

QUALITY ASSURANCE IN FEED-PRODUCING PLANTS

1. GENERAL CONCEPTS

To assure consistent product quality and safety, feed producers must have standard protocols in place for self-regulation of all of the processes in their feed-producing plant (feed plant). This includes written procedures, maintenance of records, and periodic audits. Quality assurance protocols are not only important for the implementation of TSE control measures, but also for measures to control or prevent other diseases and feed-related animal health problems.

In addition to self-regulation, most national or local governments have auditing or inspection regulations for feed plants, including a standard protocol for official sampling of feeds for testing. Audits should also follow standardized written protocols and adequate records should be kept.

A basic concept is that all contaminants (non-desired products and substances, including prohibited or restricted products or substances) are excluded from all feed products at each processing step. In the context of TSE control, these contaminants and prohibited materials or products include some ingredients of animal origin and feeds or feed components containing these ingredients. The exact definition of what materials are considered contaminants (and prohibited) depends on the feed ban in place, but generally includes MBM or other meals derived from ruminants or mammals. The definition also depends on the species of animals for which the feed is intended (e.g. pets, ruminants, poultry, non-ruminants, aquatic species).

Because these materials may not be considered contaminants (and prohibited) outside the context of TSE control, and/or may be allowed for certain groups of animals, they may be legally present in feed plants. Therefore, the term “cross contamination” is often used for these prohibited materials, while ‘contamination’ is used for materials that should not be in any feed materials (e.g. rodents, birds, rodent droppings, toxins, mould).

In the feed plant, separation of feeds must be assured and cross contamination must be prevented in areas and on equipment at each processing step, according to the specific feed ban in place and other national regulations. Major steps where contamination and cross contamination may occur include:

- Unloading of the raw materials
- Storage of the raw materials
- Transport of the raw materials
- Conveying
- Cleaning
- Drying
- Milling
- Mixing
- Pelleting
- Bagging
- Transport between each step
- Transport of the final product

- Loading and unloading of the final product
- Storage at and between each step and storage of the final product, including storage in silos and other containers at feed plants and on the farm
- Feeding equipment including buckets, bags, and feed troughs on the farm

2. IMPORTANT POINTS FOR QUALITY ASSURANCE

In the following sections of this chapter, important points are outlined for quality control in feed mills and plants producing premixes and mixed feed according to the protocols of the Swiss feed control authorities (ALP, 2004). The implementation of feed bans and the manufacturing process itself are described in separate chapters in this course manual.

2.1. Working areas and production equipment

In general, working areas and production equipment should be constructed, arranged, operated, and maintained so that:

- usability is optimized and the risk of failures is minimized;
- they remain clean and dry;
- thorough cleaning and the elimination of foreign bodies is possible;
- adequate access is available for maintenance;
- contamination, cross contamination, and other quality-reducing effects are minimized;
- the exclusion of rodents and other pests is optimized;
- the maintenance and cleaning materials used are appropriate for feed production (i.e. non-toxic).

Moreover, the production equipment should be designed in such a way, that the first in first out (FIFO) principle is maximized for all materials (e.g. raw materials, single component feeds, mixed feeds, premixes, feed additives) in order to optimize quality.

Working areas and production equipment should be exclusively dedicated to the production of feed, and clearly separated from other production activities. When plants produce multiple categories of feeds, including feeds that contain substances that are prohibited in other categories of feeds, the production processing lines for the different feed categories should be completely separated.

The potential high-risk areas for cross contamination are specific to individual plants and equipment types, and therefore must be identified separately for each plant. The likelihood of contamination and cross contamination increases if all aspects of the production process are not optimized, including arrangement, material flow and equipment maintenance.

The production process for premixes and mixed feeds should be described in a flow chart. The flow chart should show that the production equipment used for production of feeds containing prohibited materials is separated from that used for production of other categories of feed, depending on the feed ban in place, as described in the "Overview: Implementation of TSE measures" chapter in this course manual. In this context, the production processes are divided in the following general steps:

- delivery/cleaning
- storage of the additives and starting materials
- grinding
- dosage/weighing
- mixing/homogenizing

- pressing
- storage
- shipment

Additional plant-specific procedures (e.g. heat treatment) should be integrated where appropriate.

In order to reduce the risk of contamination and cross contamination, protocols should be implemented to assure that all working areas and equipment that directly effect product quality are regularly cleaned and professionally maintained. The protocols should define frequency, method and the responsible person(s). Protocols to prevent the introduction of pathogenic organisms should also be implemented. Protocols should also describe implementation of appropriate pathogen eradication measures.

A preventive pest eradication protocol, including responsibilities and specific tasks, should be defined and implemented. The entire manufacturing facility, including all storage areas, should be included in the plan. If necessary, the administrative and non-production areas should also be included. When active eradication of pests is required, the treatment date, pest type, treatment area, treated materials, eradication materials used, quantity of the eradication materials used, waiting period, and signature of the responsible person should be recorded.

Working areas and production equipment that are of critical importance for product quality should be subjected to appropriate and regular inspection. Inspection should be in accordance with a standard protocol provided by the manufacturer or an independent qualified person should conduct the inspection.

2.2. Personnel

The manufacturer must employ sufficient personnel with adequate knowledge and experience for the relevant production process.

The management person responsible for the production must fulfil the required job specifications (see section 2.3 of this chapter). A proxy person must be designated to be responsible if and when the responsible person is unavailable. The proxy person may either be recruited from the personnel of the same sector or be external to that sector.

An organization chart and staff appointment protocol must be developed that indicate the qualifications and responsibilities of management personnel. The chart must be presented to the competent authorities mandated with inspections. All management personnel must be informed in writing about required tasks, responsibilities and competences, especially with every employment change. In addition, management personnel must be provided with a description of professional and disciplinary authorities for their respective branch of production.

Position descriptions must also be developed for each other position, and must include the professional training and required skills, assigned tasks, the specific areas of responsibility (e.g. to stop production or shipments, to allow usage or withdrawal/recall of goods, to release/clear goods for shipment), and a designated proxy person.

2.3. Production process

A qualified person must be identified to be responsible for production. This person must be certified in the specific production area and have sufficient knowledge of animal feed legislation, process engineering, and animal nutrition and should meet the following qualifications:

- has successfully completed an education as agronomist or animal feed specialist, or has another equivalent education;
- understands what constitutes appropriate working area and production equipment, and understands their constructional, technical, and hygienic maintenance and operation;
- understands the current production flow chart and operation protocols from delivery through shipment;
- understands when to use authority to stop and/or block production (and must have this authority);
- is familiar with the critical points of the facility and of the production process and is able to control these points using appropriate measures;
- is familiar with the contents and requirements of a quality control system and is able to implement such a system in the responsible plant;
- is able to design documentation in such a way that complete traceability is possible;
- knows when to authorize a recall (and must have this authority) and what aspects should be included;
- has knowledge of the legislative guidelines or has access to a competent third person in case of questions;
- has sound knowledge of animal feed science and of animal nutrition or has access to a competent third person in case of questions;
- is aware of his/her responsibilities and is able to transfer information to all involved personnel in order that they understand the specific production requirements.

The manufacturer must guarantee that the various production processes are conducted in accordance with the written protocols and flow charts. These protocols allow the critical points¹ of the production process to be defined, audited and controlled. All technical and organizational possibilities must be optimized to avoid contamination, cross contamination and other errors.

A critical point can be defined as a step in the production process that, if no targeted control is applied, could lead to a loss or significant deviance from the required target state of product quality and safety. Critical points in the production process can be determined using:

- the HACCP² method;
- the plant specific flow chart;
- an analysis of possible places for contamination or cross contamination;
- the required examinations concerning accommodation and production equipment;
- plant-specific protocols for the single production processes;
- plant-specific technical and organizational preventive measures to prevent contamination, cross contaminations and errors.

¹ The term "critical point" in the production process is used in the context of HACCP methodology, which is accepted as an analysis method for food safety in the food processing industry.

² HACCP is described in the "Quality control concepts, hygiene, and HACCP in the meat industry" chapter in the *Capacity Building for Surveillance and Prevention of BSE and Other Zoonotic Diseases* project course manual *Management of transmissible spongiform encephalopathies in meat production* (FAO, 2007).

Critical points generally include addition of ingredients to the premixes, addition of premix to the feed, the weighing scale, the measuring devices, the mixer and the chronological order of the production steps. Often, other points are also included.

A thorough analysis of the potential for contamination, cross contamination and sources of other errors from delivery through shipment must be made. Moreover, component residues must be quantified step-by-step over the entire production process (using estimates or true observations). Single production steps cannot be summarized by addition, because important information regarding the location of component residues will be lost and because the potential individual causes must be understood in order to implement controls. Using a step-by-step analysis it will become evident which technical and organizational plant-specific preventive measures must be taken and documented.

2.4. Quality control

A qualified person (or persons) must be identified who is responsible for quality control. This person must at least meet the job specifications required for the person responsible for production (see section 2.3 of this chapter). Additionally, this person is responsible for the development of a written quality control plan and for its implementation.

There must be a control laboratory at the disposal of the manufacturer, and that laboratory must be sufficiently equipped with staff and equipment to be able to guarantee and verify the consistency of the premixes and the mixed feeds containing premixes with specifications previously defined by the manufacturer. This laboratory may be internal or external. Specifications that must be guaranteed and verified in the laboratory include:

- type, concentration and homogeneity of feed additives;
- type, concentration and homogeneity of all substances (including unwanted substances) in the mixed feed;
- maximum allowed concentrations of unwanted substances.

Both internal laboratories that perform analyses for third parties and external laboratories that are appointed for the quality controls must be accredited and work according to validated methods. Internal laboratories working exclusively for the plant are not required to be accredited but must also work according to validated methods. The method by which analysis results are verified must be recorded.

A written quality control plan must be developed and implemented that includes, in particular, the critical points of the production process, the procedure for sampling (see section 5 of this chapter) and sampling frequency, the methods and frequencies of the analyses, and the validation of the specifications of ingredients, as well as procedures in case of dispute regarding these specifications.

Criteria for determining the frequency with which single nutritional substances, feed additives and unwanted substances must be verified include:

- probability of exceeding or not reaching tolerance values;
- effect on animal production if tolerance values are exceeded or not reached;
- effect on animal health if tolerance values are exceeded or not reached;
- probability of residues in food products if tolerance values are exceeded or not reached.

Results and methods of analysis of all verified nutritional components, feed additives and unwanted substances must be recorded in writing.

All feed additives, premixes, raw materials and single component feeds, intermedi-

ate products and final products must be characterized using written specifications. Differentiations must be made between specifications that characterize quality (e.g. energy content) and specifications that characterize safety (e.g. content of unwanted substances). Specifications must be verified by the manufacturer (for premixes or mixed feeds) and/or by the supplier (for feed additives or premixes). When specifications are verified through certification or other documentation, the recipient may also require test results within an appropriate time interval.

Disposition must be described in case of non-conformity with the specifications. Deviances in quality and deviances in safety must be differentiated. In case of deviances in safety, the feed manufacturer must block the affected products. In case of deviances in quality, the feed manufacturer may decide what is done with the deficient products, and must at least record:

- first measures conducted after identification of the deviances;
- long-term corrective measures;
- verification of the efficacy of the conducted corrective measures.

In addition to samples collected for testing, samples from every batch of premixes and mixed feeds must be retained and stored in amounts defined by the manufacturer's protocols to enable trace back of each product. Samples from every identified production sector (continuous production) or from appropriate time intervals (exclusive production for own needs) must be collected. These samples must be sealed and labelled so as to be easily identifiable. Storage conditions must prevent abnormal changes to the sample's composition and other adverse effects, and samples must be available for the competent authorities for a certain specified amount of time, for example three months past expiry date.

The procedure for collecting and retaining these samples should be in accordance with these procedures for other samples (as described in section 5 of this chapter). Additionally, the following points must be considered:

- the samples must be at least 250 grams;
- every sample must have its original label and traceability must be guaranteed;
- the storage rooms must be dry, not exposed to heat, and safe from pests.

2.5. Storage

Materials should be stored according to a written plant specific storage concept, which should be made available to company personnel. Raw materials containing high levels of unwanted substances or materials that are destined for detoxification, as well as premixes and mixed feeds that comply with specifications, must be stored in appropriate containers or rooms that have been constructed and maintained in such a way that good storage conditions are guaranteed.

Storage conditions must not produce negative changes to quality (e.g. heating, generation of condensation) during storage. Measures to prevent generation of condensation are:

- storing sufficient amount of dry products;
- cooling and air circulation;
- circulating grains.
- avoiding direct sunlight and indirect heat (e.g. via exposed outside walls).

Contamination of stored products must be prevented by avoiding use of damaged containers or storage rooms, or of damaged lids, sliders and air circulation systems.

During cleaning and maintenance, all storage rooms and containers must be checked for damage, especially if storage rooms are also used for other purposes.

Storage rooms and containers must be closed whenever possible, and only persons authorized by the manufacturer should have access. This is especially applicable when products are stored in freely accessible areas on the plant grounds or in areas outside the plant grounds.

Measures must be taken to avoid access by rodents and other pests and, if necessary, appropriate eradication protocols must be implemented. Products must be stored in such a way that they are easy to identify and misidentification and/or cross contamination between different products is excluded.

The concept should describe how and where which products are stored to exclude misidentification or cross contamination between different products. If a plant manufactures and/or stores multiple categories of livestock feed or is otherwise storing animal materials prohibited for livestock, then those feeds or materials prohibited for certain categories of animals must be clearly identified and must be stored separately from non-prohibited feeds to reduce the risk of cross contamination. Clear identification of stored products means:

- For final products: identification of containers with appropriate labels according to instructions for declaration or unique identification of products and silos that allow full back tracing within the operating system of a feed plant.
- For raw materials, feed additives and premixes: labelling on bags and containers or plant specific codes, with bulk storage entries in specific silos or specifications on the overall silo operating system or plant specific codes.

3. DOCUMENTATION AND DATA

The manufacturer must have a documentation system at its disposal to define and control critical points in the production processes and to develop and implement the quality control plan. The manufacturer must maintain complete records of the appropriate audits and other controls. These records must be stored so that the history of each produced batch can be back traced and that the responsible person can be identified if complaints arise after distribution.

The manufacturer must document the correct implementation of all defined preventive measures. For this purpose a documentation protocol must be created which at minimum describes all specifications and records for complete traceability (see below), the person(s) responsible for the filing and archiving of documents, the place at which records are filed and archived and the duration of filing and duration of storage of records.

At minimum, the following protocols and records must be available:

- specific production flow chart and current process overview for the plant;
- analysis of possible carryover between individual batches;
- guidelines for cleaning and maintenance of the working area;
- guidelines for cleaning and maintenance of production equipment;
- preventive and active pest eradication plans;
- accreditation of external laboratory;
- accreditation of internal laboratory (and validation of any methods that are not included in the accreditation);
- methods for the verification of the results from analyses;
- sampling procedure and protocols;



- methods of analyses;
- frequency of the required analyses;
- specifications of the individual products produced in the feed plant (e.g. nutritional);
- disposition protocols for products that do not comply with the specifications;
- guidelines regarding sampling and storage of retained samples;
- guidelines regarding sampling and storage of stored products, including preventive measures to avoid generation of condensation;
- overview of required documentation;
- delivery receipts, invoices, batch records;
- checklist for recall action;
- organization of proxies;
- organization chart;
- job descriptions for management and other positions.

In order to assure the reliable tracking and tracing of products, the following data should be maintained within the manufacturer's documentation system.

For all feed components purchased: the type, manufacturer's batch number and production date, volume delivered, and delivery date of the product, as well as the name and address of each supplier, including the delivery receipt or invoice indicating this information.

For all materials produced: the type, original batch number, and volume of all components used and, for components not produced internally, the names and addresses of suppliers and all information listed for materials purchased (above), as well as the type, production date and volume produced.

For feed additives and premixes used in further production internally: the type and original batch number, the volume used and, for the resulting product, the type, production date, resulting batch number (including the internal batch record indicating this information) and, in case of continuous production, the point of introduction into the production line.

For feed additives, premixes, and mixed feeds sold: the type, batch number, volume, and delivery date of the product, as well as the name and address of the receiver of the product (trader or end user), including the delivery receipt or invoice indicating this information.

4. COMPLAINTS AND PRODUCT RECALL

Each legitimate and illegitimate negative comment from a customer must be considered a complaint and a cause must be sought for the problem with the product. The manufacturer must record and verify complaints systematically, including at minimum the following points:

- person filing the complaint;
- product that is the subject of the complaint (including batch number, production or delivery date), problem description, entry date of complaint and signature of the person who has accepted the complaint;
- immediate corrective measures or action taken;
- re-audit in which the effectiveness of the conducted corrective measures are verified;
- final audit.



The manufacturer must be able to implement a systematic evaluation quickly so that products can be recalled if indicated. Documents reviewed must include:

- production or batch records;
- delivery receipts and invoices;
- analysis reports and certificates;
- cleaning and maintenance records;
- records of proper storage of products;
- records of test equipment checks (e.g. verification of scales, temperature measures, humidity measures);
- standard recipes and handling protocols (date of use, date of withdrawal);
- product flow protocols for starting materials, feed additives and premixes.

If a product is recalled, it is important to be able to quickly and specifically identify affected customers. The method for identification of affected customers must be defined in writing, and a checklist developed. The checklist must also include how the recalled products will be processed. The manufacturer must maintain written records of the destination of recalled products. Before decisions can be made regarding future use or resale of these products, they must undergo a thorough quality check.

5. SAMPLE COLLECTION

The collection of appropriate samples of feed ingredients or finished compound feed is an important aspect of feed control, both for domestically produced and imported feeds. The individual samples collected may then be tested using different laboratory analyses, in order to:

- detect prohibited components, particularly those of animal origin;
- verify that ingredients, pesticides, drugs and medications are used properly;
- determine that the feed is of composition, quantity or quality as represented by the label;
- have banked samples available for trace back.

Sampling and testing may be carried out in the context of official feed control or within the quality assurance programme of a feed production plant, and results of the testing may serve as a basis or justification for defending against legal actions or proving liability. The verification of production or import documents must be done in parallel with the sample collection. When the sample collection is carried out by a feed inspector, he must assure that an employee of the plant is present during his visit.

The sampling protocol(s) for both feed plants and for governmental control programme must be available in a written form. The protocol must allow for sampling at regular intervals, and assure that the samples taken are homogeneous and representative of the entire batch. The protocol includes at least the following details:

- device with which the samples are taken;
- method of collection;
- timing of sample collection (moment in production);
- number of samples per batch;
- distribution of the samples;
- type of product for which the procedure is valid;
- frequency of collection;
- frequency of audits.

When sample collection is part of an overall feed plant inspection, document verifica-



tion starts with the shipping documents for the receipt of raw materials. Assuring proper ingredient identity and quality is important for its appropriate use in feed production. Already at this stage, sampling and testing can reveal commingling or cross contamination with prohibited components of animal origin during transport. Representative samples should be taken before unloading and retained for several months after the feed in which the ingredient is used is manufactured and delivered to the buyer (as part of the overall quality assurance programme). The process for sampling of raw materials and finished products is similar, and must include bagged, bulk, and liquid feed.

The amount of material to be collected each time for each purpose (e.g. import control, feed plant inspection) must be determined and written in the respective protocol. Generally, at least five to ten separate samples of each different ingredient and finished feed should be collected each time, to total between 500 to 1 000 grams of each material. Although it is often difficult, an effort should be made to obtain a sample that is representative of the entire batch of material. For example, to attempt to sample feeds from different time points during the production of the batch, bags should be selected for sampling that have been stored in slightly different locations, i.e. five bags should not be selected that are stacked right next to each other.

For bagged products, it must first be determined how many bags must be sampled (at approximately 100 grams sampled per bag) to achieve the final sample volume required by the protocol. Then, a standard slotter bag trier (sampling device) is inserted in the upper section of the bag (slot in the downward position). The slot of the sample probe must be larger than the largest particle of the feed being sampled. The sample is taken by rotating the trier until the slot is on the upper side of the trier, which is then removed.

For bulk feed, the sample collection is best carried out while loading or unloading, i.e. at railcars, trucks or trailers. With a stream cutter, a minimum of 10 cuts at equal intervals are taken to provide approximately one kg total sample. With stationary bulk feed (generally in a vehicle), only a limited access is possible. Depending on the accessible surface of the feed, a minimum of 10 probe samples are taken from different compartments.

For liquid ingredients, it is best to obtain samples at periodic intervals during unloading. It is advisable to discard the first several litres of material before sampling, to account for any solids or contaminants that may have collected in the tank.

The person responsible for sample collection must be careful to prevent any contamination during the process. Separate equipment must be used for each sample, samples sealed individually (e.g. using bag triers), and general hygiene rules (e.g. for clothing and handling of feed) must be respected during the process.

At the collection site, the samples may be assessed for their colour, texture, odour and moisture content. The presence of foreign objects may be assessed and the temperature determined for liquid fats and molasses. The detailed label of the feed ingredient/finished feed should be attached to each sample. Further analyses are generally performed in the laboratory.

6. SUMMARY OF TSE-RELEVANT CONCEPTS

- The overall quality assurance systems in feed plants, including separation of production lines, are important to minimize the risk for cross contamination with prohibited materials.

- The exact implementation requirements for separation of materials will be dependent on the specific feed bans in place.
- Sample collection for detection of prohibited material of animal origin is important for import control as well as for feed plant quality control. It must be done at regular intervals and include raw ingredients as well as finished products.

7. REFERENCES

ALP (Agroscope Liebefeld-Posieux). 2004. <http://www.alp.admin.ch/>

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

LABORATORY ANALYSIS FOR THE DETECTION OF PROHIBITED MATERIALS IN LIVESTOCK FEED

1. GENERAL CONCEPTS

Implementation of an effective feed ban is a crucial component of any national BSE control program. In order to effectively enforce any feed ban in place, representative samples from both imported and domestically produced feeds must be routinely collected and tested (Gizzi *et al.*, 2003). Sample collection is covered in the “Quality assurance in feed-producing plants” chapter in this course manual.

Unfortunately, it is not possible to directly detect prions in samples of feed and feed components. Therefore, laboratory methods must focus on detecting the presence (intentional or inadvertent) of prohibited materials, primarily MBM. Because of the differences in the scope of feed bans implemented in different countries, the test used must be appropriate to the specific result required.

For example, in various countries fish meal, poultry meal, and/or pure meals from non-ruminant animal species (e.g. equine, porcine) may still be allowed for feeding of pigs, fish and/or poultry, as well as ruminants in some cases. In other countries, all animal proteins are banned for all food-producing animals. Therefore, the most appropriate test for a particular country might be one that detects any animal material (including fish), any terrestrial animal protein (i.e. poultry and mammals), any mammalian material or any ruminant material. If all intra-species feeding is banned (as in the EU; EU, 2002)¹, the test will need to distinguish among material from different mammalian species. Moreover, the presence of animal material inadvertently included in feeds (such as rodents and birds) must be considered. Although this material is not considered to pose a TSE-related risk, its presence might affect the validity of the test result as well as indicate other problems with the quality and safety of the feed.

In addition, a quantitative test method may or may not be required depending on whether the feed ban in a country includes an allowable level of a prohibited material, or whether any detectable prohibited material is unacceptable.

Furthermore, different laboratory tests differ in their ability to distinguish material that has undergone processing. For example, the MBM produced by rendering at specific conditions of heat, pressure and time may no longer test positive for material of interest (usually protein or DNA) when certain tests are used. Therefore, both when choosing the testing method and when interpreting the test results, the processing conditions (or possible conditions) for imported and domestic MBM must be taken into account.

For example in the EU, with the exception of the intra-species feed ban, current legislation only requires distinction between material from fish and material from terrestrial animals for in feed for most livestock. In addition, all rendering plants in the EU states should be processing at standard parameters (133 °C/3bar/20minutes; see the “Rendering of animal by-products” chapter in this course manual for further

¹ The intra-species ban requires determination of the species of origin for all feed components except blood and milk, but will only be relevant if and when the current mammalian to livestock ban is lifted (EU, 2002).

discussion). Therefore, tests must be able to distinguish between fish meal and MBM processed under these conditions. No quantification is required, as in the EU detection of any prohibited material in a sample means that feed does not comply with the feed ban. Therefore, tests or testing protocols for all feed materials produced both in the EU states and imported from other countries into the EU must be able to appropriately address these questions.

2. AVAILABLE TEST METHODS

Different tests make use of different approaches for detection of prohibited materials in feed. In optical microscopy, animal tissues such as bones, muscle fibres, hairs, and feathers are directly identified. With other methods, various animal proteins, peptides, lipids, DNA, volatile materials or specific organic molecules are identified. The primary test methods currently used are described in the following sections.

2.1. Optical microscopy

Optical microscopy (OM) is the direct identification of animal tissues by typical physical structure. It involves concentrating the materials by both sedimentation and flotation and then evaluating them at different magnifications with both the stereomicroscope and the compound microscope (Plates 1-5). The sediment contains mineral particles including bones and teeth, and the flotation contains organic particles, which are mainly plant products but include meat particles and feathers.

Technically, the OM methodology is relatively simple (EU, 2003). However, in practice, OM technicians must have a high level of expertise that only comes from extensive experience in observing MBM samples under the microscope.

Sedimentation/flotation: About 10 grams of feed material are first dissolved in an organic solvent (tetrachlorethylene) to concentrate the minerals in the sediment and the organic components in the flotation. This separates the material as follows:

Sediment: Minerals, salts, phosphate, magnesium

Animal components: terrestrial animal bone fragments, fish bone fragments, scales, teeth

Flotation: Plant material

Animal components: meat particles (muscle fibres), feathers, hairs

Then, by sieving and weighing, three fractions of different particle diameter (>1 mm, between 1 and 0.35 mm, and <0.35 mm) are obtained.

Observation with the stereomicroscope: Preparations of both the sediment and the flotation are made from the fraction of particles greater than 1 mm diameter. These large particles of bone fragments (sediment) or meat particles (flotation) may be visualized in the stereomicroscope at a magnification of 50x.

Observation with the compound microscope: The sediment is prepared with a clearing agent (phenoglycerin) and the flotation is treated with potassium iodide (to stain the proteins orange-yellow). The preparations are then observed at a magnification of 50-400x with the compound microscope. Generally, three preparations are screened, one preparation of the <0.35 mm fraction and two preparations of the fraction between 1 mm and 0.35 mm.



Plate 1

Terrestrial animal (mammal or bird) bone fragment in sedimentation fraction of compound feed sample, as seen with the stereomicroscope. Magnification 50x, particle size >0.315 mm

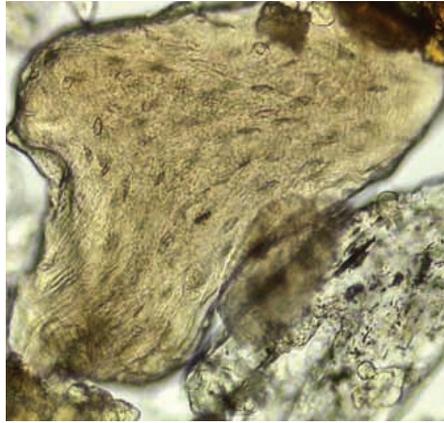


Plate 2

Terrestrial animal (mammal or bird) bone fragment in sedimentation fraction of compound feed sample, as seen with the compound microscope. Magnification 100x, particle size <0.315 mm

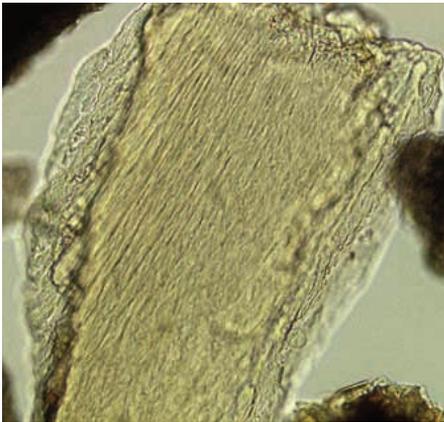


Plate 3

Fish bone fragment in sedimentation fraction of compound feed sample, as seen with the compound microscope. Magnification 100x, particle size <0.315 mm

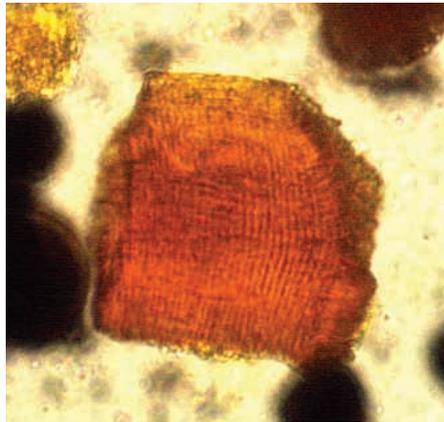


Plate 4

Muscle fragment (fish or terrestrial animal) in flotation fraction of compound feed sample, as seen with the compound microscope. Magnification 400x, particle size <0.315 mm

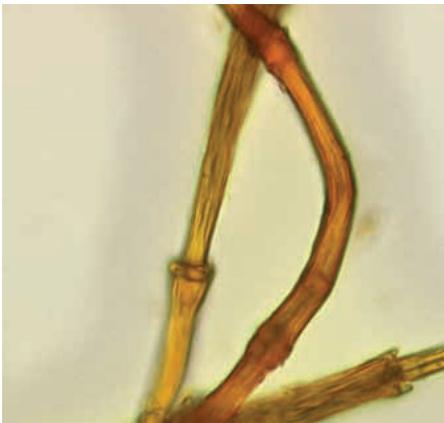


Plate 5

Feather fragment in flotation fraction of compound feed sample, as seen with the compound microscope. Magnification 400x, particle size <0.315 mm

2.2. Immunoassay

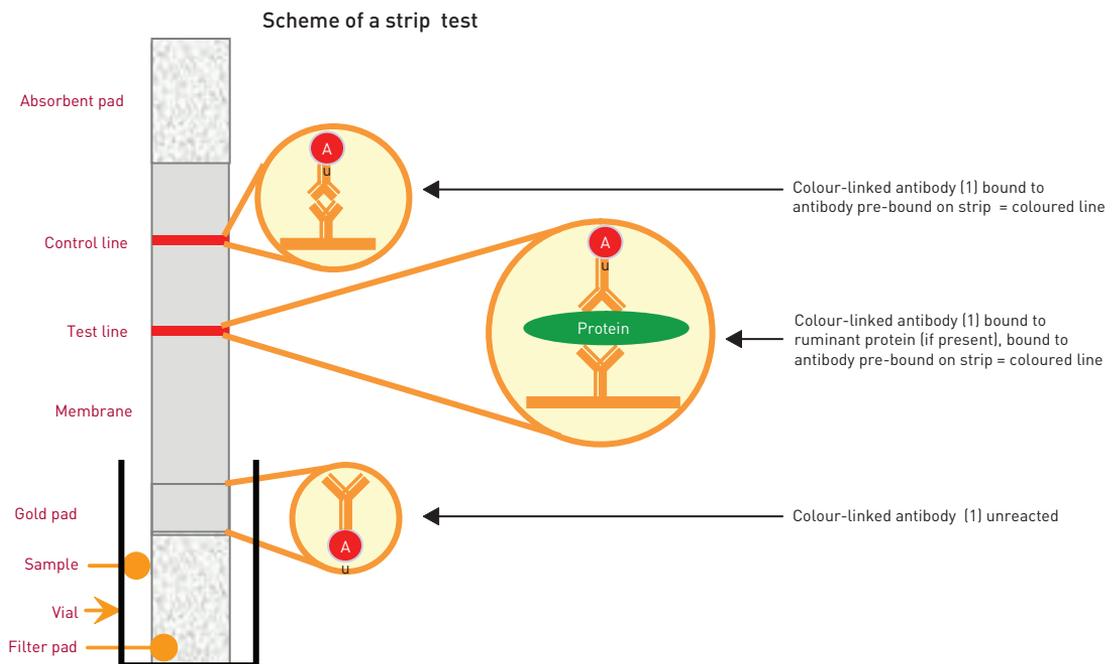
Immunoassay is a method to identify a specific protein in a sample. It involves extraction of the protein, reaction of the protein of interest with a specific antibody and then amplification and visualization of the response signal.

However, because many materials to be tested have undergone processing, immunoassays must overcome the problem of maintaining test sensitivity and specificity in the face of the degradation of proteins that occurs during heating. Furthermore, even when not fully degraded, the conformation of proteins often changes with heat treatment. Therefore, the immunoassay must employ antibodies that detect either heat stable antigenic sites or antigenic sites on heat stable proteins, i.e. those that remain after the application of heat treatment and/or other appropriate processing parameters. Therefore, a parallel application of the immunoassay methodology is to control and audit the adequacy of processing parameters at the rendering plant by measuring the presence or degradation of various proteins (Pallaroni *et al.*, 2001).

The first method developed for detection of MBM using molecular biology techniques was an immunoassay for detection of ruminant proteins in rendered material heated to greater than 130 °C (Ansfield, 1994), and an improved immunoassay for detection of ruminant and porcine proteins heated to greater than 130 °C at 2.7 bar in compound animal feeds was later described (Ansfield *et al.*, 2000). However, the usefulness of these immunoassays was somewhat restricted by their tedious protocols which required performance in specialized laboratories.

FIGURE 1

Basic schematic of a lateral flow immunochromatographic assay (strip test) for detection of specific proteins in feed



Source: Oliviere Fumière, Walloon Agricultural Research Centre, Gembloux, Belgium.

TABLE 1. Selected lateral flow immunochromatographic assays, including manufacturer information

Company	Web site	Test name
NEOGEN® Corporation	www.neogen.com	ReVeal®
Strategic Diagnostics Inc.	www.sdix.com	FeedCheck TM
CIBITest GMBH & Co. KG	www.cibitest.de	FLORIDA
ELISA Technologies, Inc.	www.elisa-tek.com	MELISA-TEK TM Ruminant Kit

More recently, a new type of commercial immunoassay has been developed, the so-called "strip test" (Figure 1), most commonly used to identify ruminant protein. These single-step lateral flow immunochromatographic assays are simple to handle and allow the testing of many samples in a relatively short time. Most of these tests detect the presence of Troponin I (a heat stable ruminant muscle protein) in the sample. These immunoassays are not quantitative, and give either a positive or negative result with a detection limit at 1% to 5% for MBM that has been rendered at the standard processing parameters (133 °C/20min/3bar).

2.3. Lateral flow immunochromatographic assays

The test procedure of the lateral flow immunochromatographic assays (strip tests) normally includes weighing out of approximately 10 grams the sample, adding extraction solution, short heating of the sample in boiling water or washing (depending on assay), placing the test strip into the sample tube and reading of the results within several minutes.

The strips have two possible visualization zones: A reaction line that appears only if ruminant protein is present and a control line that forms to validate that the strip is working properly. Specific test procedures differ for the different tests, and further details are available from the manufacturers (Table 1).

2.4. Polymerase chain reaction

Polymerase chain reaction (PCR) is a method to identify specific DNA in a sample. It involves grinding of the sample and extraction of the DNA, followed by amplification of those DNA sequences that are specifically of interest and visualization of the amplified DNA fragments (Brambilla *et al.*, 2004; Kingombe *et al.*, 2001; Matsunaga *et al.*, 1999).

2.5. Near infrared spectrography

Near infrared spectrography (NIRS) is a method to identify spectrographic vibrations of groups of organic molecules (e.g. O-H, C-H, N-H) indicating specific materials of interest. It involves initial calibration and validation of the system, preparation and irradiation of the sample, and analysis of the answer spectra using specific mathematical equations (Murray *et al.*, 2004).

2.6. Near infrared microscopy

Near infrared microscopy (NIRM) is also a spectrographic method to identify specific isolated particles. It involves concentration of bone material by sedimentation (similar to OM), irradiation and analysis of a pure spectrum for each particle, and classification

of the particles. In this test, minerals show no vibration spectra (Murray *et al.*, 2004; Baeten *et al.*, 2004).

2.7. Near infrared camera

Near infrared camera (NIRC) is similar to the NIRM but faster, as it can analyse 500 particles in five minutes. The equipment is expensive, and there is a high cost per analysis (Baeten *et al.*, 2004).

2.8. High performance liquid chromatography

High performance liquid chromatography (HPLC) is a method to detect specific dipeptides that indicate the presence of muscle tissue (e.g. carnosine). However, with this method it is not possible to differentiate between muscle from terrestrial animals and that from fish. This test method is described by Schönherr (2002).

2.9. Electronic nose

Electronic nose is a recent approach for screening raw materials by odour, but is not yet commonly used. This test method is described by Campagnoli *et al.* (2004).

No international standards exist for performing these tests, as different international working groups have set their own standards for the different methods. For example, the European Feed Microscopists Working Group (IAG) sets standards for OM, and the EU project on methods to detect MBM (Stratfeed, 2004) has set standards for OM, PCR, NIRS, and NIRM.

3. COMPARISON OF TESTS

Each method has advantages and disadvantages (Table 2: Von Holst and Boix, 2004). OM is based primarily on the detection of bone material and is not affected by the processing parameters of the sample. In OM, terrestrial animal bones (Plates 1 and 2) can be distinguished from fish bones (Plate 3) in the sedimentation fraction, but bone material from mammals and poultry cannot be separated. This also means that with OM it cannot be determined if bone material is from rodents or birds that may be inadvertently present in feed components. Muscle (Plate 4) in the flotation indicates the presence of animal material (terrestrial animal or fish) in the sample, but the origin cannot be distinguished more specifically. The identification of feathers (Plate 5) in the flotation is indicative of the presence of poultry or other avian material, but feathers must be distinguished from plant material, which may look similar.

The PCR is species specific, but is also sensitive to contamination and interfering ingredients that may affect the validity of the result. It may be appropriate as a confirmatory method. Over the past few years, both molecular biological methods (PCR and immunoassay) have improved in their ability to detect heat-treated MBM, as the tests now utilize the detection of very short DNA target sequences and more stable protein fragments, respectively.

A relatively high number of samples can be processed in a short time with both immunoassay and NIRS. Therefore, these could be appropriate screening methods, especially as they do not require toxic reagents. However, although immunoassay has a very low level of false negative results, they both have relatively low sensitivities, which is not optimal for screening tests.

TABLE 2. Comparison of characteristics of the main testing methods

Test characteristic	Optical microscopy	PCR ¹	Immunoassay	NIRS ²	NIRM ³
Terrestrial animal/fish differentiated	yes	yes	yes	yes/no	yes
Species identification possible	no	yes	yes	no	no
Limit of detection (% of feed)	< 0.1%	~ 0.5%	~ 1 %	3 - 5 %	< 0.1%
Sample size required (in grams)	5 - 20 g	0.1 - 1 g	10 g	5 - 100 g	0.2 - 10 g
False negative and positive rate	very low	low	very low	high	very low
Interference from allowed ingredients (milk, blood)	no problem	problem	problem	no problem	no problem
Interference by heat/processing (MBM)	no problem	some problem	some problem	no problem	no problem
Matrix dependent	no	yes	no	yes	no
Particle size	no problem	no problem	no problem	no problem	problem
Risk of erroneous result due to contamination	very low	high	low	low	low
Quantitation possible	no	no	no	yes	yes
Reagent toxicity	yes	yes	no	no	yes
Required expertise of analyst	high	high	low	low	low
Number of possible samples/day/analyst	~ 10	~ 10	100-200	100-200	3-5
Existing facilities usable	yes	yes	yes	yes	no
Initial cost of instrumentation	average	average	low	average	average
Cost of analysis per sample	average	average	low	low	average

Note:

¹ Polymerase chain reaction

² Near infrared spectrography

³ Near infrared microscopy

Source: adapted from Von Holst and Boix (2004)

NIRM has a high sensitivity and is similar to OM with the advantage that it can quantify the material in the sample. The NIR camera is expensive, but also gives a quantitative result. Besides being quantitative, both have the advantage of being able to determine different ingredients in one analysis.

Given these characteristics of tests, the decision on what test to use must practically consider:

- the ban in place (and therefore the question to be answered);
- the number of samples to be tested;
- the equipment, infrastructure and technical personnel available.

4. SUMMARY OF TSE-RELEVANT CONCEPTS

- Laboratory testing of domestic and imported feeds and feed components to identify prohibited materials is necessary for effective implementation of feed bans.
- Tests differ in the extent to which they can identify the category of animal a protein is derived from.
- The method(s) chosen must be able to provide information appropriate to the specific feed ban and other measures in place.

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BSE RISK AND TRADE IN ANIMAL PRODUCTS AND LIVESTOCK FEEDS

1. GENERAL CONCEPTS

Currently, there is a trend for more and more countries to participate in international or regional trade in agricultural products. The economic and quality-of-life benefits of increased trade, especially for developing countries, can be enormous (World Bank, 2005). However, the risks to global public and animal health also increase with increased movement of these products. The spread of BSE is an excellent example of these increased risks. This chapter presents aspects of trade in agricultural products, such as assessment of BSE risk, available data and other issues of global trade.

2. THE EFFECTS OF BSE ON GLOBAL TRADE

Clearly, the world supply of animal protein has been disrupted by the appearance of BSE. Trade in animal protein, including meals made from processing of mammalian protein (e.g. meat and bone meal/MBM) as well non-mammalian protein (e.g. poultry meal, fish meal) is already more restricted than in the past, and could become more restricted as countries become more aware of risk of BSE and other diseases that may be spread through livestock feeds. However, as economies grow and the demand increases for meat and other animal products, the demand also increases for high-quality proteins for manufacture of livestock feeds. Globalization of markets can ease some of the effects of this increased protein demand but global trade also introduces new risks. Moreover, animal welfare, environmental issues and an increased consumer awareness of food safety all must be considered when trading in agricultural products.

Consequently, additional sources of high-quality alternative protein will need to be established or reconsidered, (as described in the “Protein use in livestock feeding” chapter in this course manual) and assurances for the safety of non-prohibited animal protein sources improved. This will include assuring full traceability and strengthening of compliance with international regulations, which will require increased technical capacity in many exporting countries. Ultimately, all of these factors, in parallel with the TSE-related feed bans, will play a role in the types of proteins produced and traded in the world.

3. ASSESSING THE RISKS OF BSE IN TRADE

In principle, all trade decisions must be based on an assessment of the exporting country's actual risk for a specific commodity and disease, as well as the domestic situation (Heim and Mumford, 2005). WTO, through the OIE (the international standard setting body for animal health issues) and the Codex Alimentarius (the international standard setting body for foods and feeds), requires a risk assessment to be made anytime agricultural trade restrictions are in place that are more stringent than these international standards (WTO, 1994). Without an assessment scientifically justifying their restrictions, WTO member countries are not in compliance with Agreement on the Application of Sanitary and Phytosanitary Measures.

For example, in most cases it is not logical to prohibit import of a product from a country where a disease is endemic if the disease is also endemic domestically, or if the domestic situation precludes spread or establishment of the disease (e.g. an insect-borne disease in a country where a required vector does not exist, or BSE in a hypothetical country that raises no ruminant livestock).

Similarly, it is not logical to ban products from a country where a disease has been reported, if the importing country has a similar domestic risk (even if cases have not been reported). There are many examples of countries implementing import bans or restrictions after a trading partner reports a first BSE case, even though the importing country has an equivalent BSE risk. It is also clear that BSE exposure of a country probably happened 5-15 years before any cases are seen, and therefore implementation of bans against long-time trading partners does not necessarily affect current risk (Heim and Mumford, 2005). It must also be considered whether there is a large discrepancy between BSE control measures implemented in the importing and exporting countries.

In addition, it is not logical to ban products that pose negligible risk. The OIE has determined that milk and milk products, protein free tallow, and certain other products pose negligible risk, irrespective of the BSE risk of the exporting country (OIE, 2005). Deboned beef (under certain conditions) can also be traded, even from countries with a risk of BSE (OIE, 2005a). Therefore, there should be no restrictions on importing these products, even when the exporting country has a non-negligible BSE risk. However, restriction of some products for human consumption (e.g. processed meat pies, minced meat) from countries with a risk but insufficient control measures (e.g. no SRM ban) can be justified, as these could pose an immediate public health risk.

The impact of all these scenarios on BSE risk through trade would be taken into account through the process of national risk assessment in conjunction with assessments of the risk of importing specific products. National risk assessments are currently available for some countries, as described in the "Introduction to TSEs and BSE" chapter in this course manual.

4. TRADE DATA: QUALITY AND QUANTITY

Sufficient valid trade data of high quality for any commodity can be extremely difficult to collect and compile. Countries engaging in trade generally maintain their own national records of import and exports, although the level of detail varies considerably. Examination of records often shows discrepancies between import records and export records for a single transaction between two countries, and records of transactions between neighbouring countries may not be even minimally complete. Moreover, no single international institution is responsible for compiling these data into a single global database.

In developing countries, the problem with trade data is often augmented for political or infrastructural reasons, or both. Many developing countries still do not have an adequate record-keeping system in the agricultural sector. In developing countries (as well as in many developed countries) much of the available agricultural data are incomplete in terms of commodities included, range of variables included, and geographical coverage of the country. Furthermore, even when data are available, their reliability may be questionable.

When spread of infectious diseases is considered, tracking of imports and exports becomes crucial. In the case of BSE, which carries public and animal health conse-

quences, the import of risky products must be examined to evaluate a national exposure risk for importing as well as exporting countries, as described above. Unfortunately, due to the long incubation period of this disease, data on imports beginning in the mid-1980s through today must be available for examination.

4.1. Data sources

FAO is one source of trade data. FAO compiles information on various aspects of food and agriculture from member countries throughout the world to support their programmes and activities. The data are analysed and interpreted and made generally available (FAOSTAT, 2004). Data compiled by FAO that are relevant to this discussion include agricultural production, agriculture and food trade, food aid and exports of cereals by source and destination.

FAO collects its data through questionnaires sent annually to member countries, accessing websites of the countries, national and international publications, country visits made by FAO statisticians, and reports by representatives in member countries. In the absence of reliable sources or when information is not available, figures are estimated on the basis of trade data from trading partners. In the case of entirely missing data, statisticians estimate certain data points if other parameters are sufficiently available.

In addition to these data, some data from EU member countries are obtained and compiled through Eurostat (2004). The United Nations Statistics Division (UN, 2004) has extensive publications not only related to trade statistics, but also regarding other trade standards and issues. In some cases, official trade data can be supplemented with trade information and data from other national or international agencies or organizations, as well as from unofficial sources.

4.2. Problems encountered in gathering and interpreting trade data

Systems for trade reporting. Countries may report data on imports and exports in different ways. For example, they may have different systems to describe imported commodities used for domestic consumption and those re-exported to other countries. Most countries report general trade data, which do not discriminate between goods used and goods re-exported without entering the country, i.e. exports from customs warehouses and free zones or ports. This distinction is important in considering whether a commodity (such as MBM) has been used within a country or exported onward to a third country, but depending on the system used the information may not be ascertainable. In addition, it may not be ascertainable whether a particular import contained material produced only in the exporting country, or also included products originating elsewhere. Consequently, it is difficult to evaluate the BSE risk for re-exported products, especially those that have been held for long periods prior to shipment.

Classification and definitions. Additional problems regarding trade data are the discrepancies that exist in classification and definition of traded commodities (as mentioned in the "Protein use in livestock feeding" chapter in this course manual). In 1988, many countries adopted the third revision of the United Nations Standard International Trade Classification (SITC; UN, 1998) or the Harmonized Commodity Description and Coding System of the Customs Cooperation Council (HS; CCC, 2004) causing some confusion. In an attempt to maintain comparability with the system(s) up to 1987, FAO has opted to continue using Revision 2 of the SITC, while attempting to adjust the new classification to the old one.

However, problems and confusion remain. For example, in the classification of meat, certain national statistics include only grouping at the three-digit (SITC Revision 2) level, e.g. fresh, chilled or frozen (code 011), or dried, salted or smoked (code 012). In this case it has been necessary to redistribute the data from the three-digit groups among the four-digit level subgroups (SITC Revision 3), by taking into account the information from trading partners, which may or may not be available or reliable. As another example, within the FAOSTAT classification, meat meal (code 1173) is defined as "Flours, meals and pellets of meat and offal (including of marine mammals); greaves and tankage. Used for feed." (FAOSTAT, 2004). Thus, this classification code does not allow determination of different by-product types or species of origin (e.g. mammalian, avian, aquatic) and therefore the possibility for trace back is limited.

Unreported movement. In some regions, the movement of considerable numbers of animals into neighbouring countries remains unrecorded, either intentionally or unintentionally. To obtain more representative data of international trade in live cattle, estimates of unrecorded trade have been made and incorporated. Further, black market movement of cattle and other agricultural products, as well as international crisis aid (cattle, food and supplies, which in many cases are differently classified), are difficult to estimate.

Re-export. Examination of import and export records from countries indicates that re-export of BSE risk products, including cattle, mammalian protein, and feeds containing mammalian protein has been common. Therefore, for example, risky products exported from West Europe to East Europe were often re-exported to the Near East or beyond. Moreover, trading companies buy and sell products all over the world and shipments may change owners several times. Although it is known that many of these transactions occur only on paper or electronically, while the actual product remains in storage, the number of transactions makes the ability to track and trace the products, and therefore determine the country of origin, increasingly challenging (Brian Cooke, FEFAC, Personal communication, 2005).

Post-production contamination. Due to the complexity of global trade, there is often opportunity for products with no inherent risk to become contaminated with BSE (or other disease agents) during shipment or storage. Countries must assess these risks and conduct import audits to assure the safety of all imported products.

5. ASSESSING BSE EXPOSURE RISK

5.1. What we know

It is well known that before BSE was recognized in the world, global trade in live cattle and bovine products including MBM and feeds containing MBM was widespread, especially from industrialized areas such as the EU and North America (Tables 1 and 2). Even after BSE was recognized and it was determined that transmission occurred through livestock feed containing the infective agent, trade in these risky products continued. For example, export of mammalian protein continued from the EU even after it was prohibited from being fed to ruminants in 1994, and continued until export was banned from the UK in 1996, Portugal in 1998, and the rest of the EU in 2001 (EU, 2001).

In an attempt to provide some general estimate of BSE challenge to countries and regions worldwide, available import and export data were compiled for the highest risk years (i.e. 1988 to 2000) for the most risky products for BSE (live cattle in Table 1 and in MBM in Table 2). These data confirm that there was considerable trade in both live cattle and MBM before the bans on exports from Europe were implemented. It is very likely

TABLE 1. Cattle exports from western Europe by importing region

Importing region	Cattle imports (total number of animals)		
	1988 to 1990	1991 to 1995	1996 to 1999
Eastern Europe	48 648	192 561	157 411
Near and Middle East	80 476	916 851	803 639
North Africa	209 593	1 095 021	366 949
Sub Saharan Africa	5 718	3 136	969
Central America	369	418	975
North America	31	637	147
South America	2 030	8 780	1 149
Central Asia	0	741	667
East Asia	450	346	601
South Asia	648	1 828	188
Southeast Asia		633	1 912
Oceania	216	189	152

Source: FAO (2004).

that some of the MBM was used in importing countries for the direct feeding of ruminant livestock, and imported MBM was certainly present in feed manufacturing plants with the potential for contamination of ruminant feed supplies. This imported MBM, or feeds containing this MBM, may still be stored in these countries, in feed plants or on farms, or it could even still be circulating globally through unregulated channels.

5.2. What we don't know

Up to and including the present time, many countries have assumed that there is no BSE risk when importing from countries that have not reported BSE cases. However (as described in the "Introduction to TSEs and BSE" chapter in this course manual) this is clearly not the case, as the risk of BSE being present in countries outside Europe is still largely unknown because:

1. The long incubation time means that some level of infectivity can be present for many years before clinical cases appear in a country; and
2. Countries vary greatly in their surveillance and reporting of diseases such as BSE.

Therefore, the BSE agent may still be silently amplifying in many, as yet undetermined, countries that continue to export risky products. This, in addition to the lack of a clear ability to establish exactly what was traded, when, and by whom, means that most countries throughout the world have, knowingly or unknowingly, received some level of exposure to the BSE agent through imports. Further, continued domestic amplification and trade may continue to spread risky products worldwide, despite continued tightening of regulations (Heim and Mumford, 2005).

These specific considerations are discussed more fully in the "Introduction to transmissible spongiform encephalopathies" chapter in this course manual, but the situation emphasizes the need for all countries to undertake a national BSE risk assessment so that the global BSE situation can be validly addressed.

TABLE 2. Trade matrix for meat and bone meal from 1996 to 1999 by importer (horizontal axis) and exporter (vertical axis), in metric tonnes

Exporting Country	World Trade in Meat and Bone Meal (1996 – 1999)										
	Importing Country										
	North Africa	Central America	North America	South America	East Asia	Southeast Asia	East Europe	United Kingdom	West Europe	Near and Middle East	Oceania
North America	19 715	337 070	276 467	17 679	462 241	341 903	41	779	6 262	5 036	2 503
South America		47	668	68 527	212 587	951	40		4 195		
East Asia			250	0	72 440	1 927	9	221	1 528		3
Southeast Asia					326	53					
United Kingdom	3 386	86	103	2	8 212	11 269	3 678		43 396	11 526	43
West Europe	270 483	1 826	5 381	212	197 892	93 642	1 320 672	113 462	1 743 059	126 952	42
Oceania		155	85 331	1 074	404 374	203 377	960	618	1 703	162	8 224

Source: FAO (2004).

6. SUMMARY OF TSE-RELEVANT CONCEPTS

- It is crucial for importing countries to evaluate the BSE status of exporting countries, as well as to consider the risks of imported products. Risk assessments are already available for some countries and international recommendations are available from the OIE describing under which conditions the trade of commodities pose a negligible risk.
- In order to assess risk and justify trade restrictions, it is required that countries perform their own national BSE risk assessment.
- Given the difficulties in assessing the risks it must be assured that all imported products comply with domestic regulations through examination of records or accreditation of sources, as well as border checks, testing and audits.

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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.

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/animath/bse/index.html>

FAO. BSE pages: <http://www.fao.org/ag/AGInfo/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
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Scheaffer RL, Mendenhall W, Ott L. 1990. Elementary Survey Sampling. Duxbury Press, Belmont CA.

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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>

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	European 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 Organisation for Animal Health
OM	Optical Microscopy
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 <i>PRNP</i> , 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



WB

Western blot

WHO

World Health Organization

WTO

World Trade Organization

Additional definitions can be found in

- the OIE *Terrestrial Animal Code*, Chapter 1.1.1. http://www.oie.int/eng/normes/MCode/en_chapitre_1.1.1.htm
- the FAO/WHO Codex Alimentarius "Current official standards". http://www.codex-alimentarius.net/web/standard_list.do?lang=en

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
- Transmissible spongiform encephalopathies management in livestock feeds and Feeding
- Transmissible spongiform encephalopathies Management 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 Management of transmissible spongiform encephalopathies in livestock feeds and feeding and it is targeted at governmental and industry personnel 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.