.5g NaCl (MW 58.44) in 800ml double –distilled water. Adjust pH to 7.6 with concentrated HCl. Dilute to 1 liter, mix well, then add 1ml Tween 20 and mix gently to avoid foaming.

Prepare 37mM Ammonium Hydroxide Water Solution: Add 2.5 ml of 15N ammonium hydroxide to 1 liter of water. Hold at room temperature in capped bottle to prevent evaporation.

Western blotting technique for detection of PrP^{Sc}

Large number of unfixed or frozen brain or spinal cord samples can be screened with Western blotting method. This technique is easy and rapid to perform and potentially more sensitive than the histopathological evaluation. Digestion with protease allows a distinction between normal PrP^C and PrP^{Sc} i.e. PrP^C is completely digested while PrP^{Sc} is cleaved at the N-terminus leading to a fragment of reduced molecular weight (PrP 27-30). Detection of this fragment by Western blotting can be done rapidly in hundreds of samples per day for surveillance purposes and to examine sub-clinical cases in slaughtered bovine on a routine basis without causing delays to the meat processing industry. Sampling of the correct brain region and the method used for protein extraction are important factors for correct diagnosis. This method is useful in BSE surveillance, and rapidly identifies animals - infected with BSE.

Prionics Western blotting (PWB) kit (Bio-Rad) to detect bovine and ovine disease-specific, protease-resistant prion protein (PrP^{Sc})

- Protinase-k treated samples are run on a 13% SDS - PAGE and immunoblotted with a rabbit anti-PrP polyclonal antibodies (78295, Dr. R. Kascsak, Inst. Of Basic Research and Developmental Disabilities, Staten Island, NY, USA).

- Followed by incubation with alkaline phosphatase labelled goat anti rabbit antibody and development with NBT and BCIP.
- Or Prionics western blotting kits (Bio-Rad) is also used for BSE surveillance.

Test procedure

1. **Preparation of the brain sample and isolation of the BSE prion protein:** The test sample is a small piece of obex, a defined region of the brain stem. The homogenized brain sample is incubated with an optimized reagent mixture consisting of digestion enzymes and a buffer solution (I). This solution degrades the normal prion protein. Only the BSE-specific prion protein remains in the test sample.
2. **Separation of proteins by gel electrophoresis and immunological detection of BSE prion protein:** The proteins in the sample are then separated according to size by gel electrophoresis (II) and transferred to a special blot membrane for detection (III). The BSE-specific prion protein on the membrane is detected with specific antibodies and is visualized on a digital file or on a film (follow the instruction of the kit).

Note:

1. Always use 1 N NaOH or 2 N NaOH for decontamination in case of BSE.
2. Disposal of tissue/ containers should be done as per the recommended procedure.

APPENDIX

Table: Proforma for recording history of bovine

SPECIES		BOVINE
BREED	BOVINE	
	BUFFALOES	
AGE		
SEX		
PLACE OF ORIGIN		
HEALTH STATUS		
EXTERNAL APPEARANCE & FITNESS FOR WORK		
HEALTH STATUS OF ANIMALS FROM FIELD CONDITION		

Table 1: Different microscopic observation of brains

Microscopic observations	Total numbers of brains showed the lesions
1. BLOOD VESSELS	
Congestion	
Hemorrhages	
Proliferate thickenings	
Serous Vasculitis	
Perivascular collection of MN cells	
Inflammatory reaction/Other cells	
Blood vessel wall thickening	
Thin wall of blood vessels	
Proteinaceous material in vasculature	
Vasculature disrupted in size	
Perivascular cuffing	
Necrosis/Odema	
2. NEURONS	
Neuropil (Intactness)/vacuolation	
Neuronal degeneration (Chromatolysis)	
Satellitosis	
Neuronophagia	
Neuronal loss	
Glial cells/Inflammatory cells	
Neuronal Vacuolation (Microvacuolation in gray matter)	
3. FIBRE TRACT	
Demyelination	
Vacuolation (White matter)	
Glial cells proliferation (microglial cells)	
4. TUMOR CELLS	
5. PARASITIC CYST/LESIONS	
6. NO SIGNIFICANT LESIONS/NORMAL BRAIN	

JOINT DIRECTOR

REPORTING SCIENTIST/OFFICER

Table: Histopathological Interpretation of Bovine Brains:

HISTOPATHOLOGICAL OBSERVATIONS	NO. OF BRAINS	PERCENTAGE OF LESIONS (%)
Chronic Encephalopathy (CE)	-	
Vasculo Encephalopathy (VE) (Multi Etiological Origin)		
Rabiform Encephalopathy (RE)		
Bacterial/Viral or Infectious Encephalitis (IE)		
Low Grade Encephalitis (LE)		
Tumors/Parasitic lesions		
No histopathological lesions (Normal Brain)		
Total No. of Brains screened for BSE		
Total No of Brain Collected		
Brains to be processed for HP		

Bovine Spongiform Encephalopathy sample submission proforma									
Ref:		Sp. No.						Date:	
Species:	Breed:	Age	1-	2-	4-	7-	>9	Sex:	Identity:
		Years	2	4	7	9		M/F	
History of animal									
Sick (Clinical suspect)	Down/Nonambulatory/ Recumbent (Causality slaughter)		Died in premises or during transport or at an abattoir (Fallen stock)				Routine slaughter (>36 months of age)		
Date & time of Death					Date & Time of PM				
Owner Name & Address:					Referred by & Address:				
Tel.:					Tel.:				
Mobile:					Mobile:				
E-Mail:					E-Mail:				
I. Neurological History/ Neurological Examination of animal (Tick Yes/No)									
1. Excessive and asymmetrical ear movements					Yes/No				
2. Apprehension and vigorous kicking					Yes/No				
3. Swaying of pelvic limb, unable to stand					Yes/No				
4. Over reaction to visual or auditory or touch stimuli					Yes/No				
5. Sudden jerking movements of limbs, body parts or whole body					Yes/No				
6. Head, neck shaking and head tossing, head bobbling					Yes/No				
7. Sneezing, snorting					Yes/No				
8. Excessive licking, nose wrinkling					Yes/No				
9. Tooth grinding					Yes/No				
10. Tremors					Yes/No				
11. Excessive vocalization					Yes/No				
12. Others: Bellowing/Salivation/Pyrexia					Yes/No				

II. Disease course: Unusual clinical signs for prolonged period or shorter period.		
III. Treatment: if any		
Sample detail (tick if appropriate)		
1. Nature & content of specimen:		
2. Brain sample:	Brainstem/ Whole brain/Half brain/ or others	
3. Area of Brain:	Cerebral hemispheres/Cerebellum/obex/Medulla oblongata/mid brain, diencephalon, other	
4. Examination Desired:		
5. Preservative or Transport media used	10% formalin/Cold chain (without preservative)	
List of neurological diseases/disorders confused with BSE		
1. Metabolic/Endocrine disorders: Hypomagnesaemia (Grass tetany)/ Hypocalcaemia / Nervous ketosis /Hepatic encephalopathy, etc.		
2. Nutritional disorders: Polioencephalomalacia (grain fed)/ Cerebrocortical necrosis, Thiamine/vitamin B1 deficiency /Copper deficiency, etc.		
3. Infectious/Inflammatory conditions:		
↳ Viral: Rabies/Tick encephalitis.		
↳ Bacterial: Epidural abscesses (e.g. salmonella)/listeriosis		
↳ Fungal: Aspergillosis		
↳ Parasitic: Cysts or migration (sarcocystis/coenuriasis)		
↳ Other infectious: Myeloencephalitides (Miscellaneous agents)		
4. Toxic Syndromes: Tetanus, Botulism/Miscellaneous plant toxins: Mycotoxicoses/Urea poisoning/Ammonia toxicosis/Organophosphate and other insecticide toxicity/Mercury poisoning/lead poisoning/Salt poisoning.		
Herd Details	Species	Breed
No. of Affected		
No. of Died		