BIOSAFETY

1. GENERAL CONCEPTS

Microorganisms can be classified according to their pathogenicity for humans and animals. According to this classification, precautions must be taken when handling them. These precautions are necessary primarily to protect the people handling these agents, but also to protect the general human population and livestock from accidental exposure. Several guidelines have been published on the classification system for and the handling of microorganisms. An internationally well accepted guideline is the WHO *Laboratory biosafety manual* (WHO, 2003). This manual defines risk groups, biosafety levels (BL) of laboratories, the requirements for risk assessments and the requirements for each of the biosafety levels.

WHO defines four risk groups, classified 1 to 4, which are based on the risk a specific microorganism poses for humans or animals. Risk groups are:

1) Poses no or a low risk for individuals (laboratory personnel) and the community.
2) Poses a moderate individual risk and a low community risk. Effective treatment or prevention is available, and spread of the microorganism is ordinarily limited.
3) Poses a high individual risk and a low community risk. Effective prevention or treatment is available, but spread of the microorganism is not ordinarily limited.
4) Poses a high individual and a high community risk. Prevention and treatment are not available, and the microorganism is readily transmitted.

The classification of microorganisms is based on four criteria: pathogenicity, transmission, preventive measures and treatment as follows:

Pathogenicity is the ability of an organism to invade a host and to cause disease. Microorganisms with a high pathogenicity are more likely to be classified in a higher risk group and microorganisms with a low pathogenicity are more likely to be classified in a lower risk group.

Transmission of microorganisms can, for example, be through direct contact, be water-borne or be air-borne (by aerosol). When transmission of a microorganism is limited to direct contact, the possibility of becoming infected is relatively low. Air-borne microorganisms have a higher possibility of infecting hosts. Also, the chance of becoming infected is higher for laboratory personnel who work in close contact with microorganisms than for the community. The more easily microorganisms can be transmitted the more likely they are classified in a higher risk group.

Preventive measures against microorganisms include vaccination. There can be a difference in the need for preventive measures between laboratory personnel and the community. There are no vaccinations available against some microorganisms, and these microorganisms are generally classified in a higher risk group.

Treatment. If no treatment is available for infection with a microorganism, that microorganism is classified in a higher risk group.

Although WHO defines the risk groups and the criteria, it does not classify the different microorganisms into risk categories. It does give the requirements for a risk assessment, based on which microorganisms can then be classified. Single countries

or regions (e.g. the EU) should perform such risk assessments and establish a national or regional risk group classification for each microorganism of interest.

Depending on the classification of the microorganism, precautions should be taken to protect laboratory workers and the community from possible infection, for which WHO has defined four biosafety level categories. These categories correlate somewhat with the risk group categories, but also reflect what is being done with the microorganism in the laboratory.

BL 1: This level is the basic safety level for laboratories. Agents (microorganisms) handled at this level do not ordinarily cause human disease. Both research and teaching can be done in this level laboratory.

BL 2: This level is the second basic safety level for laboratories. Agents handled at this level can cause disease in humans, but their potential for transmission is limited. Both diagnostics and research can be done at this level.

BL 3: This level requires a containment laboratory. Agents handled at this level can be transmitted by aerosol and can cause serious infections. At this level special diagnostics and research can be done.

BL 4: This level requires maximum containment, and may be considered a laboratory for dangerous pathogens. Agents that are handled at this level may pose a high risk of life-threatening disease, may be transmitted as an aerosol, and may have no associated vaccine or therapy available. These agents are often considered exotic to a country.

2. BIOSAFETY FOR TSEs

In 2000, the EU published a directive regulating the protection of workers from risks related to work-related exposure to biological agents (EU, 2000), based on the WHO guidelines. In this directive, a new risk group is defined for BSE and related animal TSEs. Characteristics of the BSE agent include a limited risk for laboratory personnel and the community; however aerosol transmission cannot be excluded (though it has never been described). This new risk group is called 3**, which means risk group 3 with some alleviations. Scrapie, on the other hand, is still classified as risk group 2. According to the Swiss Expert Committee for Biosafety (2006), different BLs are required when handling BSE materials, depending on the type of material. For example, histology and IHC on formic acid inactivated BSE material can be performed in a BL 1 laboratory, and for routine BSE diagnostics the laboratory should be BL 2 with some additional measures. A reference laboratory for TSE must be BL 3, but some modifications are allowed. Attention should be paid to the fact that BSE laboratory requirements often differ between countries. In general, the following criteria should be considered:

- All BSE laboratories should have a separated working area with documented restricted access through double doors. The anteroom created by the two doors can also be used as a changing room. The outside door should be labelled with a "biohazard" sign.
- A standard vector control programme (e.g. for ants, flies) should be in place for the laboratory.
- All surfaces within the laboratory must be resistant to acids, bases and disinfectants because all work spaces should regularly be decontaminated, which can only be accomplished using strongly oxidizing substances.
- The laboratory should have a class 2 biosafety cabinet (BSC) with a vertical upward air flow system and a filter that filters out the smallest possible particles.

This airflow system ensures that aerosols, possibly containing BSE-infected particles, are removed to reduce the chance of exposure of laboratory personnel. To maintain the upward airflow, minimal equipment should be placed in the BSC.

- To prevent cross contamination, a complete set of devices and instruments should be available, solely used for BSE diagnostics.
- An autoclave that can achieve a temperature of 134 °C at 3 bar of pressure should be present, optimally in the laboratory, but at minimum within the same building.
- Laboratory personnel should put on protective clothing prior to entering the BSE laboratory. This clothing can be put over the normal clothing. Standard protection includes disposable overalls or gowns, protective gloves, protective glasses or face shields, and dedicated laboratory shoes or shoe covers. This clothing should not leave the laboratory except for final disposal, and then only after autoclaving for decontamination.
- When working in the BSC, personnel should wear a second pair of gloves and protective sleeves to prevent any contact between normal clothing and BSE materials. This second pair of gloves should be used only while working in the BSC and should be disposed of directly afterwards.
- To ensure a standard level of quality and safety, the BSE laboratory should follow good microbiological technique (GMT); (WHO, 2003) guidelines and implement a quality assurance programme.
- Eating, drinking and smoking should not be allowed in the laboratories.
- What has been done when, by whom and based on which instructions should be documented fully in writing, allowing back-tracing when necessary and facilitating accurate job assignments.
- Disposable materials have the advantage that they do not have to be decontaminated and re-used, and therefore reduce the chances for cross contamination.
- The number of sharp objects used should be minimized to reduce breakage and possible injuries.
- Equipment on the work surfaces should be minimized to provide adequate space for placing samples and decrease the risk of accidents.
- The BSC should be used to prevent aerosols in the working area, even when opening a test tube or during centrifuging.
- Pipetting should always be done using a pipetting balloon or an automated system, never by mouth.
- All materials that have been in contact with BSE samples should be considered contaminated. This implies that everything that leaves the laboratory should be decontaminated before disposal. Decontamination is a very important step when working with BSE infected materials. Materials that have been in contact with infectious material must either be disposed of directly in the trashcan (which is decontaminated before disposal) or must be decontaminated when leaving the BSC. Materials can be grouped in four groups and must be decontaminated accordingly.
  1. *Solid waste.* Solid waste should be collected in closed trashcans, which can be opened by foot to prevent having to touch the trashcan when disposing of materials. Within the trashcan there must be an autoclavable trash bag, which has to be labelled "biohazard". The trashcan should be emptied at regular

intervals, and transport to the autoclave must be validated by documenting who disposes of what in which way and along which route. Critical control points along this route can be identified, analysed and adapted accordingly. Solid waste should either be autoclaved at 134 °C, at 3 bar for one hour (or under other internationally accepted circumstances) or incinerated.

2. *Liquid waste*. Liquid waste must be incinerated or autoclaved under the same conditions as solid waste whenever possible. However, practically, not all liquid waste can be incinerated or autoclaved. In this case, liquid waste can be incubated with 2 N NaOH for one hour. It is important that the final concentration is 2 N, therefore the amount of NaOH added should be adapted to the concentration of the liquid waste.
3. *Instruments*. Whenever possible instruments should be autoclaved according to the procedure mentioned above. If this is not possible, they should be immersed for one hour in 4% NaOCl or in 2 N NaOH. Both media are strongly oxidizing, however, so depending on the instruments this decontamination procedure can be detrimental to them.
4. *Equipment and surfaces*. The only way to decontaminate large equipment and surfaces is to bring them in contact with paper towels soaked with 4% NaOCl or 2 N NaOH for one hour. Afterwards, they should be rinsed with water for neutralization. The laboratory itself (floor, walls, shelves, etc.) should be decontaminated at regular intervals and the BSC working area should be decontaminated after each use using 4% NaOCl or 2 N NaOH.

Although extensive information on biosafety has been presented in this chapter, it is clear that situations not described here may still arise. Using this background on how to handle BSE infected materials and equipment will likely allow the handling of other situations to be deduced.

It is important to remember that PrP^{Sc} is entirely resistant to many standard disinfection protocols.

3. REFERENCES

EU. 2000. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC)

WHO. 2003. *Laboratory biosafety manual*. Second edition (Revised). Reference number WHO/CDS/CSR/LYO/2003.4. Geneva

Swiss Expert Committee for Biosafety. 2006. www.efbs.ch

BOVINE SPONGIFORM ENCEPHALOPATHIES DIAGNOSTIC METHODOLOGY

1. BSE DIAGNOSIS AT THE TSE REFERENCE LABORATORY, BERNE

The NeuroCenter at the Veterinary Faculty of the University of Berne is the Swiss National Reference Laboratory for TSEs in animals. The NeuroCenter mainly focuses on BSE, and is registered as an official BSE Reference Laboratory by the World Organisation for Animal Health (OIE). In this role, it is therefore responsible for the diagnosis of BSE at both the national and international levels and regularly confirms the diagnosis of suspected cases of BSE from countries other than Switzerland. The NeuroCenter is also responsible for the evaluation of new BSE tests.

The BSE Reference Laboratory uses two types of tests for BSE diagnosis. The first group of tests includes histopathology and immunohistochemistry (IHC). For these tests, formalin-fixed brain sections are embedded in paraffin and, for histopathology, stained with haematoxylin and eosin (H & E). The sections can then be examined for the presence of BSE-specific lesions. For IHC, the abnormal prion protein associated with BSE (PrP^{Sc}) is labelled with a specific antibody to improve diagnostic specificity. Optimal brain fixation and tissue processing are time consuming procedures, and a minimum of ten days is required to obtain good IHC results. An advantage of these tests is that other neurological diseases can also be detected. More detailed information on histopathology and IHC is given in section 3 of this chapter.

The second group of tests are the rapid tests. For the rapid tests, fresh, non-formalin fixed brain material is used. Several rapid tests are available. More detailed information on rapid tests is given in section 4 of this chapter.

1.1. Confirmation of cases and data gathering

In Switzerland, each suspected case of BSE (identified either clinically or by screening test) must be confirmed by the NeuroCenter before it is officially registered as a BSE case. For all clinically suspected BSE cases, the unopened head of the animal must be sent to the BSE reference laboratory for testing as quickly as possible to prevent post mortem artefacts. Additionally, in Switzerland, all emergency slaughter cattle and fallen stock have to be screened using a rapid test. In these cases, the caudal brainstem is removed at the slaughterhouse by qualified personnel and sent to a laboratory authorized to perform BSE rapid tests. All positive and un-interpretable samples are then forwarded to the BSE Reference Laboratory for confirmation. Data, including the location of origin, the animal identification, the date on which the animal was sent for slaughter, and the history and clinical signs (if known) are included with the samples. Detailed sampling procedures are given in section 2 of this chapter.

2. SAMPLE COLLECTION

2.1. Brain removal (clinical suspects)

All animals clinically suspected of having BSE should be killed with an intravenous injection of a concentrated barbiturate solution, following sedation or by some other

humane means. Optimally, several representative areas of the brain should be examined; therefore the whole head of the animal should be removed and sent to the laboratory. At the laboratory, the brain should be removed as soon as possible for further testing.

Before handling the head, protective clothing should be worn to prevent any direct contact with potentially-infective materials or inhalation of aerosols possibly containing the BSE agent. Standard protective clothing consists of the following:

- a long disposable gown with long sleeves;
- protective gloves in combination with extra protective sleeves;
- protective glasses or face shield in combination with a mouth cover;
- dedicated laboratory boots or shoes.

For removal, the head is placed with the ventral surface (i.e. jaw) up. The soft tissues that could get in the way are removed with a sharp knife. For the next steps, an electric saw can be used. First, the front part of the head is removed by making a transverse cut between the incisors and the premolars (to prevent the saw blade from being damaged by the incisors). Afterwards the head (and brain) is split longitudinally exactly on the midline, starting at the foramen magnum. At this point it is important to make sure that all brain structures, especially the medulla oblongata, have been correctly split into two, approximately equal, parts. Otherwise, a very sharp knife or sharp scissors can be used to create approximately equal halves. One half of the brain is then immediately placed in a container with a large volume of 10% buffered formalin (for histopathology and IHC). The formalin must be changed after seven days and fixation duration is ideally two weeks. Fixation can be accelerated by placing the formalin container on a shaker, but the minimum fixation time remains five days. After fixation, the whole brain is sliced into about 5-mm-thick transverse sections and is subjected an approximate examination. After macroscopical examination, the medulla oblongata, cerebellum, midbrain, thalamus, parietal/occipital cortex, hippocampus, frontal cortex and basal nuclei are selected and trimmed, then placed into cassettes for further processing.

The remaining half of the brain is first sampled for rapid tests and then frozen at -20 °C or -80 °C.

2.2. Brainstem removal (risk animals and routine screening at slaughter)

In cases of emergency slaughter, fallen stock or routine screening, only the caudal brainstem (medulla oblongata) is removed for testing without opening the skull. The head is separated from the body between the atlas and the foramen magnum, and then placed with the ventral surface up. In some slaughterhouses the presentation of the head may be different, but in an cases the caudal end of the brainstem should be visible through the foramen magnum. A specially designed spoon is used to remove the brainstem through the foramen (Figure 1). The spoon is inserted ventrally (along the top edge, as the head is resting upside down) between the brainstem and the *dura mater* and advanced approximately 7 cm while carefully moving to the left and the right to sever the cranial nerves on both sides. Damage to the brainstem can be avoided by keeping the spoon close to the bone. Then, the spoon is bent downwards to cut the brainstem from the rest of the brain. The spoon is kept in a downward position while being gently pulled out of the skull through the foramen magnum, bringing the caudal brainstem with it. The area of interest within the brainstem, the obex region, is then available for testing by histopathology, IHC and rapid tests.

FIGURE 1

Removal of the medulla oblongata

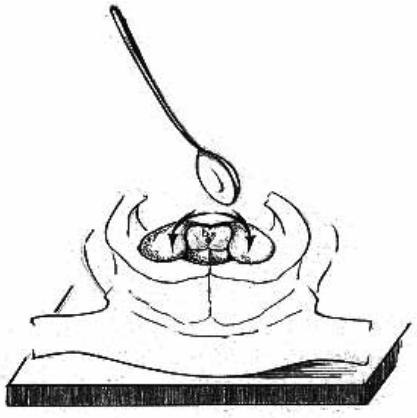
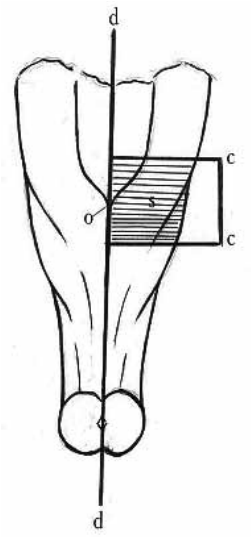


FIGURE 2

Tissue selected for testing for BSE (histopathology and rapid tests), (s), includes the obex region (o)



The medulla is then split longitudinally (Figure 2), and one half is put in formalin for histopathology and IHC while the other half is reserved and sampled for rapid tests. The obex region is targeted for sampling for all tests. The fresh tissue remaining after sampling for rapid tests is then frozen at -20 °C or -80 °C.

3. NEUROPATHOLOGY AND IMMUNOHISTOCHEMISTRY

3.1. Preparation of the formalin-fixed brain

Each of the eight formalin-fixed brain areas (or brainstem removed from risk animals and routine screening animals) is placed in a cassette. These cassettes are placed in

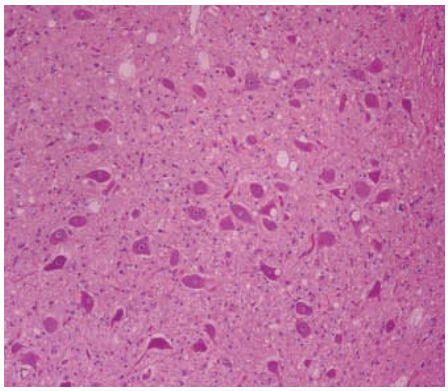
98% formic acid for one hour, then removed and replaced in formalin for 3-4 hours. After this time the cassettes are embedded in paraffin. The embedded brain samples are sliced into 4-5 μm thick sections and placed on glass slides. These sections are used for neuropathology and IHC.

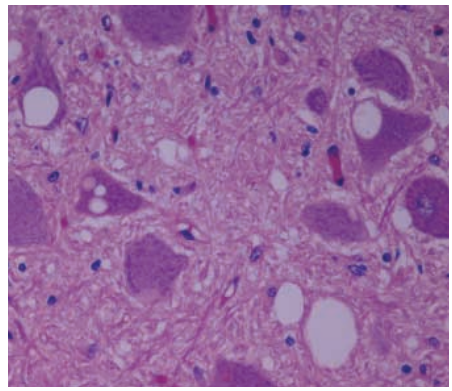
3.2. Neuropathology

For neuropathology, sections are stained with standard H & E stain using standard procedures.

The neuropathology of TSE has five characteristic features:

1. No macroscopic (gross) changes or lesions are present. Therefore, it is not possible to analyse a BSE sample without a microscope.
2. Spongiform changes of the brain tissue. These mostly occur in the grey matter and are bilaterally symmetrical (although this symmetry is normally unrecognized, since only one half of the brain is examined). Spongiform changes occur in several predilection areas, especially the dorsal nucleus of the vagus nerve (Plates 1 and 2), solitary tract nucleus, nucleus of the spinal tract of the trigeminal nerve and olivary nucleus (Figure 3). However, these are not the only areas where spongiform changes can occur. The intensity of changes is variable, but there is no correlation between the intensity of the changes and the appearance of clinical signs in live animals. Cattle with strong clinical signs have been found to have no spongiform changes of the brain tissue, and cattle without any clinical signs could have a high density of vacuoles.
3. Neuronal vacuolation. As with spongiform changes, neuronal vacuolation occurs in certain predilection areas, including the dorsal nucleus of the vagus nerve and the vestibular nuclei. However, it can also occur outside these areas. The number and size of vacuoles in a neuron are variable. Some have only one large vacuole, some several small ones, and some have combinations of both. The vacuoles are mostly empty, and often there is no additional neuronal change to the affected neuron. It is important to realize that neuronal vacuolation can be normal in certain areas, and that it is not a sign exclusive to BSE.



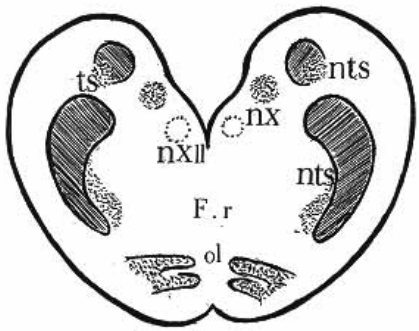


Plates 1 and 2:

Severe spongiform changes in the dorsal nucleus of the vagal nerve of a BSE-positive cow. Magnification of Plate 1 100X, magnification of Plate 2 400X.

FIGURE 3

Transverse section of the obex region of the medulla oblongata: tracts and nuclei important for TSE neuropathology are identified



Legend:
ts: solitary tract
nts (top): nucleus of the solitary tract
nts (centre): nucleus of the spinal tract of trigeminal nerve
nX: dorsal nucleus of the vagus
nXII: hypoglossal nucleus
F.r.: reticular formation
ol: olivary nuclei

4. Neuronal degeneration. Neuronal degeneration can occur in the brainstem and thalamic nuclei.
5. Gliosis (i.e. hyperplasia and/or proliferation of astrocytes and occasionally of microglial cells). Gliosis can either be associated or not associated with spongiform changes of the brain tissue and the presence of PrP^{Sc}. The reason for this is not known at present. Gliosis is normally mild in BSE cases, but usually severe in scrapie cases. Glial fibrillary acidic protein (GFAP) staining may be useful to show the presence of astrogliosis.

Differential diagnosis of spongiform encephalopathy in the bovine brain

After identification of the histopathological features present in a sample, BSE must be differentiated from other neural diseases showing similar lesions. The name "encephalopathy" refers to the fact that the disease is primarily degenerative and, apart from gliosis, does not show any inflammatory changes. The term "spongiform" is purely descriptive and is sometimes used interchangeably with other terms, such as *vacuolation, spongiosis, spongy degeneration* or *microcavitation*. Vacuolation of the neuropil can be seen in many different diseases and even in normal brain, so possible causes of spongiform changes must be differentiated.

Normal vacuolation: Intraneuronal vacuoles can be found in clinically healthy cattle, mostly in the red nucleus or in the nucleus of the oculomotor nerve; they are not associated with any PrP^{Sc} accumulation and are considered incidental findings.

Pathological vacuolation: Vacuoles can be seen in many different toxic and/or metabolic disorders, in some congenital/inherited diseases and in some infectious diseases.

TABLE 1. Differential diagnoses for clinical BSE suspect cases at the Swiss BSE Reference Laboratory in 1999 (after confirmation of negative BSE status) by percent of total samples showing various pathological changes. (Of 47 BSE suspect samples submitted to the BSE Reference Laboratory in Berne in 1999, 7 were BSE positive and 40 were negative.)

Pathological change	Percent
No morphological changes (lesions) in brain	35.2
Listeriosis	21.0
Bovine sporadic meningoencephalomyelitis	16.7
Brain edema	4.7
Polioencephalomalacia	4.3
Bacterial encephalitis (undetermined etiology)	3.6
Cerebellar atrophy	3.2
Brain neoplasias	3.2
System degenerations	3.2
Hepatoencephalopathy	1.4
Neuroaxonal dystrophy (Weaver syndrome)	1.0
Gliosis (undetermined etiology)	1.0
Miscellaneous	1.5
Total (n = 40)	100.0

The following list gives examples of possible differential diagnoses (but is not meant to be exhaustive):

Toxic/metabolic disorders: hepatic encephalopathy
renal encephalopathy
polioencephalomalacia with cerebrocortical necrosis (thiamine deficiency, lead poisoning, water deprivation)

Inherited diseases: congenital errors of amino acid metabolism (such as maple syrup urine disease and citrullinemia)

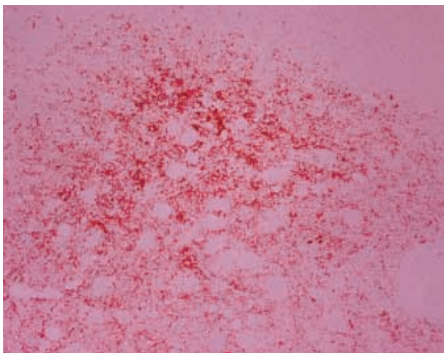
Infectious diseases: rabies (Classically a non-suppurative polioencephalomyelitis and ganglionitis and may cause only very minimal inflammatory changes in cattle. Vacuolar changes may be encountered, not necessarily in association with the inflammation. Negri bodies, which in cattle are mostly found in Purkinje cells, are pathognomonic for rabies.)

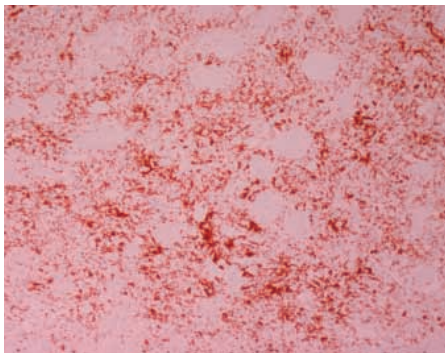
Vacuolation due to post mortem artefacts: Vacuoles may be seen as a result of autolysis, inadequate fixation, freezing and problems in tissue processing (particularly when the samples are kept in 70% alcohol for more than 36 to 48 hours).

To diagnose BSE in clinically suspect cases, the whole brain can be examined for histological lesions. Table 1 shows the results of brain examination of such cases for the year 1999 in Switzerland, as an example.

3.3. Immunohistochemistry

After evaluating the histopathology of the samples according to the five characteristic features (described in section 3.2), IHC can be used to increase the specificity of diagnosis by directly identifying the accumulation of prion protein (PrP^{Sc}). This is accomplished through labelling of PrP^{Sc} in the sample with specific antibodies. In Plates 3 and 4,





Plates 3 and 4:

PrP^{Sc} deposition in the dorsal nucleus of the vagal nerve of the same BSE-positive cow as in Plates 1 and 2. Magnification of Plate 3 100X, magnification of Plate 4 400X.

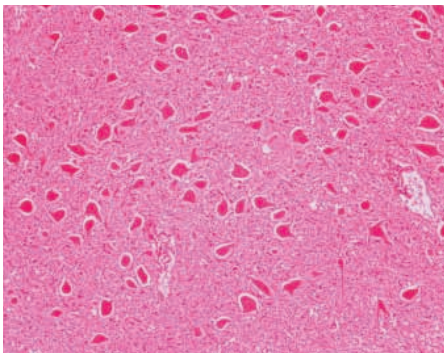


Plate 5:

Very few spongiform changes in the dorsal nucleus of the vagal nerve of a BSE-positive cow; the clear spaces around the neurons are artefacts. Magnification 100X

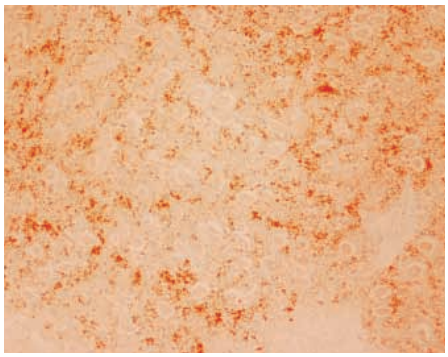


Plate 6:

Corresponding area to Plate 5, stained by IHC: clear deposition of PrP^{Sc} in the neuropil. Magnification 400X

IHC stains of brain sections of the same BSE-positive cow presented in Plates 1 and 2 clearly show accumulation of PrP^{Sc}, and confirm the diagnosis provisionally made with histopathology. Histopathologic examination of the brain section in Plate 5 might yield a questionable result, especially if the pathologist did not have much experience with BSE diagnostics. However, examination of the IHC preparation (Plate 6) from the same cow allows a definitive diagnosis of BSE to be made.

However, the antibodies used are unable to differentiate normal PrP protein (PrP^{C}) present in the brain cells from abnormal PrP^{Sc}. Therefore, the enzyme proteinase K must first be used to destroy the PrP^{C} differentially, while leaving the proteinase K resistant core of PrP^{Sc} unaffected. This ensures that any PrP detected will be PrP^{Sc}. This requirement is explained further in the rapid test section (section 4) of this chapter.

In addition, a step to demask the appropriate epitope of the proteinase K resistant core of PrP^{Sc} is required, otherwise the conformation of the protein prevents the antibody from binding. Demasking can be accomplished by denaturation of the protein or by using non-specific proteases.

In the IHC protocol used at the Swiss BSE Reference Laboratory, the antibody C15S is

used for the detection of PrP^{Sc}. The IHC protocol was optimized for this antibody, which is a polyclonal rabbit antiserum raised against a peptide of the bovine PrP sequence GQGGTHGQWNKPS. This sequence is located near the N-terminal of the PrP^{Sc} proteinase K resistant core. This antibody can be used for IHC as well as for ELISA tests, and has specificity is against bovine, feline and ovine proteins. Many other antibodies, mostly monoclonal, are now commercially available and can be obtained for use with various testing protocols.

For all IHC analyses, a positive and a negative control should be run together with the BSE samples to rule out any procedural errors. The control samples must be treated in exactly the same way as the actual samples. All analyses should be performed in duplicate.

4. RAPID BSE TESTS

Since 1997, tests have been developed to analyse BSE suspect materials rapidly. The EU and several individual countries have intensively validated these tests. Which rapid tests are licensed and approved in various countries throughout the world is variable. Tests approved in the EU (as of 14 June 2006) are given in Table 2.

All existing and licensed BSE rapid tests have several things in common. First, all tests use material from the brainstem, implying that these tests are post mortem tests. The samples must be taken from the obex region (described in section 2 of this chapter) in order to maximize sensitivity of the tests. Second, all tests are currently based on the same principles of homogenization, proteinase K digestion (with the exception of the IDEXX HerdChek BSE Antigen EIA), and detection. Although the principles of these steps are similar between tests, there are significant differences in the execution.

Performance

According to external evaluations (Moynagh and Schimmel, 1999; EU, 2006) the ten tests currently approved in the EU (Table 2) all have excellent sensitivity (100%) and specificity (100%), when IHC is taken as the reference (gold standard) method.

TABLE 2. BSE post mortem tests approved in the EU (as of June 2006)

Name	Year of approval	Producer	Principle
Prionics Check Western	2001	Prionics, Switzerland	Immunoblot
Bio-Rad TeSeE	2001	Bio-rad, France	Sandwich ELISA
Prionics Check LIA	2003	Prionics, Switzerland	Sandwich ELISA
InPro CDI-5	2003	InPro, San Francisco, USA	Conformation dependant immunoassay
CediTect BSE	2006	Cedi diagnostics, Netherlands	ELISA
IDEXX HerdChek BSE Antigen Test Kit	2006	IDEXX, Maine, USA	ELISA
Institut Pourquier Speed`it	2006	Institut Pourquier, Montpellier, France	Sandwich ELISA
Roboscreen Beta Prion BSE EIA	2006	Roboscreen Leipzig, Germany	Sandwich ELISA
Roche Applied Science Prion Screen	2006	Roche, Basel, Switzerland	Sandwich ELISA
Prionics Check PrioStrip	2006	Prionics, Switzerland	Lateral flow immunoassay

Special devices

Although the required materials are primarily included in the test kits, the presence of special devices and equipment in the laboratory is a prerequisite for testing for all tests. Not all tests require the same devices, and price differences among devices are considerable.

Availability of single components

All tests are primarily supplied as kits, with the required materials for conducting a certain number of tests. However, it is likely that not all materials will be used at the same rate, especially when only limited amounts of samples are analysed. The availability of single components is then an advantage and could allow a reduction in costs, though in some cases single components are not necessarily less expensive than the whole kit. The kits differ in the availability of single components.

High throughput

Laboratories that participate in a BSE surveillance programme will have to analyse relatively large amounts of samples. In this case, it is important that the test used has a high throughput potential. This potential can, for example, be increased by automating as many steps as possible in the test procedure, as each step requiring manual handling reduces the throughput potential.

Low throughput

In contrast to BSE surveillance laboratories, BSE reference laboratories have a relatively low throughput of samples. Then it is important that the test used is also suitable for a small amount of samples. The components (e.g. buffer, antibodies) should be available or should be able to be prepared in small amounts.

Time

Although all tests discussed here are rapid tests, the time needed for analysis differs among them. The shorter the time, the faster results can be reported to the customer. This is especially important when normal slaughter animals are being tested, as carcasses are often only released from the slaughterhouse after test results are negative.

Handling

In general, tests that have fewer handling steps are easier to perform and have lower risk of human error. Automation of the test steps reduces the amount of human handling. However, the type of handling is important, as some handling steps are more complicated than others.

Interpretation

The last step of the test procedure is the interpretation of the results. Computer printouts with values designated as over or below a stated cutoff value are easy to interpret. The interpretation of a western blot (WB) result needs more experience.

Conclusions

All tests currently approved in the EU are either based on WB or ELISA technology. Although there are differences between the tests, the overall performance is compara-

ble. Great differences can be found in the handling and the versatility for high and low throughput set-ups.

Procedure after positive test results

The procedure for handling test positive results differs between the EU and Switzerland. Within the EU, initially reactive samples can be retested in duplicate using the same test starting from the homogenate. The test cannot be repeated starting from the original brain material, since this has already been processed into homogenate. If at least one of the two duplicates has a value higher than the sample cutoff, the sample is considered to be positive and the sample will be sent to the national reference laboratory for confirmation.

In Switzerland, the initial reactive samples are not retested. The initial reactive samples are sent to the National Reference Laboratory, where confirmatory tests are performed.

New developments

Work is constantly being done on the development of new rapid tests both by companies that already provide rapid tests and by new companies. New tests can be based on the refinement of an established procedure or on the replacement of procedures by completely new concepts.

All these new tests are still based on post mortem sampling as they use brain material from the obex region. Of course, the ability to diagnose BSE ante mortem would be a huge advantage, and much research is being done in this field. Reports on possible ante mortem tests are published regularly. However, none of these tests have so far passed the validation process, and an imminent breakthrough in ante mortem testing is not foreseen.

5. REFERENCES

EC, 2006. BSE diagnostic opinions, Scientific advice. http://europa.eu.int/comm/food/food/biosafety/bse/sci_advice_en.htm .

Moynagh J, Schimmel H. 1999. Tests for BSE evaluated. Bovine spongiform encephalopathy. *Nature* 400(6740),105.

APPENDIX 1

Authors and course contributors

AUTHORS AND COURSE CONTRIBUTORS

Catherine Botteron	NeuroCenter, University of Berne, Switzerland
Marcus Doherr	VetSuisse Faculty, University of Berne, Switzerland
Anou Dreyfus	FAO, Animal Production and Health Division, Rome, Italy
Christine Friedli	SAFOSO, Berne, Switzerland
Dagmar Heim	Swiss Federal Veterinary Office, Berne, Switzerland
Ulrich Kihm	SAFOSO, Berne, Switzerland
Elizabeth Mumford	SAFOSO, Berne, Switzerland
Francesco Proscia	FAO, Animal Production and Health Division, Rome, Italy
Manon Schuppers	SAFOSO, Berne, Switzerland
Torsten Seuberlich	NeuroCenter, University of Berne, Switzerland
Andrew Speedy	FAO, Animal Production and Health Division, Rome, Italy
Marc Vandevelde	NeuroCenter, University of Berne, Switzerland
Andreas Zurbriggen	NeuroCenter, University of Berne, Switzerland

Participants from the partner countries have also contributed significantly to the production and translation of the course manuals, and to many other aspects of the courses.

APPENDIX 2

Related background reading and Web links*

* These references and Web links refer to all four *Capacity Building for Surveillance and Prevention of BSE and Other Zoonotic Diseases* project course manuals. Therefore, all documents and links may not be applicable to the topics covered in this manual.

RELATED BACKGROUND READING AND WEB LINKS

TSE pages of selected ministries and other general data sources

Department of Environment Food and Rural Affairs. United Kingdom, BSE homepage: http://www.defra.gov.uk/animalh/bse/index.html

FAO. BSE pages: http://www.fao.org/ag/AGAinfo/subjects/en/health/bse/default.html

Ministry of Agriculture of New Zealand. BSE homepage: http://www.biosecurity.govt.nz/node/7650

Swiss Federal Veterinary Office. BSE homepage: http://www.bvet.admin.ch/gesundheit_tiere/01752/01804/02075/index.html?lang=de

TAFS. Position papers: http://www.tseandfoodsafety.org/startseite.htm

United States Department of Agriculture. Animal and Plant Health Inspection Service, BSE homepage: http://www.aphis.usda.gov/lpa/issues/bse/bse.html

WHO. BSE pages: http://www.who.int/zoonoses/diseases/bse/en/

International standards

OIE. Bovine spongiform encephalopathy. *Terrestrial Animal Health Code*, Chapter 2.3.13. http://www.oie.int/eng/normes/MCode/en_chapitre_2.3.13.htm

OIE. Factors to consider in conducting the bovine spongiform encephalopathy risk assessment recommended in chapter 2.3.13. *Terrestrial Animal Health Code*, Appendix 3.8.5. http://www.oie.int/eng/normes/MCode/en_chapitre_3.8.5.htm

OIE. Surveillance for bovine spongiform encephalopathy. *Terrestrial Animal Health Code,* Appendix 3.8.4. http://www.oie.int/eng/normes/MCode/en_chapitre_3.8.4.htm

OIE. Procedures for the reduction of infectivity of transmissible spongiform encephalopathy agents. *Terrestrial Animal Health Code,* Appendix 3.6.3. http://www.oie.int/eng/normes/MCode/en_chapitre_3.6.3.htm

OIE. 1994. Agreement on Sanitary and Phytosanitary Measures. *Final Act of the Uruguay Round,* Article 5. http://www.wto.org/english/docs_e/legal_e/15-sps.pdf

BSE cases and risk

EC. BSE testing results of member countries of the EU. http://europa.eu.int/comm/food/food/biosafety/bse/mthly_reps_en.htm

OIE. Number of reported cases of BSE worldwide. http://www.oie.int/eng/info/en_esbmonde.htm

OIE. Resolution No. XXVII, Recognition of the bovine spongiform encephalopathy status of member countries http://www.oie.int/eng/info/en_statesb.htm#List

SSC. 2002. Opinion on TSE infectivity distribution in ruminant tissues (state of knowledge, December 2001). Adopted by the Scientific Steering Committee at its meeting of 10-11 January 2002. http://europa.eu.int/comm/food/fs/sc/ssc/out241_en.pdf

SSC. Opinions of the Scientific Steering Committee of the EC. http://europa.eu.int/comm/food/fs/sc/ssc/outcome_en.html

Measures

EU. 2002. Regulation No 1774/2002. Laying down health rules concerning animal by-products not intended for human consumption. http://europa.eu.int/eur-lex/pri/en/oj/dat/2002/l_273/l_27320021010en00010095.pdf

European Union Guidance Document for Regulation 1774/2002. http://europa.eu.int/comm/food/fs/bse/bse48_en.pdf

FAO. 2004. *Good practices for the meat industry.* FAO Animal Production and Health Manual No. 2. Rome (also available at: ftp://ftp.fao.org/docrep/fao/007/y5454e/y5454e00.pdf).

FAO. 2004. **Protein sources for the animal feed industry.** Proceedings of the FAO Expert Consultation and Workshop, Bangkok, 29 April-3 May 2002. FAO Animal Production and Health Proceedings No. 1. Rome (also available at http://www.fao.org/documents/show_cdr.asp?url_)file=/docrep/007/y5019e/y5019e00.htm

FAO. 2007. *Management of transmissible spongiform encephalopathies in livestock feeds and feeding*. Course manual, Project *Capacity Building for Surveillance and Prevention of BSE and Other Zoonotic Diseases*. Rome

FAO. 2007. *Management of transmissible spongiform encephalopathies in meat production.* Course manual, Project *Capacity Building for Surveillance and Prevention of BSE and Other Zoonotic Diseases*. Rome

Heim D, Kihm U. 2003. Risk management of transmissible spongiform encephalopathies in Europe. *Rev Sci tech Off int Epiz* 22(1), 179-199

Heim D, Mumford E. 2005. The future of BSE from the global perspective. *Meat Science* 70: 555-562

Heim D, Murray N. 2004. Possibilities to manage the BSE epidemic: cohort culling versus herd culling – experiences in Switzerland. In: *Prions: a challenge for science, medicine and the public health system,* 2nd ed. Eds HF Rabaneau, J Cinatl, HW Doerr. Karger, Basel, Switzerland. pp 186-192

OIE. 2005. Diseases notifiable to the OIE. http://www.oie.int/eng/maladies/en_classification.htm

Render – The National Magazine of Rendering. 2004. *Rendering 101: Raw material, rendering process, and animal by-products.* http://www.rendermagazine.com/August2004/Rendering101.pdf

The BSE Inquiry. 2000. *The report. The inquiry into BSE and variant CJD in the United Kingdom,* Volume 13: Industry processes and controls, Chapter 6, Rendering. http://www.bseinquiry.gov.uk/report/volume13/chapter6.htm

Diagnostics

EFSA. 2006. EFSA Scientific reports on the evaluation of BSE/TSE tests. http://www.efsa.eu.int/science/tse_assessments/bse_tse/catindex_de.html

OIE. 2005. Bovine spongiform encephalopathy. *Manual of diagnostic tests and vaccines for terrestrial animals*, Chapter 2.3.13. http://www.oie.int/eng/normes/mmanual/A_00064.htm

Safar JG, Scott M, Monaghan J, Deering C, Didorenko S, Vergara J, Ball H, Legname G, Leclerc E, Solforosi L, Serban H, Groth D, Burton DR, Prusiner SB, Williamson RA. 2002. Measuring prions causing bovine spongiform encephalopathy or chronic wasting disease by immunoassays and transgenic mice. *Nat Biotechnol* 20(11): 1147-1150.

Surveillance

Cameron AR, Baldock FC. 1998. Two-stage sampling in surveys to substantiate freedom from disease. *Prev Vet Med* 34: 19-30

Salman MD. 2003. Animal Disease Surveillance and Survey Systems. Methods and Applications. Iowa State Press, Ames, Iowa, USA

Scheaffer RL, Mendenhall W, Ott L. 1990. Elementary Survey Sampling. Duxbury Press, Belmont CA.

Clinical examination

Braun U, Kihm U, Pusterla N, Schönmann M. 1997. Clinical examination of cattle with suspected bovine spongiform encephalopathy (BSE). *Schweiz Arch Tierheilk* 139: 35-41 (also available at: http://www.bse.unizh.ch/english/examination/htmlsklinischer.htm)

Human prion diseases

Department of Health, United Kingdom. CJD-homepage: http://www.dh.gov.uk/PolicyAndGuidance/HealthAndSocialCareTopics/CJD/fs/en

APPENDIX 3

Glossary of technical terms and acronyms*

* This glossary refers to all four *Capacity Building for Surveillance and Prevention of BSE and Other Zoonotic Diseases* project course manuals. Therefore, all documents and links may not be applicable to the topics covered in this manual.

GLOSSARY OF TECHNICAL TERMS AND ACRONYMS

AAFCO	Association of American Feed Control Officials
Ab	Antibody
AFIA	American Feed Industry Association
Animal by-products	Tissues and other materials (including fallen stock) discarded at the slaughterhouse, which generally go to incineration, burial or rendering (depending on the country)
Animal waste	Animal by-products
Ante mortem	Before death (generally refers to the period immediately before slaughter)
AP	Apparent prevalence
BAB	Born after the ban; animals with BSE that were born after implementation of a feed ban
BARB	Born after the real ban; animals with BSE that were born after implementation of a comprehensive and effectively-enforced feed ban
BSC	Biosafety cabinet
BSE	Bovine spongiform encephalopathy
BL	Biosafety level
By-pass proteins	Proteins that are not degraded in the rumen but are digested in the small intestine to provide additional amino acids
CCP	Critical Control Point: a step in a production chain that is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level and at which a control can be applied
CEN	Europan Committee for Standardization
CJD	Creutzfeldt-Jakob Disease
CNS	Central nervous system
Combinable crops	Those able to be harvested with a combine
Contaminants	Materials that should not be present in a given product; e.g. rodents, birds, rodent droppings, toxins and mould are contaminants that should not be present in any livestock feed
Control (noun)	The state wherein correct procedures are being followed and criteria are being met (HACCP context)
Control (verb)	To take all necessary actions to ensure and maintain compliance with criteria established in a HACCP (or other control) plan (HACCP context)

Core fragment	The part of PrP^{Sc} that is not digested by proteinase K (also called PrP^{Res})
Critical limit	A criterion that separates acceptability from unacceptability (e.g. during audits)
Cross contaminants	Substances carried from areas or materials where they are not prohibited to areas or materials where they are prohibited
Cross feeding	The feeding of a livestock group with prohibited feeds intended for another livestock group
CP	Crude protein
CWD	Chronic wasting disease.
DNA	Deoxyribonucleic acid; the genetic material for all living organisms except bacteria
Downer cattle	Cattle too sick to walk to slaughter (definition differs among countries)
EC	European Commission
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
Emergency slaughter	Slaughter cattle with clinical signs non-specific for BSE (definition differs among countries)
Epitope	Structural part of an antigen that reacts with antibodies
Epitope demasking	Process in which the epitope becomes available for antibody binding (for example, by denaturation)
Essential amino acids	Those that cannot be synthesized and therefore must be provided by the feed/food
EU	European Union
Fallen stock	Cattle that died or were killed for unknown reasons (definition differs among countries)
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration (United States of America)
FEFAC	European Feed Manufacturers' Federation
FIFO	First in first out; a production concept to optimize quality
Flushing batches	Batches of feed processed or transported in-between feed batches containing prohibited and non-prohibited materials, and intended to remove traces of prohibited materials from the equipment
FMD	Foot-and-mouth disease
FN	False negatives; truly-diseased animals that test negative on a diagnostic test
FP	False positives; truly non diseased animals that test positive on a diagnostic test
FSE	Feline spongiform encephalopathy; TSE in cats, believed to be caused by ingestion of the BSE agent.

GAFTA	Grain and Feed Trade Association
GAP	Good agricultural practices
GBR	Geographical BSE risk assessment
GHP	Good hygiene practices
GMP	Good Manufacturing Practices
GMT	Good microbiological technique
Greaves	A proteinaceous by-product of the rendering process
GTM	GAFTA Traders Manual
H & E	Haematoxylin and eosin stain
HACCP	Hazard Analysis and Critical Control Points: a method to identify process steps where a loss or significant deviance from the required product quality and safety could occur if no targeted control is applied
HACCP plan	A document prepared in accordance with the principles of HACCP to ensure control of hazards that are significant for the segment of the production under consideration
Hazard	A biological, chemical or physical agent with the potential to cause an adverse health effect
Hazard analysis	The process of collecting and evaluating information on hazards and conditions leading to their presence to decide which are significant for the segment of the production under consideration and therefore which should be addressed in the control (or HACCP) plan
High quality protein	Protein sources that match the requirements of a particular species or production class well
HPLC	High performance liquid chromatography
IAG	European Feed Microscopists working group
IFIF	International Feed Industry Federation
IHC	Immunohistochemistry
Indigenous BSE case	Domestic BSE case; non-imported BSE case
M+C	Methionine plus cysteine; amino acids generally considered together, because cysteine can be derived from methionine in animals
ISO	International Organization for Standardization
Mammal	An animal that lactates; in this context, livestock excluding aquatic species and poultry
MBM	Meat and bone meal; the solid protein product of the rendering process
Medulla oblongata	Caudal portion of the brainstem
MMBM	Mammalian meat and bone meal
Monitoring	An ongoing process of specific animal health data collection over a defined period of time

Monogastric species	Animals with simple stomachs (e.g. swine, poultry, horses, humans)
MOSS	Monitoring and surveillance system
MRM	Mechanically recovered meat
NIRC	Near infrared camera
NIRM	Near infrared microscopy
NIRS	Near infrared spectrography
Notifiable disease	A disease for which there is a national legal requirement to report cases and suspects to an official authority
Obex	The point on the midline of the dorsal surface of the medulla oblongata that marks the caudal angle of the fourth brain ventricle; a marker for the region of the brain stem where some of the predilection areas for histological lesions and PrP^{Sc} deposition in BSE are located (such as the dorsal nucleus of the vagus)
OD	Optical density
OIE	World Organization for Animal Health
OR	Odds ratio
Pathogenicity	Ability of an organism to invade a host organism and to cause disease
PCR	Polymerase chain reaction
Pithing	The laceration of central nervous tissue by means of an elongated rod-shaped instrument introduced into the cranial cavity of slaughter cattle after stunning.
PK	Proteinase K; a serine proteinase that digests PrP^{C} completely but PrP^{Sc} only partially under certain conditions
Post mortem	After death
Prion	Infectious agent causing TSE
Proteolysis	Cleavage of a protein by proteases; also referred to as "digestion"
PrP	Prion protein, encoded by the gene *PRNP*, expressed by many cell types and many organisms
PrP^{BSE}	Resistant prion protein associated with bovine spongiform encephalopathy; also called PrP^{Sc}
PrP^{C}	Normal prion protein found in eukaryotic cells
PrP^{Res}	Resistant prion protein core remaining after proteolysis of PrP^{Sc} using proteinase K
PrP^{Sc}	Resistant prion protein associated with transmissible spongiform encephalopathies, including BSE
PrP^{Sens}	Normal prion protein found in eukaryotic cells; also called PrP^{C}
PV	Predictive value

Rapid test	Test systems using immunological assays that detect the presence of infectious agents in animal tissues or other materials within hours
RR	Relative risk
Ruminant species	Animals with multichambered stomachs that allow bacterial fermentation of feeds prior to intestinal digestion (e.g. cattle, sheep, goats, camellids)
Scrapie	A TSE of sheep and goats
SE	Sensitivity of a diagnostic test
Segregation	Undesirable separation of raw ingredients in a compound feed after processing
SFT	Swiss Institute of Feed Technology
Sick slaughter	Cattle with non-specific signs (definition differs among countries)
SP	Specificity of a diagnostic test
SPS Agreement	Agreement on the Application of Sanitary and Phytosanitary Measures
SRM	Specified risk materials; those animal tissues most likely to contain TSE infective material
SSC	Scientific Steering Committee of the European Commission
Strip test	Lateral flow immunochromatographic test for rapid detection of proteins in feed samples
Surveillance	Extension of monitoring in which control or eradication action is taken once a predefined level of the health-related event has been reached
TAFS	International Forum for TSE and Food Safety
TBT Agreement	Agreement on Technical Barriers to Trade
Terrestrial animal	In this context all livestock excluding aquatic species (e.g. poultry, ruminants, pigs, horses)
TME	Transmissible mink encephalopathy
TP	True prevalence
Tracing	Determining where an animal or product originated or has been
Tracking	Following an animal or product forward through the system
TSE	Transmissible spongiform encephalopathy
UK	United Kingdom of Great Britain and Northern Ireland
USA	United States of America
vCJD	Variant (or new variant) Creutzfeldt-Jakob disease of humans; believed to be caused by ingestion of the BSE agent

APPENDIX 4

Project summary

PROJECT SUMMARY

This course is a part of the project *Capacity Building for Surveillance and Prevention of BSE and Other Zoonotic Diseases*. The aim of the project is to build capacity, establish preventive measures and analyse risks for bovine spongiform encephalopathy (BSE), so that, ultimately, partner countries are able either to prove themselves to be BSE-free or are able to decrease their BSE risk to an acceptable level. Governmental and private veterinary services, diagnostic laboratories, and the livestock, food and animal feed industries will be strengthened and supported, and technical capacity built at every step along the food production chain. In the future, the knowledge gained during this project could be used by the countries to establish similar programmes for control of other zoonotic food-borne pathogens.

The project is funded by Swiss governmental agencies and utilizes expertise available in Switzerland and worldwide and infrastructure available from the Food and Agriculture Organization of the United Nations (FAO) to assist the governments of the partner countries to achieve the project's aim. The executing agency is Safe Food Solutions Inc. (SAFOSO) of Berne, Switzerland.

The direct project partner in each country is the National Veterinary Office. The countries commit and pay a salary to at least one individual, situated in the National Veterinary Office, to act as a National Project Coordinator (NPC), commit three trainees per course and provide the necessary infrastructure for implementation of the project in the country. The NPC is responsible for coordinating the activities of the project within the country, including offering training courses, identifying and organizing trainees, and promoting communication between the project, the government, the scientific community in the country, the livestock and food industries, and the public. Other commitments by the countries include providing paid leave time for employees to attend courses, providing infrastructure and facilities for in-country courses, providing historical and current data (surveillance data, animal movement data, import/export records) and the staff required to identify those data, and providing adequate staff for and facilitating the initial needs assessment and final comprehensive risk assessment.

A National Project Board in each of the participating countries regularly evaluates the operational progress and needs of the project, and provides a regular venue for communication among the project team, national partners and stakeholders. This Board is comprised of the NPC, representatives of the national government, a project representative, the local FAO representative, and local stakeholders from private industry and the veterinary community.

ACTIVITIES OF THE PROJECT

1. The specific needs of each participating country are assessed.
2. Comprehensive courses to "train the trainers" are provided in Switzerland (or elsewhere) to selected participants to improve understanding of the epidemiology of and relevant risk factors for BSE and to develop specific knowledge and skills for implementing appropriate controls.

Three trainees from each country, as well as the NPC, travel to Switzerland (or elsewhere) to participate in each course.

The courses are:

- Diagnostic Techniques for Transmissible Spongiform Encephalopathies
- Epidemiology, Surveillance and Risk Assessment for Transmissible Spongiform Encephalopathies
- Management of Transmissible Spongiform Encephalopathies in Livestock Feeds and Feeding
- Management of transmissible spongiform encephalopathies in meat production

Each course is preceded by an introduction to BSE covering the background of transmissible spongiform encephalopathies, BSE, biosafety, general concepts of epidemiology and risk assessment, and risk communication. Each course also includes discussion of aspects of risk communication that are relevant to the topic being presented.

Only those motivated individuals who will be implementing the relevant information into the national BSE programme, who have some experience (e.g. ability to use a microscope, veterinary training) and have adequate English skills, are accepted.

After each course, the relative success of the course is evaluated focusing on the success of the training methods and effectiveness of the knowledge transfer rather than on the learning of the individual trainees. Therefore, no written test is given, but close contact is maintained with the trainees after they return to their countries, and their progress and success in implementation of their training into the national BSE programme is followed and evaluated in the field.

3. Each of the TSE-specific courses is then offered as an in-country course in the native language, and is organized by the trainees and the National Veterinary Offices with technical support from the project. In-country courses use the same curriculum and expected outcomes as the original courses, and are provided with support, technical assistance and materials (translated into their own language). The introductory TSE and biosafety course curriculum is also presented. At least one expert trainer assists in presenting these courses. Participants are chosen according to strict selection criteria, but the number of participants and the frequency and location of courses given depends on the needs of the country and the type of course.
4. The knowledge gained through the courses should then be integrated by the partner country through development and implementation of a national BSE control programme. The programme is promoted and supported by the countries to ensure the sustainability of the system. Contact, technical support and follow-up with the countries is ongoing throughout the project.
5. Information campaigns to improve BSE awareness are targeted to national governments, producers and consumers.
6. Partner countries are supported in the submission of a comprehensive national BSE risk assessment to the World Organisation for Animal Health (OIE) in order to document their BSE status to the international community.

To support countries with economies in transition and developing countries in the control and prevention of bovine spongiform encephalopathy (BSE), the project Capacity Building for Surveillance and Prevention of BSE and Other Zoonotic Diseases, involves collaboration between FAO, SAFOSO and National Veterinary Offices in partner countries, and is funded by the Government of Switzerland. The aim of the project is to build capacity, establish preventive measures and analyse risks for BSE. Partner countries are thus enabled to decrease their BSE risk to an acceptable level or demonstrate that their risk is negligible, and thereby facilitate regional and international trade under the SPS agreement of the WTO. The project includes comprehensive training courses to improve understanding of the epidemiology of and relevant risk factors for BSE and TSE and to develop specific knowledge and skills for implementing appropriate controls.

This manual is a supplement to the training course on Diagnostic Techniques for Transmissible Spongiform Encephalopathies and it is targeted at veterinary diagnosticians who will contribute to the development and implementation of the national BSE surveillance and control programme, and to the BSE risk assessment for the partner countries.