CAPACITY BUILDING FOR SURVEILLANCE
AND PREVENTION OF BSE AND OTHER ZOONOTIC DISEASES

MANAGEMENT OF TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES IN LIVESTOCK FEEDS AND FEEDING

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CAPACITY BUILDING FOR SURVEILLANCE
AND PREVENTION OF BSE AND OTHER ZOONOTIC DISEASES

course manual

MANAGEMENT OF TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES IN LIVESTOCK FEEDS AND FEEDING

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FOREWORD

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*, involving collaboration between the Food and Agriculture Organization of the United Nations (FAO), Safe Food Solutions, (SAFOSO, Switzerland) and national veterinary offices in partner countries, and 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 BSE risk is negligible, and thereby facilitate regional and international trade under the Agreement on the Application of Sanitary and Phytosanitary Measures (SPS) of the World Trade Organization (WTO). A brief project summary is included as an appendix to this course manual.

Activities of the project:

- The specific needs of partner countries are assessed.
- Four comprehensive courses to "train the trainers" are provided to selected participants to improve understanding of the epidemiology of and relevant risk factors for BSE and Transmissible spongiform encephalopathies (TSE) and to develop specific knowledge and skills for implementing appropriate controls.
- In a third step, in-country courses are held by trained national personnel in the local language and are supported by an expert trainer.

FAO has the mandate to raise levels of nutrition and standards of living, to improve agricultural productivity and the livelihoods of rural populations. Surveillance and control of diseases of veterinary public health importance contribute to this objective. SAFOSO, a private consulting firm based in Switzerland, is providing the technical expertise for this project.

This manual is a supplement to the training course *Management of transmissible spongiform encephalopathies in livestock feeds and feeding,* which is given within the framework of the project. This practical course 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.

The information included in the manual is not intended to be complete or to stand on its own. For further reading, specific references are included at the end of the chapters. General background material and Web links, and a glossary of terms and frequently used acronyms are included as appendices.

The preparation of this manual was a collaborative effort of the trainers of the *Management of transmissible spongiform encephalopathies in livestock feeds and feeding* course offered in Switzerland and the project staff. The content of the manual reflects the expertise and experience of these individuals. FAO and SAFOSO are grateful to the professionals preparing the manual and to the Government of Switzerland for funding this public-private partnership project in support of safer animal production and trade.

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COURSE OBJECTIVES

Upon completion of the lectures and exercises of the course on *Management of transmissible spongiform encephalopathies in livestock feeds and feeding*, of the project *Capacity Building for Surveillance and Prevention of BSE and Other Zoonotic Diseases,* the participants should:

- basic information on BSE and TSEs, including transmission, pathogenesis, risk factors, and epidemiology;
- rendering and inactivation of TSE agents;
- categorization of animal by-products and knowledge of the risks of animal by-products in animal feed;
- modern process technology in feed and premix manufacturing plants, including control of cross contamination with animal by-products;
- international and national regulations in feed manufacturing, including guidelines for the use of animal by-products;
- quality management in feed manufacturing;
- sampling strategies for testing of feed and principles of the tests;
- inspection of feed plants, including BSE controls;
- global market for animal feed, including assessment and control of the risks.

INTRODUCTION TO TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

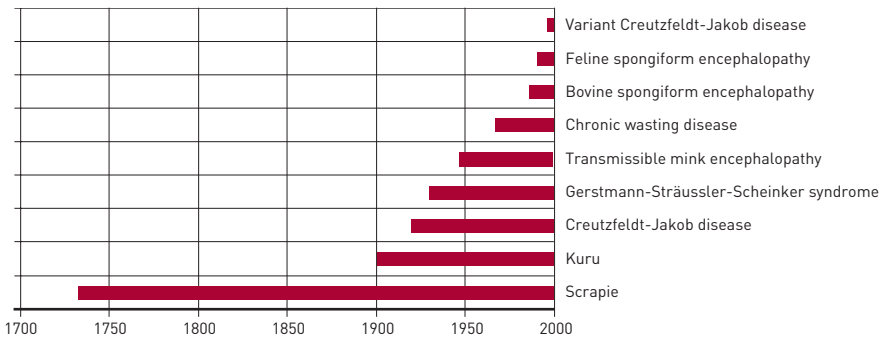
1. TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

Transmissible spongiform encephalopathies (TSE) are a class of neurodegenerative diseases of humans and animals characterized by spongiform degeneration of the brain and the associated neurological signs. TSEs are slowly developing and uniformly fatal.

Diseases include kuru, Gerstmann-Sträussler-Scheinker syndrome and Creutzfeldt-Jakob disease (all in humans), scrapie (in sheep and goats), feline spongiform encephalopathy (FSE; in cats), bovine spongiform encephalopathy (BSE; in cattle), chronic wasting disease (CWD; in cervids) and transmissible mink encephalopathy (TME; in mink). Most of these TSEs had already been reported before the first detection of BSE. (Figure 1) (Lasmezas, 2003).

FIGURE 1

Year in which the various TSEs were first reported



The TSE with the longest history is scrapie, which was recognized as a disease of sheep in Great Britain and other countries of western Europe more than 250 years ago (Detwiler and Baylis, 2003). Scrapie has been reported in most sheep-raising countries throughout the world with few notable exceptions (e.g. Australia, New Zealand).

Transmissible mink encephalopathy (TME) was first described in 1947. It is a rare disease of farmed mink and has been recorded in countries including the United States of America (USA), Canada, Finland, Germany and the Russian Federation. Contaminated feed is suspected to be the main source of TME infection.

Chronic wasting disease (CWD) in captive and free-roaming North American deer and elk was first described in the 1960s. Initially, cases were only reported in captive deer and elk in Colorado (USA), but CWD in captive and/or free roaming deer, elk and moose has now been reported in several other states in the USA and in areas of Canada. The origin of CWD is still unknown.

Scrapie, kuru, Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker syndrome, TME, and CWD are believed to be distinct from BSE. However, strain typing has indicated that some other TSEs are caused by the same strain of the TSE agent that causes BSE in cattle. Only four years after the initial BSE cases had been diagnosed in cattle in the United Kingdom of Great Britain and Ireland (UK), BSE in domestic cats (feline spongiform encephalopathy / FSE) was first reported. Almost all of the approximately 100 FSE cases diagnosed worldwide occurred in the UK. The most widely accepted hypothesis is that the affected domestic cats were exposed to BSE infectivity through contaminated commercial cat feed or fresh slaughter offal that contained brain or spinal cord from bovine BSE cases. Several large cats kept in zoos were also diagnosed with FSE. These included cheetahs, lions, ocelots, pumas and tigers. All of the large cats that were diagnosed with FSE outside the UK originated from UK zoos. It is suspected that these large cats acquired the infection by being fed carcasses of BSE-infected cattle.

Not long after BSE was diagnosed in cattle, sporadic cases of BSE in exotic ruminants (kudus, elands, Arabian oryx, ankole cows, nyala, gemsbock and bison) were diagnosed in British zoos. One zebu in a Swiss zoo was also BSE positive. In the majority of these cases, exposure to animal feed produced with animal protein (and therefore potentially containing BSE infectivity) was either documented or could not be excluded.

Moreover, there has long been concern that sheep and goats could have been exposed to BSE, because it has been experimentally demonstrated that BSE can be orally transmitted to small ruminants (Schreuder and Somerville, 2003). In 2005, the first case of BSE in a goat was confirmed in France (Eloit *et al.*, 2005), though there have been no confirmed BSE cases in sheep to date. It is difficult to distinguish between scrapie and BSE in sheep, as differentiation is currently not possible by clinical or pathological means.

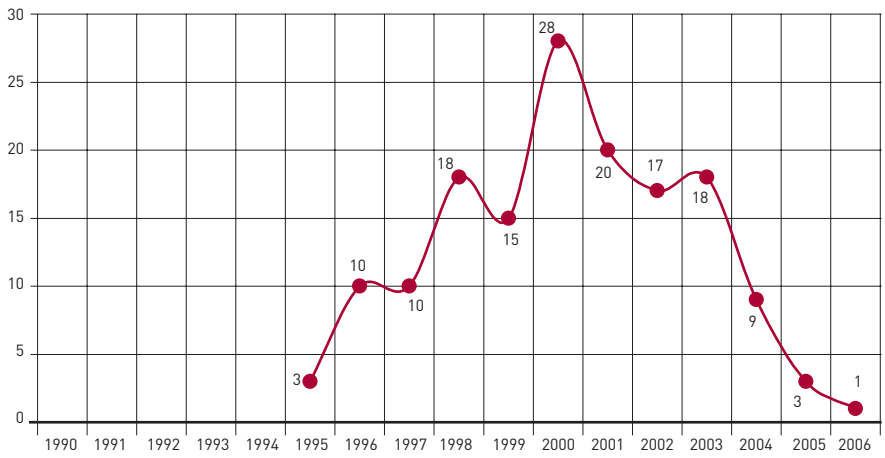
Several TSEs have been reported to occur in humans, including two forms of Creutzfeldt-Jakob disease (sporadic CJD and variant CJD [vCJD]), Kuru, Gerstmann-Sträussler-Scheinker syndrome, as well as fatal familial insomnia. Of these, only vCJD has been associated with BSE. Sporadic CJD was first identified in 1920 as an encephalopathy occurring almost exclusively in elderly patients worldwide. The incidence of sporadic CJD is approximately 0.3-1.3 cases per million individuals per year, and is similar in most countries. The duration of the disease is approximately six months. Approximately 80-89% of CJD cases are believed to be sporadic, 10% are familial (a result of a heritable mutation in the PrP gene), and the remainder are believed to be iatrogenic.

Variant CJD was first reported in March 1996 in the UK (Will *et al.*, 1996). In contrast to sporadic CJD, patients are young (average age 29 years) and the duration of the disease is longer (average 22 months). Epidemiologically, little is known about vCJD. In some cases the disease was seen in geographical clusters, and there are indications that special consumption patterns may have played a role. Genetic factors may also play a role in infection, as patients with clinical disease have been homozygous for methionine at codon 129 of the prion protein gene. In Europe, this genotype accounts for approximately 30% of the population.

The expected course of the vCJD epidemic is difficult to predict, since important variables such as human exposure rate, the infectious dose, the incubation period and human susceptibility are largely unknown. The predictions initially ranged from a few hundred to a few million expected cases. However, the lower predictions are more prob-

FIGURE 2

Number of vCJD cases in the UK over time



Source: Department of Health UK (2006)

able based on the current incidence of vCJD cases (Figure 2).

The link between BSE and vCJD is commonly accepted. Initially, the temporospatial association of the outbreaks suggested a causal relationship. Experimentally, inoculation of the BSE agent into the brains of monkeys produces florid plaques histologically identical to those found in the brains of vCJD patients. In addition, the agents associated with BSE and vCJD are similar, both by glycotyping (evaluating the glycosylation pattern) and by strain typing, whereas the prions associated with other TSEs (such as sporadic CJD, scrapie and CWD) are different.

2. BOVINE SPONGIFORM ENCEPHALOPATHY

2.1. Origin and spread of BSE

BSE was first diagnosed in cattle in the UK in 1986 (Wells *et al.*, 1987). Extensive epidemiological studies have traced the cause of BSE to animal feed containing inadequately treated ruminant meat and bone meal (MBM) (Wilesmith *et al.*, 1988). Although elements of the scenario are still disputed (e.g. origin of the agent; Wilesmith *et al.*, 1991; Prince *et al.*, 2003; SSC, 2001a), it appears likely that changes in UK rendering processes around 1980 allowed the etiological agent to survive rendering, contaminate the MBM, and infect cattle. Some of these infected cattle would have been slaughtered at an older age and therefore would have been approaching the end of the BSE incubation period. Potentially, they had no clinical signs or the signs were subtle and went unrecognized, although the cattle would have harboured infectivity levels similar to those seen in clinical BSE cases. The waste by-products from these carcasses would then have been recycled through the rendering plants, increasing the circulating level of the pathogen (which by now would have become well adapted to cattle) in the MBM, thus causing the BSE epidemic.

In 1989, the first cases outside the UK, in the Falkland Islands and Oman, were identified in live cattle that had been imported from the UK. In 1989, Ireland reported the first non-imported ("native" or "indigenous") case outside the UK, and in 1990 Switzerland

reported the first indigenous case on the European continent. Indigenous cases were then reported in many countries throughout Europe. In 2001, Japan reported the first indigenous case outside Europe, and this case has been followed by indigenous cases in Israel and North America.[1]

2.2. Epidemiology

Cattle testing positive for BSE have ranged from 20 months to 19 years of age, although most of the cases are between 4 and 6 years of age. A breed or genetic predisposition has not been found. Most cases of BSE have come from dairy herds, likely due to differences in feeding systems when compared with beef cattle. Additionally, beef cattle are typically younger at the time of slaughter. Because the average incubation period is four to seven years, infected beef cattle will generally not live long enough to develop clinical signs.

There is no experimental or epidemiological evidence for direct horizontal transmission of BSE, and there is still controversy regarding the potential for vertical transmission. No infectivity has thus far been found in milk (TAFS, 2007; SSC, 2001b), ova, semen, or embryos from infected cattle (SSC 2002a, 2001c; Wrathall, 1997; Wrathall *et al.*, 2002). Some offspring of BSE cases in the UK were also infected, and a cohort study of UK cattle concluded that vertical transmission could not be excluded. However, the role of variation in genetic susceptibility or other mechanisms in this conclusion is unclear, and no offspring of BSE cases have been reported with BSE outside the UK. If some amount of maternal transmission does occur, it is clearly not enough to maintain the epidemic, even within the UK.

2.3. Pathogenesis

In the early 1990s, infectivity studies of BSE in cattle were ongoing. At that time, experimental inoculation of tissues from BSE-infected cattle into mice had only identified infectivity in brain tissue. Therefore, definition of specified risk materials (SRM; those tissues most likely to be infective) was based on scrapie infectivity studies. Scrapie replicates primarily in the lymphoreticular system, and scrapie infectivity has been found in numerous lymph nodes, tonsils, spleen, lymphoid tissue associated with the intestinal tract and placenta. During the later preclinical phase, infectivity is found in the central nervous system (CNS). In addition, scrapie infectivity has been detected in the pituitary and adrenal glands, bone marrow, pancreas, thymus, liver and peripheral nerves (SSC, 2002b).

The first results of BSE pathogenesis studies, in which calves were intracerebrally inoculated with tissue from BSE field cases and from cattle experimentally infected by the oral route, became available in the mid-1990s (Wells *et al.*, 1996; 1998). In cattle experimentally infected by the oral route, BSE infectivity has been found in the distal ileum at specific intervals during the incubation period, starting six months after exposure (Wells *et al.*, 1994). Furthermore, CNS, dorsal root ganglia and trigeminal ganglia were found to be infective shortly before the onset of clinical signs. Recently, low levels of infectivity early in the incubation period have been detected in the palatine tonsil. In one study, sternal bone marrow collected during the clinical phase of disease was infective; however, this result has not been reproduced (therefore it may possibly have been due to cross contamination) (Wells *et al.*, 1999; Wells, 2003).

[1] Current through January 2007.

2.4. TSE agents

Although some controversy still exists regarding the nature of the BSE agent, most researchers agree that a resistant prion protein is the cause of the disease. Research has shown the agent to be highly resistant to processes that destroy other categories of infectious agents, such as bacteria and viruses, and no nucleic acid has been identified.

In eukaryotic species, most cells contain a normal prion protein, termed PrP^{C} (superscript "C" for "cellular"). This protein is normally degradable by proteases. TSEs are thought to be caused by an abnormal, infectious form of PrP^{C}, in which the steric conformation has been modified and which is highly resistant to proteinase degradation. This infectious form is most commonly termed PrP^{Sc} (initially for "scrapie"), but may also be referred to as PrP^{BSE} or PrP^{res} (for the portion that is "resistant" to a specific proteinase, proteinase K). Because prion protein is very closely related to the normal cellular PrP^{C} protein, it does not induce the production of antibodies in infected animals.

The role of PrP^{C} in normal animals is still under discussion. Genetically modified mice lacking the gene for PrP^{C} (and expressing no PrP^{C}) can be experimentally produced, but these mice have no obvious physiological changes that can be attributed to lacking the protein. They cannot, however, be infected experimentally with TSE agents.

3. MEASURES FOR CONTROL AND PREVENTION

3.1. Aims of measures

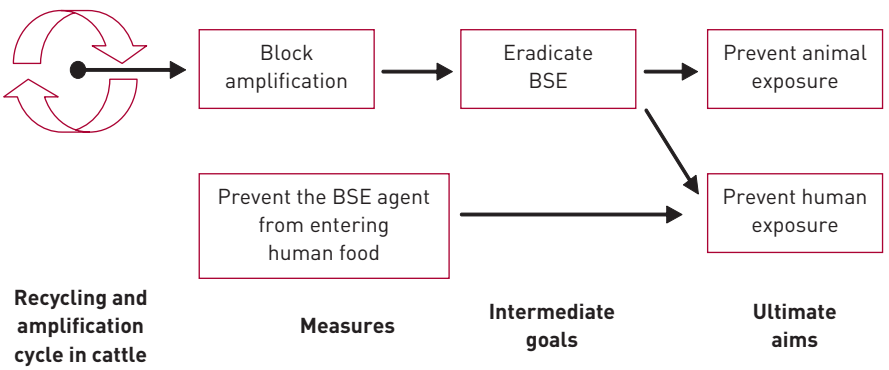
The ultimate aims of BSE control and prevention programmes are to reduce exposure risk both to cattle and to humans (Figure 3). Two levels of measures must therefore be considered:

- those that block the cycle of amplification in the feed chain;
- those that prevent infective material from entering human food.

As a result of the prolonged incubation period, it may be more than five years between effective enforcement of measures and a detectable decrease in the number of BSE cases, i.e. before the effect of the measures is seen. This interval may be even longer if the measures are not enforced effectively, as is usually the case for some time after implementation.

FIGURE 3

Reducing BSE exposure risk to both cattle and humans



Risk management for BSE is not globally harmonized. In Europe, the member states of the European Union (EU) have common rules for the implementation of measures, and other countries in Europe and countries wanting to join the EU are adapting their measures accordingly. However, the implementation of these measures still varies considerably from one country to another.

3.2. Measures to protect animal health

Feed bans

Recognition of MBM as a source of infection led to bans on feeding MBM to ruminants in order to break the cycle of cattle reinfection (DEFRA, 2004a; EC, 2004; Heim and Kihm, 1999). Implementation of a "feed ban" may mean different things in different countries. Feeds containing MBM of ruminant or mammalian origin might be banned, or the ban might include all animal proteins (i.e. mammalian MBM, fishmeal and poultry meal). The ban might prohibit feeding of the materials to ruminants or to all livestock species, or might entirely prohibit use of the material.

In some countries, a feed ban of ruminant MBM to ruminants was implemented as the first step. The ban was then often extended to mammalian MBM due to the difficulty in distinguishing between heat-treated MBM of ruminant origin and MBM of other mammalian origin. This extended ban was generally easier to control and enforce.

Even when no MBM is voluntarily included in cattle feed, there is still a risk of recycling the agent through cross contamination and cross feeding. Experience has shown that small amounts of MBM in feed are sufficient to infect cattle. These traces may result from cross contamination of MBM-free cattle feed with pig or poultry feed containing MBM, e.g. from feed mills that produce both types of feed in the same production lines, from transport by the same vehicles or from inappropriate feeding practices on farms. Apparently, using flushing batches as a safeguard against such cross contamination in feed mills is not sufficient. The traces of MBM in cattle feed that have been detected in European countries are most often below 0.1%, which seems to be enough to infect cattle. Therefore, as long as feeding of MBM to other farmed animals is allowed, cross contamination of cattle feed with MBM is very difficult to eliminate. Dedicated production lines and transport channels and control of the use and possession of MBM at farm level are required to control cross contamination fully. In most European countries, a ban on feeding MBM to all farm animals has now been implemented.

More detailed information on measures for livestock feeds can be found in subsequent chapters of this course manual.

Rendering parameters

Rendering of animal by-products (e.g. bovine tissues discarded at the slaughterhouse) and fallen stock into MBM, which is then fed to ruminants, can recycle the agent and allow amplification. When rendering processes are properly applied, the level of infectivity is reduced. It has been determined that batch (rather then continuous) rendering at 133 ºC and 3 bars of pressure for 20 minutes effectively reduces infectivity (providing that the particle size is less than 50 mm) although it does not completely inactivate the agent (Taylor *et al.*, 1994; Taylor and Woodgate, 1997; 2003; OIE, 2005a). Therefore, using these parameters does not guarantee absolute freedom from infectivity in the MBM, especially when material with high levels of BSE infectivity enters the rendering process.

More detailed information on measures for rendering can be found in subsequent chapters of this course manual.

Specified risk materials

Specified risk materials (SRM), are tissues that have been shown (or are assumed) to contain BSE infectivity in infected animals, and that should be removed from the food and feed chains (TAFS, 2004a). If these materials are removed at slaughter and then incinerated, the risk of recycling the pathogen is markedly reduced. In addition, in order to remove infectivity further from the feed chain, carcasses from high-risk cattle (e.g. fallen stock) should also be treated as SRM. Countries define SRM differently, and definitions sometimes change as new information becomes available; however, most definitions include the brain and spinal cord of cattle over 30 months (Table 1).

3.3. Measures to prevent human exposure

The above measures to protect animal health indirectly protect human health by controlling the amplification of the BSE agent. The most important direct measures for preventing human exposure to the BSE agent in foods are described in the following pages.

Ban of SRM and mechanically recovered meat for food

Excluding SRM and mechanically recovered meat (MRM) from the human food chain effectively minimizes the risk of human exposure and is the most important measure taken to protect consumers (TAFS, 2004a). MRM is a paste derived from compressed

TABLE 1. A summary of designated SRM in Europe (as of October 2005)

Species and tissue	European Union	UK and Portugal	Switzerland
		Age	
CATTLE			
Skull (including brain and eyes)	>12 months	-	>6 months
Entire head, (excluding tongue)	-	> 6 months	>30 months
Tonsils	All ages	All ages	All ages
Spinal cord	>12 months	>6 months	>6 months
Vertebral column *(including dorsal root ganglia but NOT vertebrae of tail or transverse processes of lumbar and thoracic vertebrae)*	>24 months	>30 months	>30 months *(includes tail)*
Intestines and mesentery	All ages	All ages	>6 months
Spleen	-	>6 months	-
Thymus	-	>6 months	-
SHEEP AND GOATS			
Skull (including brain and eyes)	>12 month	>12 months	>12 months
Spinal cord	>12 months	>12 months	>12 months
Tonsils	>12 months	>12 months	All ages
Ileum	All ages	All ages	All ages
Spleen	All ages	All ages	All ages

carcass components from which all non-consumable tissues have been removed. These carcass components include bones as well as the vertebral column with the spinal cord and dorsal root ganglia often attached. The MRM is then used in cooked meat products, such as sausages and meat pies, and, if ruminant material is included, is regarded as a major BSE risk factor.

BSE detection at slaughter

Measures for minimizing risks for human health require the identification and elimination of clinically affected animals before slaughter, which can only be achieved through an adequate surveillance programme including an ante mortem inspection specific for BSE. Because the SRM from clinically affected animals is known to contain infectivity, removal and destruction of these animals **prior** to entering the slaughterhouse have two clearly positive effects:

- The risk of infective material entering the food and feed chains is reduced.
- There is less contamination of the slaughterhouse, and less potential for cross contamination of normal carcasses.

In addition, most countries in Europe have been conducting laboratory testing of all slaughter cattle over 30 months of age (or even younger) for BSE since 2001 (TAFS, 2004b).

The benefits of testing regular slaughter cattle are:

- It identifies the very few positive animals that may not yet be showing clinical signs.
- It decreases the risk of contaminated material entering the food chain in those countries where other measures (e.g. ante mortem inspection, SRM removal) may not be effectively implemented.
- It could increase consumer confidence in beef and beef products.
- It may allow import bans to be lifted (although some imports bans may be in violation of WTO rules).

The drawbacks are:

- It is extremely expensive.
- It may give a false sense of security to consumers.
- It may diminish the incentive to implement effectively and enforce other, more effective measures (such as ante mortem inspection).
- It could lead to increased contamination within slaughterhouses due to processing of a greater number of positive carcasses if other measures are not implemented.

All currently available methods for diagnosing BSE rely on the detection of accumulated PrP^{Sc} in the brain of infected animals. Therefore, cattle must have already been slaughtered before confirmation of disease status can be made, potentially increasing the risk of contamination of carcasses with an infectious agent. To prevent this, identification and removal of clinically affected animals by the farmer or veterinarian during an ante mortem inspection are optimal control steps.

Measures to avoid cross contamination of meat with SRM

It has been shown that the use of certain types of captive bolt guns to stun cattle prior to slaughter causes brain tissue to enter the blood stream that could, be disseminated throughout the carcass (including muscle). Therefore, pneumatic bolt stunning and

pithing are now forbidden by many countries in Europe and elsewhere. Hygienic measures taken in the slaughterhouse to reduce potential contamination of meat with SRM are also important.

More detailed information on SRM removal and other meat production issues can be found in the *Capacity Building for Surveillance and Prevention of BSE and Other Zoonotic Diseases* project course manual entitled *Management of transmissible spongiform encephalopathies in meat production* (FAO, 2007a).

3.4. On-farm measures

Classical control measures for infectious diseases (biosecurity, quarantine, vaccination) do not generally apply to BSE. Given all available evidence, the BSE agent is not transmitted horizontally between cattle but only through feed, primarily ingestion of contaminated MBM during calfhood. When a BSE case is detected, it has been shown that other cattle within that herd are unlikely to test positive for BSE, despite the likelihood that many calves of similar age to the case all consumed the same contaminated feed.

However, some on-farm strategies, primarily those that focus on feed as a source of infection, and some culling programmes do contribute to the control and eradication of BSE. Culling strategies vary among countries, and often change over time. Some different culling strategies that have been applied include (SSC, 2000; 2002c).

- the index case only
- all cattle on the farm where the index case was diagnosed
- all cattle on the farm where the index case was born and raised
- all cattle on the index case farm and on the farm where the index case was born and raised
- all susceptible animals on the index case farm (including sheep, goats and cats)

} Herd culling

- "feed-cohort" (cattle that could have been exposed to the same feed as the index case)
- "birth-cohort" (all cattle born one year before or one year after the index case and raised on the same farm)

} Cohort culling

While herd culling may be a politically expedient means of increasing consumer confidence and facilitating exports, it is unlikely to be an efficient risk management measure (Heim and Murray, 2004). There are significant problems in implementing such a strategy. Farmers see it as a radical approach because it results in a considerable waste of uninfected animals. Although there may be sufficient compensation for culled animals, farmers may not believe it is reasonable to cull apparently healthy, productive animals. In addition they are likely to lose valuable genetic lines and/or their "life's work". For these reasons, farmers may be less willing to notify suspect cases if culling of their entire herd could result.

Evidence from a number of countries indicates that, in those herds where more than one case of BSE has been detected, the additional case(s) were born within one year of the index case. As a result, culling a birth cohort is a more rational risk management strategy as it focuses on those animals within a herd that have the greatest chance of having BSE. Even so, depending on the initial level of exposure and the original size of the cohort, it is likely that relatively few additional cases of BSE will be detected in the

birth cohort of a herd index case. Cohort culling is, however, likely to be much more acceptable to farmers when compared with herd culling.

3.5. Import control

The best means of preventing the introduction of BSE is to control the import of certain BSE risk products from countries with BSE or countries that are at risk of having BSE. Most countries do not ban imports of potentially infective materials until the exporting country has reported their first BSE case. This is usually too late, however, because the risk already existed before the first case was detected. Materials that should be considered risky for import (unless appropriate safety conditions are met) include any mammalian derived meals (including MBM and other protein meals), feed containing MBM, live cattle and offal. Import of beef and beef products for human consumption, including processed beef products, whole cattle carcasses and bone-in beef, should also be controlled, especially for the exclusion of SRM. Deboned beef meat is generally considered as non-risky for import.

3.6. Enforcement

Although implementation of each measure decreases the overall risk of exposure, combining measures decreases the risk more profoundly (Heim and Kihm, 2003). For example, feed bans implemented in conjunction with an SRM ban for feed have a stronger impact. Moreover, measures must be effectively implemented and enforced. Simply issuing a regulation or ordinance without providing the necessary infrastructure and controls will not achieve the desired goals. Education of all people involved is required at all levels and in all sectors in order to improve understanding and capacity, and thus improve compliance.

4. CLINICAL SIGNS OF BSE

In contrast to many BSE cases pictured in the media, most cattle with BSE have subtle signs of disease. Signs are progressive, variable in type and severity, and may include depression, abnormal behaviour, weight loss, sensitivity to stimuli (light, sound, touch) and gait or movement abnormalities. Other signs that have been noted in some BSE cases include reduced milk yield, bradycardia and reduced ruminal contractions (Braun *et al.*, 1997).

Differential diagnoses for BSE include bacterial and viral encephalitides (e.g. borna disease, listeriosis, sporadic bovine encephalitis, rabies), brain edema, tumors, cerebrocortical-necrosis (CCN), cerebellar atrophy, metabolic diseases and intoxications, as well as other causes of weight loss and neurological abnormalities.

Because none of the clinical signs are specific (pathognomonic) for the disease, a definitive clinical diagnosis cannot be made. With experience, however, farmers and veterinarians can become efficient at early identification of BSE suspects. These suspicions should always be confirmed through laboratory testing.

5. DIAGNOSIS OF BSE

5.1. Biosafety

Microorganisms are classified by the World Health Organization (WHO) according to their pathogenicity for humans and animals. According to this classification, precautions must be taken when handling these agents primarily to protect the people

handling them and also to protect the general human population and livestock from accidental exposure. Depending on the classification of the microorganism, precautions also must be taken to protect laboratory workers and the community from possible exposure and infection. Thus, WHO has defined four biosafety level (BL) categories for laboratories. These categories correlate somewhat with the WHO risk group categories, but also reflect what is being done with the microorganism in the laboratory.

The most internationally well-accepted guideline on the classification system for and the handling of microorganisms is the *WHO Laboratory biosafety manual* (WHO, 2003). This manual defines the risk groups, the requirements for risk assessments, and the requirements for each of the laboratory BLs.

In 2000, the EU published a directive based on the WHO guidelines, which defines a new risk group for BSE and related animal TSEs based on BSE agent characteristics (e.g. limited risk for laboratory personnel and the community, inability to exclude aerosol transmission). 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.

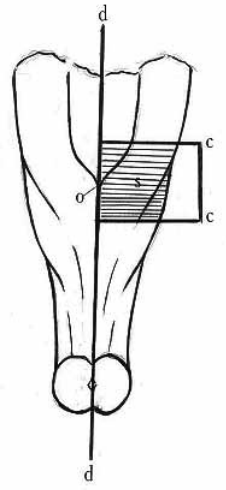
According to the Swiss Expert Committee for Biosafety, different biosafety levels are required when handling BSE materials, depending on the type of material (Swiss Expert Committee for Biosafety, 2006). For example, histology and Immunohistochemistry (IHC) on formic acid-inactivated BSE material can be performed in a BL 1 laboratory, and routine BSE diagnostics can be performed in a BL 2 laboratory 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 among countries.

5.2. Sample collection

Because both the highest concentration of PrP^{Sc} and the most prominent related lesions tend to be located in the area of the obex region of the brainstem (Figure 4), sampling this region optimizes sensitivity, regardless of the diagnostic test method used. If this

FIGURE 4

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



region is not sampled correctly, false negative results may be obtained. This requires that individuals collecting samples are familiar with the anatomy in this region.

All animals clinically suspected of having BSE should be examined post mortem. Optimally, several representative areas of the brain of clinical suspects are examined; therefore, the whole head of the animal should be removed and sent to the laboratory. As well, this allows tests to be performed for other differential diagnoses. At the laboratory, the brain is removed as soon as possible for further testing and one half is fixed in formalin (for histopathology and IHC). The remaining half of the brain is first sampled for rapid tests and then frozen at -20 °C or -80 °C.

In cases of emergency slaughter, fallen stock or routine screening, only the caudal brainstem (medulla oblongata) is generally removed for testing, without opening the skull. The caudal end of the brainstem should be visible through the foramen magnum after separation of the head, and a specially designed spoon can be used to remove the brainstem (including the obex region) through the foramen. The brainstem is then split longitudinally, and one half fixed in formalin for histopathology and IHC while the other half is reserved and sampled for rapid tests. The fresh tissue remaining after sampling for rapid tests is then frozen at -20 °C or -80 °C.

For neuropathology and IHC, tissue is fixed in formalin, inactivated with formic acid, and then embedded in paraffin. The embedded brain samples are sectioned and placed on glass slides. For neuropathologic examination, sections are then stained with standard haematoxylin and eosin (H & E) stain.

5.3. Neuropathology and immunohistochemistry

Visualization of typical neuropathologic changes requires that the tissue structure be intact. Therefore it may not be possible to evaluate even slightly autolytic samples (e.g. samples from fallen stock or cadavers, samples improperly fixed for transport). Freezing of samples also destroys the tissue structure.

After characterization of the histopathologic features present in a sample, BSE must be differentiated from other neural diseases showing similar lesions. 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 a normal brain, so possible causes of spongiform changes must be differentiated (e.g. normal vacuolation vs pathological vacuolation vs vacuolation from post mortem artifacts). "Encephalopathy" refers to the fact that the disease is primarily degenerative and, apart from gliosis, does not show any inflammatory changes.

After neuropathologic examination, IHC can be used to identify PrP^{Sc} directly, in the sample by labelling it with specific antibodies. In some cases, IHC may allow a definitive diagnosis of BSE to be made when questionable or even no neuropathologic changes are seen.

However, because the normal PrP protein (PrP^{C}) present in the brain cells has the same amino acid sequence as PrP^{Sc}, antibodies normally used in IHC detect both PrP^{Sc} and PrP^{C}. Therefore, in order to be able to determine if there is any PrP^{Sc} present, the two proteins must first be differentiated. Proteinase K is an enzyme that causes total proteolysis of normal PrP^{C}, although PrP^{Sc} is resistant to proteolysis by proteinase K to a large extent. Only small parts at the beginning and at the end of PrP^{Sc} are digested and the remaining part, generally referred to as the core fragment or PrP^{res}, is still

detected by the antibodies. Therefore, proteinase K is used in IHC to digest totally the PrP^{C} present in the sample, ensuring that any PrP detected will be PrP^{Sc}. Without this step, samples could yield a false positive result because of the detection of normal PrP^{C}. Similarly, incomplete digestion could lead to false positive results.

For most antibodies used in testing, the respective epitope on PrP is not accessible in the native PrP conformation. Therefore, an additional step to demask the appropriate epitope on PrP^{res} is required. Demasking can be accomplished by denaturation of the protein or by using non-specific proteases.

5.4. Rapid BSE tests

Tests are available to analyse BSE suspect materials rapidly (OIE, 2005b). Which rapid tests are licensed and approved in various countries throughout the world are variable and lists are constantly being updated (European Food Safety Authority, 2005).

All currently licensed BSE rapid tests have several things in common. First, they use material from the brainstem, i.e. they are post mortem tests. Second, current rapid tests are 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 among tests, there are significant differences in the execution. The materials and procedures are specific to each test system and test performance is validated under these specific conditions, thus protocols cannot be modified or interchanged among tests.

Initially, the sample of central nervous system (CNS) material must be homogenized with a specific buffer containing stabilizers and detergents. After homogenization, proteinase K is used to digest the PrP^{C} (with the exception of the IDEXX HerdChek BSE Antigen EIA) and the epitope is demasked. Then, the proteinase K resistant fragment of PrP^{Sc}, if present, is detected with specific monoclonal or polyclonal antibodies using western blot or enzyme linked immunosorbent assay (ELISA) technology.

Although there are differences between the tests, the overall performance (sensitivity and specificity) is comparable. Great differences can be found in the handling and the versatility of the tests for high and low throughput laboratory set-ups.

5.5. New developments

Work is constantly being done on the development of new rapid tests. New tests may be based on the refinement of an established procedure or on the replacement of procedures by completely new concepts.

All 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 has so far passed the validation process, and an imminent breakthrough in ante mortem testing is not foreseen.

Diagnosis of TSEs is covered in depth in the *Capacity Building for Surveillance and Prevention of BSE and Other Zoonotic Diseases* project course manual *Diagnostic Techniques for transmissible spongiform encephalopathies* (FAO, 2007).

6. SURVEILLANCE SYSTEMS

6.1. Objectives of surveillance

The two major objectives for BSE surveillance are to determine whether BSE is present in the country and, if present, to monitor the extent and evolution of the outbreak over time. In this way, the effectiveness of control measures in place can be monitored and evaluated. However, the reported number of BSE cases in a country can only be evaluated within the context of the quality of the national surveillance system and the measures taken. BSE risk can still exist in a country, even if no cases are found with surveillance. Surveillance aims to supplement the more comprehensive data provided by a risk assessment (Heim and Mumford, 2005).

General guidelines for disease surveillance and specific guidelines for an appropriate level of BSE surveillance for the different categories of national risk are provided in the *OIE Terrestrial Animal Health Code* (OIE, 2005 c,d). These recommendations are considered by the WTO (WTO; World Trade Organization, 1994) and the international community as the international standards.

6.2. Passive surveillance

In most countries BSE is listed as a notifiable disease, which is a basic requirement for a functioning passive (as well as active) surveillance system. However, some countries have no national passive surveillance system for BSE, or only a weak system.

Until 1999, BSE surveillance in all countries was limited to the notification of clinically suspected cases by farmers and veterinarians (and others involved in handling animals) to the veterinary authorities (passive surveillance). It was assumed that this would allow early detection of an outbreak (Heim and Wilesmith, 2000). However, because passive surveillance relies solely on the reporting of clinical suspects and is dependent on many factors, including perceived consequences on the farm and diagnostic competence, it is not necessarily consistent or reliable. Thus, although passive surveillance is a crucial component of any BSE surveillance system, it has become increasingly obvious that passive surveillance alone is not sufficient to establish the real BSE status of a country.

For a passive system to function effectively, several factors must be in place:

Veterinary structure:	The disease must be notifiable.
Case definition:	A legal definition of BSE must exist and must be broad enough to include most positive cases.
Disease awareness:	The appropriate individuals (farmers, veterinarians) must be able to recognize clinical signs of the disease.
Willingness to report:	There must be minimal negative consequences to the identification of a positive case at the farm level and measures must be considered "reasonable".
Compensation scheme:	The costs of culled animals must be reasonably compensated.
Diagnostic capacity:	There must be adequate laboratory competence.

Because these factors vary greatly, both among countries and within countries over time, the results of passive BSE surveillance systems are subjective and evaluation and comparison of reported numbers of BSE cases must be made carefully.

6.3. Active surveillance

To optimize identification of positive animals and improve the surveillance data, those populations of cattle that are at increased risk of having BSE should be actively targeted within a national surveillance system. With the introduction of targeted surveillance of cattle risk populations in 2001, a large number of countries in Europe and also the first countries outside Europe detected their first BSE cases.

Cattle with signs of disease non-specific to BSE and cattle that died or were killed for unknown reasons may be defined in different countries as sick slaughter, emergency slaughter, fallen stock or downer cows. The probability of detecting BSE-infected cattle is higher in these populations, as it may have been BSE that led to the debilitation, death, cull or slaughter of these animals. Many of these cattle may have exhibited some of the clinical signs compatible with BSE, which were not recognized. The experience of many countries in the last years has shown that, after clinical suspects, this is the second most appropriate population to target in order to detect BSE. Targeted surveillance aims to sample cattle in these risk groups selectively, and testing of these risk populations is now mandatory in most countries with BSE surveillance systems in place.

Healthy cattle	→	Routine slaughter	
Cattle with non-specific signs (e.g. weight loss, loss of production) and cattle that died for unknown reasons (on the farm, during transport)	→	Sick/emergency slaughter, fallen stock, downer cows	Risk groups
Cattle with specific signs of BSE (or suspicion of BSE)	→	BSE suspects	

The age of the population tested is also important, as the epidemiological data show that cattle younger than 30 months rarely test positive for BSE. Therefore, targeted surveillance aims to sample cattle selectively over 30 months of age in the risk populations, which may be identified on the farm, at transport or at the slaughterhouse.

However, despite the fact that correctly implemented sampling of risk populations would hypothetically be sufficient to assess BSE in a country, testing a subsample of healthy slaughtered cattle should be considered. This is needed to minimize diversion of questionable carcasses to slaughter, i.e. to improve compliance. If farmers are aware that random sampling is occurring, and when the probability of being tested is large enough, they are less likely to send suspect animals directly to slaughter.

The specific surveillance approaches vary among the different countries. The EU and Switzerland are testing the entire risk population over 24 and 30 months of age, respectively. In the EU, additionally, all cattle subject to normal slaughter over 30 months of age are currently tested, whereas in Switzerland a random sample of approximately 5% is tested. Countries outside Europe have implemented a variety of different testing systems. From the experiences gained in Europe, it is clear that it is most efficient to ensure the effective implementation of passive and targeted surveillance in risk populations rather than to focus on testing of the entire normal slaughter population.

Surveillance for TSEs is covered in depth in the *Capacity Building for Surveillance and*

Prevention of BSE and Other Zoonotic Diseases project course manual entitled *Epidemiology, surveillance, and risk assessment for transmissible spongiform encephalopathies* (FAO, 2007).

7. RISK ASSESSMENT

7.1. BSE status and international standards

For a long time, BSE was considered a problem exclusively of the UK. Even after the detection of BSE cases in several countries outside the UK, the risk of having BSE was categorically denied by many other countries. Only after the introduction of active surveillance did several "BSE-free" countries detect BSE.

Before 2005, the OIE described five BSE categories for countries, but in May 2005 a new BSE chapter was adopted (OIE, 2005d) reducing the number of BSE status categories to the following three:

- Country, zone or compartment with a negligible BSE risk
- Country, zone or compartment with a controlled BSE risk
- Country, zone or compartment with an undetermined BSE risk

According to the OIE, a primary determinant for establishing BSE risk status of a country, zone or compartment is the outcome of a science-based national risk assessment. This assessment may be qualitative or quantitative, and should be based on the principles given in the Code Chapters 1.3.1 and 1.3.2 on Risk analysis and the Appendix 3.8.5 on Risk analysis for BSE (OIE, 2005 e,f,g). The OIE Code Chapter on BSE (OIE, 2005d) lists the following potential factors for BSE occurrence and their historic perspective that must be considered in such an assessment:

***Release assessment*[2]**

- the TSE situation in the country
- production and import of MBM or greaves
- imported live animals, animal feed and feed ingredients
- imported products of ruminant origin for human consumption and for *in vivo* use in cattle

In addiction, surveillance for TSEs and other epidemiological investigations (especially surveillance for BSE conducted on the cattle population) should be taken into account.

Exposure assessment:

- recycling and amplification of the BSE agent
- the use of ruminant carcasses (including from fallen stock), by-products and slaughterhouse waste, the parameters of the rendering processes and the methods of animal feed manufacture
- the feeding bans and controls of cross contamination and their implementation
- the level of surveillance for BSE and the results of that surveillance

In addition to an assessment of BSE risk, the OIE status categorization for BSE includes evaluation of some of the measures in place in the country. According to the

[2] In 2006, the OIE BSE chapter was modified so that only BSE, and not other TSEs, is included in the exposure assessment.

OIE Code, factors evaluated in the establishment of BSE status should include:

- the outcome of a risk assessment (as described above)
- disease awareness programmes to encourage reporting of all cattle showing clinical signs consistent with BSE
- compulsory notification and investigation of all cattle showing clinical signs consistent with BSE
- examination in an approved laboratory of brain samples from the surveillance and monitoring system.

7.2. The geographical BSE risk assessment

The geographical BSE risk assessment (GBR) is a BSE risk assessment tool developed by the Scientific Steering Committee of the European Commission and based on OIE assessment criteria. The GBR is a qualitative indicator of the likelihood of the presence of one or more cattle being infected with BSE, at a given point in time in a country, and has been applied to a number of countries throughout the world. The method is a qualitative risk assessment, which uses information on risk factors that contribute either to the potential for introduction of BSE into a country or region or to the opportunity for recycling of the BSE agent in a country or region. The following questions, related to release and exposure, are answered through the GBR:

- Was the agent introduced into the country by import of potentially infected cattle or feed (MBM), and if so to what extent?
- What would happen if the agent were introduced into the animal production system, i.e. would it be amplified or eliminated?

Before the detection of the first cases in many "BSE-free" countries, the GBR showed that a risk could be present. This confirmed the concept that a serious, comprehensive risk assessment must be carried out to estimate the extent of the BSE problem in countries.

Thus, decisions on preventive measures should be based on such a detailed risk assessment, whether it is the GBR or another science-based assessment based on OIE recommendations. No country should wait until the first case occurs before taking preventive measures. There remain many countries with an unknown BSE risk. In order to minimize import risks from these countries, further risk assessments are needed to evaluate the real BSE distribution worldwide.

Risk assessment for TSEs is covered in depth in the *Capacity Building for Surveillance and Prevention of BSE and Other Zoonotic Diseases* project course manual *Epidemiology, surveillance and risk assessment for transmissible spongiform encephalophathies* (FAO, 2007c).

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OVERVIEW: IMPLEMENTATION OF TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHY MEASURES FOR LIVESTOCK FEEDS

1. GENERAL CONCEPTS

Currently, it is well accepted that cattle are exposed to the BSE agent through the ingestion of contaminated feed. Therefore, a key measure in preventing the spread and recycling of the BSE agent is to prevent ruminant-derived proteins from being fed to ruminants. Ultimately, this is the simple goal of any feed ban, however, achieving this goal is not so simple. Experience in Europe and elsewhere has shown that a series of effective measures must be implemented to assure that this goal is met.

When feed bans were first introduced in Europe, the goal of preventing ruminant material from being fed to ruminants was logically implemented in the least disruptive manner possible (i.e. ruminant to ruminant bans or mammal to ruminant bans). Later, "flushing" batches (batches of feed processed or transported in between feed batches containing prohibited and non-prohibited materials) were introduced to reduce traces of prohibited materials from the equipment and reduce cross contamination. These measures do indeed reduce the risk of exposure of cattle to the BSE agent; however, the continued appearance of animals with BSE that were born after the implementation of these initial bans (animals born after the ban, or "BAB") showed that the measures were insufficient (Heim and Kihm, 2003).

It has become clear that a complementary system of integrated feed control measures must include (1) a ban on including specified risk material (SRM; those animal tissues most likely to contain TSE infectivity, including bovine fallen stock) in feeds, (2) strict feed bans that are effectively enforced, and (3) control of cross contamination at every step along the feed production chain from transport of raw materials through feeding practices on the farm, in order to ultimately prevent exposure of ruminants to potential infectivity (Heim and Kihm, 2003). In addition, restrictions on imports of risky products must be evaluated, to prevent entry or re-entry of infective material. Enforcement and control of all measures through self-regulation and external audits is crucial. Finally, education and disease awareness must be promoted in order to promote compliance with the measures at every step along the feed production chain, including at the farm level.

Exactly which measures are implemented in a country will depend on national BSE risk (as determined by a national BSE risk assessment), economics, trade, and capacity available to effectively enforce the measures. In some cases, more restrictive measures may be easier to enforce, and therefore may be more economically justifiable but all measures ultimately aim to prevent the ingestion of the BSE agent by ruminants.

2. BAN ON SRM IN FEEDS

Because SRM potentially contains the highest BSE infectivity, it should be excluded not only from the human food chain, but also from the feed chain, at minimum for ruminant species but optimally for all animals. Rendering of SRM, even at the standard processing

parameters of 133 ºC and 3 bars of pressure for 20 minutes, does not entirely inactivate the agent (Taylor and Woodgate, 2003). Therefore removal and subsequent destruction of all SRM (including fallen stock and BSE suspect animals) is a very effective method for excluding this infectivity from the BSE recycling system and substantially reducing the risk of intentional or inadvertent exposure of ruminants. Controls at the slaughterhouse (as well as at the rendering plant in the case of fallen stock) must be in place to assure the appropriate separation and disposal of the different types of material.

3. SCOPE OF FEED BANS

Currently, different countries have different bans, i.e. different rules regarding what specific materials are prohibited in feeds and for what species but all bans aim to ultimately prevent the ingestion of ruminant material by ruminants. Feed bans directed at particular groups of animals (ruminant to ruminant, mammalian to ruminant) are generally more difficult to implement and enforce than those with a broader scope (mammalian to mammalian, or mammalian to all livestock) because, in any agricultural system, the presence of material and feeds prohibited for one species and not for others increases the risk for cross contamination or cross feeding. Moreover, available feed tests are generally more effective at distinguishing between materials that are very different (e.g. plants vs. animals, or fish vs. terrestrial animal) than at differentiating between materials from different mammalian species.

Thus, taking into consideration national TSE risk, in some countries it may be more feasible to implement a broader ban. However, broader bans have greater economic consequences, as more animal by-products must be alternately disposed of and, in some cases, substantial changes in the management of the feed production lines are required.

4. CONTROL OF CROSS CONTAMINATION

The importance of cross contamination has been emphasized with the continued appearance of BAB animals in Europe. It is known that as little as one milligram of infective brain material is sufficient to infect a calf (Danny Matthews, Veterinary Laboratories Agency, UK, personal communication, 2005). In the production of feeds with batch sizes of about one tonne, even the small amounts of cross contamination that normally occur could allow this amount of material to be present in subsequent batches. Therefore, in countries without other adequate measures (such as a ban on SRM) this amount of cross contamination could lead to exposure (and potentially infection) of cattle.

Currently, it is believed that dedicating separate production lines for feeds containing prohibited materials and those not containing prohibited materials (depending on the feed ban in place) is the most effective measure for preventing this potential exposure. Alternatively, entirely separate plants may be established. Certainly, if feed for pet animals is produced using animal-derived materials, these production lines must be separate. The extent of line separation for other livestock feeds will be dependent on the particular feed ban in place. In addition, control of cross contamination must be implemented at each step along the feed production chain from handling raw materials through production, transport, and storage, to feeding on the farm. This requires adequate record-keeping and education of personnel in all the operations involved.

5. IMPORT RESTRICTIONS

The spread of BSE to countries throughout the world has been attributed to trade in live cattle, animal by-products (primarily meat and bone meal/MBM) and feeds containing (or potentially containing) MBM. This risk is not limited to exporting countries that have reported BSE cases. Therefore, importing countries must not only evaluate their domestic risk but also the risk posed by any imports of these risky products.

6. ENFORCEMENT

In order to be effective at reducing risk, all implemented measures must be controlled and enforced through a system of controls and audits integrated at every step along the feed production chain. A system for sampling and analysis of feeds and feed ingredients to detect prohibited materials which may be present due to non-compliance with the feed ban or owing to cross contamination must also be in place, which includes testing of both domestically produced products and imports. The testing method chosen must be appropriate not only to the specific feed ban in place, but also to the process parameters that were used or potentially used in production.

These basic concepts are developed in further detail, in parallel with current general rendering and livestock feed production concepts, in the following course manual chapters. At the end of many chapters, bullet points emphasize the TSE-relevant concepts from the information presented within the chapter.

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PROTEIN USE IN LIVESTOCK FEEDING

1. GENERAL CONCEPTS

All animals require certain basic nutrients, including energy (from fats and carbohydrates), protein, minerals and vitamins. Compound feeds are formulated to provide all or most of these nutritional requirements for a particular species and/or production class of livestock. It is important to consider production class when formulating rations, because as an animal grows more energy is needed for maintenance of the larger body mass and to support an increasing proportion of fat deposition. Thus, young growing animals have greater requirements for many nutrients than do older animals. Similarly, classes of high-producing animals (such as lactating dairy cattle) will also have higher nutritional requirements.

In general, the protein requirements for animals are given as some amount of protein (or sometimes of specific amino acids) per unit of time, usually the amount required per day. Protein requirements are usually also expressed as a concentration within the ration (diet), usually as g/kg of the ration as fed. As with other nutritional requirements, protein requirements differ greatly depending on livestock species and production class. In older animals both the required protein concentration of the ration and the protein:energy ratio decline. In addition, in older animals the voluntary feed intake increases, so that the increased amount of protein required can be met with a lower protein concentration in the ration. Therefore, it is important to be specific when describing requirements for protein, as well as for amino acids. A detailed review of the protein requirements of livestock is available from Miller (2004).

When formulating rations, other nutrients and micronutrients contained in the various sources and supplements must be considered not only because of their nutritional benefit but also because, in some cases, they may be present at detrimentally high levels. These nutrients include the major minerals, calcium (Ca), phosphorus (P), sodium (Na), potassium (K), and chlorine (Cl), as well as vitamins (including vitamin B_{12}, choline and vitamin D) and essential fatty acids.

1.1. Protein requirements for monogastric animals

Although many ration formulations are described in terms of crude protein (CP; Table 1), monogastric animals do not have a requirement for crude protein as such. Instead, monogastric animals require nine to ten specific amino acids that they cannot synthesize, together with a source of amino nitrogen that can be used for the synthesis of the remaining amino acids. Therefore, the ration must be formulated in consideration of those amino acids that are *essential* and those that are *limiting*.

Essential amino acids are those that cannot be synthesized and therefore must be provided by the ration. In addition, the amino acids cysteine and tyrosine can be synthesized in the body but only from the essential amino acids methionine and phenylalanine, respectively. Consequently, cysteine and tyrosine are not absolutely required if sufficient methionine and phenylalanine are available, and so are termed *semi-essential*. However, if cysteine and tyrosine are also provided in the ration, then additional methionine

TABLE 1. Typical crude protein (CP) concentrations in rations for various classes of monogastric livestock species (in grams per kg air-dried feed)

Class	Weight/age	CP g/kg
SWINE		
Starter: 3 week weaning	5-10kg	240
5 week weaning	10-20kg	210
Grower	20-60kg	165
Finisher	60-90kg	140
Sows: lactating		176
pregnant		130
BIRDS		
Broiler: starter	0-2 weeks	230
grower	2-4 weeks	210
finisher	4-7 weeks	190
Rearing pullets	0-6 weeks	210
	6-12 weeks	145
	12-18 weeks	120
Laying hens		160
Turkey: starter	0-6 weeks	300
grower	6-12 weeks	260
finisher	12 + weeks	180
Breeding turkeys		160
FISH		
Salmonids fry	fingerlings	550
	smolt	400-460
Catfish		320-360

Source: adapted from Miller EL, 2004.

and phenylalanine are not required. Arginine is an essential amino acid for birds and fish but is synthesized in mammals as part of the urea cycle. However, the amount available for protein synthesis may be inadequate as most of the synthesized arginine is broken down to release urea. Therefore, additional dietary arginine may promote growth in young animals. In certain situations, such as in young animals and rapidly growing chicks, glycine and serine may not be synthesized in sufficient quantities, and so are termed *conditionally essential*.

Limiting amino acids are those essential amino acids for which the requirements can not be met because the amino acid compositions of the different protein sources in a ration do not match the needs of (i.e. are not balanced for) the specific production class. Not surprisingly, animal protein sources are usually better able to match the amino acid needs of animals, and so better able to meet the requirements. Protein sources that closely match the requirements of a particular species or production class well are termed *high quality* proteins for that class. In attempting to formulate a balanced ration, the minimal aim is to meet the requirements for the first two limiting amino acids. Of course, an optimally balanced ration will meet the animal's needs for all amino acids, however this is difficult to achieve.

For poultry, methionine plus cysteine (M+C; generally considered together as cysteine can be derived from methionine) and lysine are usually the first two limiting amino acids in commonly used feed ingredients. Lysine requirements may be met by complementing lysine-deficient cereal grains with a lysine-rich legume protein such as soya. However,

as proteins from both of these sources are also deficient in M+C, the mixed ration remains M+C deficient. This is normally and most economically corrected by supplementation with synthetic methionine.

For pigs, lysine is normally the first limiting amino acid. Extensive research into dietary requirements for young pigs has determined the ideal amino acid pattern and allowed excesses of indispensable amino acids to be eliminated, resulting in a maximal overall reduction in protein required in these rations. Consequently, this can appear as a proportional increase in required lysine. As some of the animal proteins have very high levels of lysine, they are optimal for use in balancing the amino acid composition of the cereal component of these rations to achieve the ideal ratio.

In the past, most ration formulation has been based on the chemically determined amino acid content of feeds, i.e. established "book values" representative of the class rather than analyses of the individual feed batch. Some improvement in these established values may be made by determining CP content of the batch and the published regression equations of amino acid content in relation to CP. An additional problem is that, at the tissue level, the amino acids chemically determined to be present in the ration may not be available to the animal. Thus, it is also necessary to consider amino acid availability.

1.2. Protein requirements for ruminants

In the ruminant, ingested feed is fermented by microorganisms in the rumen. Volatile fatty acids absorbed from the rumen and omasum provide the major part of the metabolizable energy to the animal. The fermented digesta, as well as ruminal microflora then leave the rumen and are further digested in the abomasum (true stomach) and intestines (as in the monogastric animal). The majority of amino acids available to ruminants are provided by digestion and absorption of microbial protein in the small intestine. Because the amino acid balance of microbial protein is generally more similar to that of livestock than are cereal grains, the amino acid requirements of ruminants can be met at maintenance level by microbial protein alone. With an increase in energy supplied in the ration, extra microbial protein is produced and an increased level of production can be sustained.

However, the microbial protein yield is eventually limited by the fermented energy supply, therefore for moderate and high levels of production the microbial amino acid supply must be supplemented with dietary sources of protein, or protected amino acids that escape degradation in the rumen. The nitrogen (N) requirements of ruminants producing at higher production levels are thus twofold:

1. A source of degradable N to meet the needs of the rumenal microbial microflora. This can largely be met by non-protein N sources, such as urea, which are converted to ammonia in the rumen. However, growth of the microflora is additionally stimulated by a supply of protein peptides.
2. A source of non-degradable N that is later digested in the small intestine and provides amino acids to complement the microbial amino acids and meet tissue needs ("by-pass proteins").

Hence, important characteristics for protein supplied in the ration are the rate and extent of degradation in the rumen, which effect the protein's contribution not only to the ammonia and peptide needs of the microflora but also to the supply of amino acids for meeting tissue needs. Considerable variation in these characteristics exists among

different protein sources. Another important characteristic of proteins is the digestibility of the undegraded protein that leaves the rumen. Microbial protein has a true digestibility of 85% in the small intestine, but values for feed proteins can range from 50 to 90%. Finally, as with monogastric animals, the amino acid composition of the digested protein must also be considered.

2. PROTEIN SOURCES AND SUPPLEMENTS

Most rations, especially those for young growing or high-producing livestock classes, will require balancing with an additional protein source or protein supplement. Historically, an inexpensive source of protein for manufacture of livestock feeds has been meals made from rendered by-products of animals (Matthews and Cooke, 2003), particularly meat and bone meal (MBM). However, after the emergence of BSE and the direct implication of ruminant-derived protein in the transmission and recycling of the BSE agent, it was determined that effective control of the disease requires restrictions on the feeding of some types of animal-derived proteins to some or all classes of livestock (as described in the "Overview: Implementation of TSE measures for livestock feeds" chapter in this course manual). One important result of the implementation of the feed bans in many countries worldwide has been a dramatic decrease in the use of MBM and other animal-derived meals for livestock feeding. Consequently, new sources of protein are being sought and older sources reconsidered.

Classes of protein sources include animal-derived meals, plant protein sources, and synthetic amino acids. These sources, as well as other protein sources that must be considered in the context of TSEs, are described below.

2.1. Animal-derived meals

In addition to classic MBM, which generally contains by-products from multiple species (including ruminants), other sources of rendered animal protein include blood meal, meat meal, bone meal, and feather meal, as well as meals made from exclusively fish or poultry material. In some countries, dedicated rendering plants produce MBM from pure equine or porcine material. Animal-derived meals tend to have amino acid compositions that match the requirements of animals (as previously described), as well as being high in vitamin B12. Animal-derived meals are also high in lysine, which is important because lysine is the first limiting amino acid in feeds for many livestock classes.

Unfortunately, the definitions applied to various meals produced through rendering are not always consistent between countries, which causes some confusion. For example, if the ash content is high, this may indicate that the meal contains some amount of bone, and so it is referred to as MBM. If the ash content is lower it may be referred to as meat meal. The term "meat meal" in the context of international trade also often covers a range of products (e.g. meat meal, MBM, bone meal, blood meal, feather meal) from many species (e.g. including bovine, ovine, porcine, avian, pets, and wild/zoo animals). There can also be a wide variation in what exactly goes into the meal that is being prepared, not only between rendering plants but also between individual batches within a plant. In some cases, spillage and waste from other industries (such as the pet food industry) is also rendered. Some general descriptions are given here.

Meat and bone meal (MBM) has a well-balanced amino acid profile and, prior to the appearance of BSE, was considered an excellent source of supplemental protein for animal feeds. It is well suited for feeding monogastric animals, as it is not only a well-

balanced protein source, but also a source of available calcium, phosphorus and other nutrients (e.g. potassium, magnesium, sodium).

The digestibility of the MBM protein fraction is normally high (81 to 87 %). The protein quality is lower than that of fish meal or soyabean meal, limiting its usefulness as a CP supplement in cereal-based rations for monogastric animals. However, because the CP is less degradable in the rumen compared to many other supplemental protein sources, in ruminants it can be used to replace most other supplemental proteins. MBM has been considered a good source of by-pass protein for ruminants, and particularly valuable in rations for high-producing dairy cows.

Supplementation of rations with MBM has been successfully used to promote production in other livestock classes, including fish. Replacing soyabean meal or fish meal with MBM also may increase production in pigs, mainly because of its high lysine content.

Other meat meals and bone meals are defined according to the relative concentrations of these components, and these definitions differ widely. The usefulness of these specific products for feeding various livestock production classes will depend on their exact composition.

Fish meal has been widely used as a supplemental protein source for many years, primarily for monogastric animals. The amino acid quality of fish meal is excellent for most production classes, although the composition (e.g. CP, ash, energy) can vary depending upon the exact substrate and processing method. It is often used as a by-pass protein source for feeding lactating dairy cattle. The main constraint on the use of fish meal by the feed industry is its relatively high cost. Fish meal is generally processed separately from material of ruminant animals, therefore may be considered of negligible BSE risk if cross contamination is controlled during transport and storage.

Poultry meal is nutritionally similar to MBM, but is derived entirely from avian species. This is important in the context of BSE, because generally poultry slaughter and production is entirely separated from that of ruminant animals. Thus, if the rendering process, transport, and storage are also separated, then the risk of poultry meal being cross contaminated with ruminant proteins is extremely low.

Feather meal, horn meal, and *meal from swine bristles* are also separately available. These products are generally used as supplements for the protein creatine, but their digestibility is quite low. They are generally not considered a risk for BSE if cross contamination is controlled as above.

Blood meal may also be used as a source of protein. This product is also generally not considered a risk for BSE if specific stunning methods are forbidden, and if cross contamination is controlled as above.

2.2. Plant protein sources

Plant protein sources vary widely in their ability to meet the nutritional requirements of livestock. In almost all cases, plant sources must be combined to achieve an adequate balance of nutrients, particularly amino acids.

Soybean meal or cake is a valuable source of vegetable protein, and is widely used in rations for poultry, pigs and ruminants. The amino acid composition is comparable to that of milk protein. When using soybean meal as a substitute for animal proteins in compound feeds, however, it is important to consider that some minerals are present only in small quantities. In addition, although soybean meal is a good source of some vitamins, it lacks vitamin B12, which is likely the vitamin most commonly lacking in poultry rations.

Cottonseed meal is a valuable protein supplement for adult cattle. The presence of gossypol (a polyphenolic toxin) not only limits its usefulness for pigs, poultry and immature ruminants, but also has public health implications. Adult ruminants are protected from gossypol toxicity due to denaturation of the toxin in the rumen. Cottonseed meal has a constipating effect on cattle, which is beneficial in feeds with high molasses content. Cottonseed meal has relatively low rumen degradability and is therefore a useful source of by-pass protein.

Groundnut (peanut) cake is the material left after oil extraction of groundnuts. It is a safe feed for all classes of livestock and has a good amino acid balance. For mature ruminants there are no restrictions on the use of groundnut cake. It may be decorticated or undecorticated, and the high fibre content of undecorticated cake makes it a useful corrective for cattle feeding on grass that is low in fibre. The residual oil in groundnut cake may cause soft fat in bacon pigs; therefore, the extracted decorticated meal is preferable for pig feeding. Because of the low fibre and high protein content of decorticated groundnut meal, it is a valuable ingredient in poultry rations.

Maize gluten meal is the by-product of the wet milling of maize (corn) and has a higher protein content than most cereal grains. As it is not very palatable it cannot be fed alone, and its relatively unbalanced amino acid composition restricts its use in both poultry and pig rations, with maximum recommended levels of 10 to 16%. However, because it retains the yellow colouring pigment of the grain it is a valuable addition to poultry rations.

Other plant sources of protein include meals and cakes of coconut, palm kernel, rapeseed, lupin(e), safflower, sesame, mustard and linseed, as well as peas and beans, each with specific limitations on their use (FAO, 2004).

2.3. Synthetic amino acids

Synthetic amino acids have been used for over 40 years, starting with DL-Methionine produced by chemical synthesis in the late 1950s and 60s and L-Lysine produced by fermentation in the 1960s. In the late 1980s, L-Threonine and L-Tryptophan were introduced. With progress in biotechnology, the cost of production of each amino acid has been significantly reduced, a key factor in the expansion of amino acid use in animal feed.

2.4. Other protein sources that must be considered in the context of TSEs

With the emergence of BSE, the risks of other feed components have had to be considered and previously common feeding practices re-evaluated.

Pet food spillage often contains animal-derived proteins that may be prohibited for livestock species because in many countries pet food is exempt from aspects of feed bans. Pet food that spills or is otherwise considered unfit for feeding pets may be intentionally or inadvertently fed to livestock on the farm. Moreover, these materials are sometimes sent back through the rendering process as a by-product of pet food manufacturing.

Catering (table) waste also often contains animal-derived proteins (such as beef) that may be otherwise prohibited for livestock species. However, because all catering waste was at one time deemed fit for human consumption, it should pose no risk if other adequate TSE control measures are in place. For example, even though the feeding of ruminant protein to ruminants is prohibited by all feed bans, if SRMs are excluded from the human food chain and appropriate slaughter methods are used, then beef meat

should pose a negligible risk. This product is most important in the context of the spread of other animal diseases, such as foot and mouth disease (FMD).

Milk replacers, tallow, and other fat-based products have been suspected to be risk factors for the introduction of BSE into some countries, although this has never been proven. Fat itself is not considered to be SRM. Rendered tallow is considered negligible risk if SRM is excluded from the rendering process, and if restrictions on the content of proteins and other solids in the fats and other processing guidelines aimed to reduce the risk of BSE infectivity are complied with. Milk-based products are considered to be of negligible risk OIE, 2005).

Poultry litter includes excreta, bedding, unconsumed feed, and feathers, and has been used as a source of free nitrogen and of protein peptides in livestock feeds, primarily as an inexpensive method of disposal. Although poultry species are not known to be susceptible to TSEs, it has been suggested that prions potentially present in poultry feeds might pass through the digestive tract and be present in the litter. This could pose a very small risk if the litter was then fed to ruminants.

2.5. Impact of the BSE feed bans on animal nutrition

In Europe, and subsequently in other countries, the MBM bans have resulted in a need to find alternative protein sources for livestock feed. According to Abel *et al.* (2002), for all the protein (and amino acids) previously supplied by 2.3 million tonnes (MT) of MBM to be replaced in the EU, about 2.3 MT soyabean meal, 4.6 MT peas, 3.9 MT beans or 2.8 MT lupin(e)s would be needed. However, these authors also suggest that plant meals are inferior to animal meals with regard to various other components, and that plant meals may contain anti-nutritive factors that can negatively affect feed intake and/or nutrient availability. In some countries, differences in costs of the different sources must also be considered. However, more accurate livestock feeding systems (e.g. phase feeding) with adjusted dietary amino acid concentrations have recently been developed that allow for proteins to remain at levels similar to that contributed by MBM. Considering this, MBM bans could be regarded a minor problem in some countries in terms of ensuring amino acid supply.

An additional consequence of the feed ban, at least in the EU (which has had a total feed ban since 2001), is an overall reduction in phosphorous (P) supply, which is not compensated by the use of plant meals. An additional 100 000 tonnes of inorganic P for feed is now needed in the EU (Abel *et al.*, 2002). At present, this is supplied by increased mining of rock phosphates. Use of microbial phytase enzyme (which increases the availability of P from plant materials) in livestock rations could help to solve this problem in the future.

3. SUMMARY OF TSE-RELEVANT CONCEPTS

- From a nutritional and economic standpoint, animal by-products were considered a good and plentiful source of protein for livestock feeds.
- Because to the emergence of BSE, appropriate alternate protein sources for livestock feeds have had to be found, which is nutritionally more challenging for pig and poultry feeds than it is for ruminant feeds.
- The risks of MBM from some regions made from certain high-risk materials are well documented, but alternative protein sources (including some that are not prohibited in feeds) may also still pose some BSE risk.

- Some products that pose no inherent risk may still contain infectivity due to cross contamination during production.

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INACTIVATION OF TSE AGENTS

1. GENERAL CONCEPTS

Although there remain questions about the characteristics and pathophysiology of the prions that are the disease agents of TSEs, their infectivity is known to be highly resistant to many forms of inactivation. This resistance to inactivation is important, as the presence of infective agent in rendered animal by-products subsequently fed to animals has been the primary factor in the expansion of BSE in the world. Therefore, control of both the processing parameters for rendering of animal by-products and the subsequent use of the processed products in animal feed are crucial components of any TSE control program.

Moreover, many examples of inadvertent transmission of other TSEs through contaminated materials exist. Sporadic Creuzfeldt-Jakob disease (CJD) is documented to have been transmitted from patient to patient through the use of inadequately decontaminated neurosurgical equipment (Bernoulli *et al.*, 1997). Scrapie has been transmitted to sheep and goats through the survival of this agent in formol-treated vaccines (Agrimi *et al.*, 1999).

For effective development and implementation of controls, some fundamental facts about TSE agent inactivation must be taken in account.

2. INACTIVATION PROCEDURES

Rendering is used to manufacture meat and bone meal (MBM) from animal by-products primarily, but not exclusively, for inclusion in animal feed. The rendering process is described in the "Rendering of animal by-products" chapter in this course manual. However, BSE infectivity has been documented to remain in material after being subjected to the common rendering processes used worldwide (Taylor and Woodgate, 2003). It is this situation that is credited with the expansion of the BSE outbreak throughout Europe and the world.

Worldwide, one or more of the following inactivation procedures have generally been used for material potentially containing prions:

- batch autoclaving at 134-138 ºC for 18 minutes (Europe);
- exposure to sodium hypochlorite solution (20 000 ppm) available in chlorine for one hour (Europe);
- exposure to 1 M sodium hydroxide for one hour (USA);
- continuous autoclaving at 132 ºC for one hour (USA).

Some of these methods have been incorporated into formal recommendations on reducing TSE infectivity (OIE, 2005; EU, 2002), although there is still some question as to their actual effectiveness in certain situations. Because different research studies of inactivation and infectivity use different TSE agents and strains of agents, there are often discrepancies between study results, and therefore interpretations must be made carefully.

Inactivation procedures relying solely on heat and pressure are used on a large scale in the rendering industry, whereas chemical inactivation most often is applied to mate-

rial from diagnostic laboratories or from research. In many laboratories a combination of both procedures is applied. These two procedures are briefly discussed below.

2.1. Heat/pressure inactivation

Although heat is commonly used in an attempt to inactivate prions, it has been reported that dry heat may not be very effective. In one study, dry heat at temperatures up to 180 ºC for one hour did not inactivate the ME7 strain of scrapie agent and some infectivity remained even after exposure to dry heat at 160 ºC for 24 hours. However, a one-hour treatment of dry heat at 200 ºC was effective with certain TSE strains (Taylor *et al.*, 1996)

In more recent studies carried out by Brown *et al.* (2000) it was reported that traces of scrapie infectivity could still be detected after brain tissue infected with 263K (another strain of scrapie agent) had been exposed to dry heat at 600 ºC for 15 minutes. It has been speculated that this is due to the persistence of an inorganic skeleton of the infective prion even after heating to 600 ºC, and that this skeleton might still be able to trigger the conversion of normal cellular prion protein (PrP^{c}) into the infective prion form (PrP^{sc}). Complete burning of material at temperature above 1 000 ºC is generally effective for complete inactivation.

However, when moisture is added the effectiveness of heat inactivation improves. It has been demonstrated that small amounts of TSE agent can be inactivated in batch rendering systems when exposed to 134 ºC and high moisture content for 14-18 minutes (Kimberlim *et al.*, 1983). However, further studies with larger samples showed that the inactivation might not be complete. It has been suggested that larger samples more realistically represent the actual volume of potentially TSE-infected tissue that is disposed of by slaughterhouses (as well as by human or veterinary healthcare systems and other agricultural systems) and that subsequently requires inactivation. Also, partial drying of small amounts of infected tissue onto glass or metal surfaces within the autoclaving or rendering system should be taken into account when defining effective standards for inactivation of TSE agents by heat, as these materials might harbour additional infectivity.

Other experiments have investigated the inactivation of different scrapie strains, different amounts of infectivity, and different exposure durations at temperatures between 134 and 138 ºC (Taylor and Woodgate, 2003). These data suggest that the thermostability of some scrapie agents varies with the heating procedure, and inactivation is dependent on both temperature and holding time. Therefore, simply increasing the temperature and keeping the holding time constant will not necessarily linearly improve the inactivation. Consequently, both parameters should be analysed together.

In summary, most experimental evidence confirms that application of the conventional rendering parameters of 133 ºC at 3 bars of pressure for 20 minutes reduces the infectivity of a TSE agent in rendered material by an average of three logs. However, other aspects of the rendering process are important to optimize the inactivation. Particle size should be no greater than 50 mm (OIE, 2005) and a batch rather than continuous process should be used. Finally, complementary measures (e.g. an SRM ban) should be in place to exclude high concentrations of TSE infectivity from initially entering the system (Heim and Kihm, 2003).

2.2. Chemical inactivation

Experiments investigating the inactivation of various TSE agents using chemicals have also been published. In one study, two years of exposure of the BSE agent to formol saline had little effect on reducing infectivity (Fraser *et al.*, 1992). TSE agents also resist inactivation by formalin and other aldehydes, although BSE infectivity was inactivated by 30 minutes of exposure to a solution of sodium hypochlorite (NaOCl) containing 16 500 ppm of available chlorine (Taylor *et al.*, 1994).

Studies with BSE-infected bovine brain and scrapie-infected rodent brain showed that treatment with 1 or 2 M sodium hydroxide (NaOH) for up to two hours did not completely inactivate these agents, and permitted the persistence of up to four logs of infectivity (Taylor *et al.*, 1994). The detection of residual scrapie infectivity after treatment with 1 M NaOH for one hour and the survival of CJD infectivity after exposure to 1 or 2 M NaOH have also been reported (Ernst and Race, 1993).

Effective inactivation of TSE agents can also be achieved by combining the autoclaving procedure and the exposure to NaOH. It has been shown that the 22A strain of scrapie is inactivated by autoclaving at 121 ºC for 30 min in the presence of 2 M NaOH (Taylor *et al.*, 1997). There are practical problems with this procedure, such as the potential exposure of operators to splashing with NaOH, and the potential deleterious effect on the autoclave and the materials within (e.g. surgical instruments).

Although these chemical methods are not practical for inactivation of TSE agents in large volumes of animal by-products, they could be applied to decontamination in laboratory situations or when surgical instruments must be sterilized prior to reuse. This is of considerable importance, since major concerns exist in human medicine about inactivation of TSE agents in instruments used in general surgery, as well as neurosurgery.

3. FUTURE DEVELOPMENT OF INACTIVATION IN RENDERING

It is widely accepted that the conventional techniques used for rendering animal by-products considerably reduce infectivity, but do not completely inactivate TSE agents. Therefore, it will be most important in the future to define the parameters for handling, storage and use of rendered material. To optimize inactivation, high-risk material must be excluded from any rendering process where the products could be included in animal feed.

As described above for inactivation generally, the specific inactivation effect during rendering is defined by multiple factors such as temperature, moisture content, particle size, TSE strain, chemical agents, binding surfaces and time. Results under laboratory conditions have to be adapted to rendering on a large scale. Because there is no generally accepted inactivation curve for all types of inactivation, it is at least questionable to apply results from one rendering system to another without further investigation. These issues are explored further in the "Rendering of animal by-products" chapter in this course manual.

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RENDERING OF ANIMAL BY-PRODUCTS

1. GENERAL CONCEPTS

When considering the meat industry, it is often forgotten that a substantial proportion of each slaughtered animal does not enter the human food chain. During the normal slaughter and processing of animals, 33-43% of the live animal weight is removed and discarded as inedible waste (by-products). The actual percentage depends mostly on the species, and is highest for ruminants, moderate for swine, and lowest for poultry. Moreover, it also depends on the country, with industrialized countries using the lowest percentage of each animal for human consumption. These discarded animal by-products (e.g. fat trim, meat trim, viscera, bone, blood, feathers in poultry) must be safely disposed of, as they may represent a risk for human and animal health. In addition, some animals die on the farm, have to be euthanized for various reasons, or are judged unfit for human consumption. The safe disposal of these animals (generally called fallen stock or downer animals) is an important issue with regard to TSE control.

In many countries, animal by-products are collected and then processed by the rendering industry into high-quality fats (tallow) and proteins (meat and bone meal/MBM and other protein meals). Historically, MBM has been an excellent source of supplementary protein due to its well-balanced amino acid profile, as described in the "Protein use in livestock feeding" chapter in this course manual. In the past, renderers in the former 15 countries of the EU processed about 16 million tonnes of animal by-products per year, while those in North America processed nearly 25 million tonnes per year. Argentina, Australia, Brazil and New Zealand collectively processed another 10 million tonnes per year and the increasing production in Asia represents between 10 and 14 million tonnes per year. From an economic point of view, the rendering industry has traditionally been quite profitable, as the rendered products were sold for use by the animal feed and oleochemical industries around the world. The total value of the finished rendered products worldwide, before the implementation of the current TSE-related feed bans, was estimated to be between US$6 and US$8 billion per year (Hamilton, 2002).

2. THE RENDERING PROCESS

The term "rendering of animal by-products" is used to describe many different processes applied to raw animal by-products. In this course manual, rendering refers to the pressure heating of animal by-products to make products for further use, including use in animal feeding. Rendering can take place as part of the activities of a specific slaughterhouse or at a separate plant where animal by-products from multiple sources are collected.

After arrival and collection at the rendering plant, animal by-products are moved onto a conveyer belt, passed through a metal detector, and transported to a crusher. There, the raw material is crushed to reduce particle size. Then the material is pressure sterilized in the sterilizer. After sterilization a viscous mass remains, which is passed through a filter to separate liquids and solids. In an energy-intensive process step, the solid particles are then dried and ground, yielding a coarse-grained powder with a high

animal protein content (animal meal). The liquid phase contains the fat fraction. Using centrifugal forces or presses, the fats are separated from the other liquid products and can be further processed.

In this process, temperature, pressure, and time are all important. The heating and pressure must be applied in the sterilizer for the entire time, i.e. the time it takes to reach the correct temperature and pressure should not be included in the processing duration. Also, the system used for the flow of raw material being processed in the sterilizer is important. Systems where the materials are processed in batches (batch systems) are optimal for achieving the appropriate conditions, whereas continuous (i.e. flow-through) systems may not be. In addition, the maximal size of the particle is important, as penetration and therefore inactivation improves with smaller particle size. Therefore, most countries require a reduction of the particle size before heat treatment to less than 50 millimetres (OIE, 2005).

The rendering parameters can vary according to legislative requirements. In Europe, the rendering parameters for most animal by-products (including all BSE risk material) require a minimum heating at the OIE-recommended parameters of 133 °C at 3 bars of pressure for 20 minutes (OIE, 2005). Less stringent parameters only can be applied to certain categories of materials that represent minor risk to human and animal health (EU, 2002).

Worldwide, the minimum parameters for the processing of animal by-products vary substantially, and are generally substantially lower than recommended by the OIE. Heat requirements may be below 100 °C and pressure may only be required in certain situations. Often, particle size is not controlled, and continuous systems are used.

3. GOALS OF THE RENDERING PROCESS

Irrespective of the ultimate use made of the rendered products, two goals of rendering are to:

- reduce the volume of animal by-products through the separation of the water fraction from the remaining material;
- minimize the animal and public health risk from possible pathogens through the pressure sterilization process.

Unprocessed animal by-products contain approximately 60-65% water, as determined by the relative amount of meat (higher water content) and bones (lower water content). The heat used in the rendering process removes most of the moisture, thereby reducing the volume, which is important when considering disposal. The individual percentages of the final animal meal and fat fractions vary according to the proportion of meat and bone in the raw animal by-products, but the combined final weight is normally approximately 35% of the raw animal by-product weight. The final volume of the animal meal fraction is approximately 23% and the fat fraction approximately 12% of the raw animal by-product volume. Globally, the rendering process reduces the total animal by-product volume from 60 million tonnes of raw material to about 8 million tonnes of animal proteins and 8.2 million tonnes of rendered fats (Hamilton, 2002).

The heat of the rendering process also sterilizes the by-products, as the processing temperatures are usually more than sufficient to kill bacteria, viruses and many other microorganisms. A recent study showed that rendering at 133 °C at 3 bars of pressure for 20 minutes eliminated *Clostridium* Spp., *Listeria* Spp., *Campylobacter* Spp.and *Salmonella* Spp. in raw animal tissues (Trout *et al.*, 2001). Such high temperatures are

also effective in killing the anthrax bacterium (*Clostridium anthracii*) and destroying the foot and mouth disease virus. However, rendering does not completely destroy the prion causing BSE (see the "Inactivation of TSE agents" chapter in this course manual), which has led to the various bans on including rendered animal protein in livestock feeds in Europe and in many other countries (as discussed throughout this course manual).

4. OTHER OPTIONS FOR ANIMAL BY-PRODUCT DISPOSAL

With implementation of the MBM bans in livestock feed, the disposal of animal by-products, either as such or as rendered meals, becomes an extremely large problem for all countries. What ultimately happens to these materials and products will depend on what raw material was used as well as the regulations of the country.

Some alternatives currently being applied or considered are incineration, co-incineration (e.g. in the cement industry, in waste incineration, or in fertilizer processing), burial, disposal in landfills, use in biogas production or composting. Most European countries are using some form of incineration. However, incineration requires initial rendering (primarily to reduce the volume) and storage of the rendered material before incineration. Direct incineration of raw material is possible, but cannot be used on a large scale. Recent estimates by the European Fat Processors and Renderers Association give the annual incineration capacity in the EU as 2.5 million tonnes, while the quantity to be incinerated is put at 3.6 million tonnes (EU, 2001).

Abel *et al*. (2002) also note the production of greenhouse and noxious gases produced by incineration. They calculate that combustion of one kilogram (kg) of MBM causes release of about 1.4 kg of combined carbon dioxide (CO_2), carbon monoxide (CO), and some further trace gases including nitrous oxide (N_2O) and sulphur dioxide (SO_2). Of these, N_2O is the most dangerous because its global warming potential has been estimated at 310 times that of CO_2 and because of its ozone depleting potential in the stratosphere.

Composting and other biological methods of raw material disposal do not achieve the high temperatures necessary to make the material microbiologically safe without prior heating. In addition, burial, disposal in landfills, and storage of dry material pose unacceptable environmental risks as they are subject to incursion of birds and other animals, which may then spread disease agents out into the environment.

Regardless of method, the costs of disposal may be very high. They are certainly higher than before TSE-related feed bans were imposed, when the rendering industry generally paid to purchase the raw animal by-products from farmers and slaughterhouses and still produced products at a profit. The data of Abel *et al*. (2002) show that the total costs of the alternative use or disposal of 3.6 million tonnes of MBM varies from €1.0-1.8 billion. On average, every kg of MBM not used in livestock feed incurs a cost of about €0.32, or nearly twice the 1999 supply price of MBM. Expressed differently, for every kg of MBM not used in livestock feed, there is an overall economic loss of about €0.14.

5. ANIMAL BY-PRODUCT LEGISLATION IN THE EUROPEAN UNION

A regulation laying down rules concerning animal by-products not intended for human consumption was adopted by the EU in October 2002, and applied on 1 May 2003 (EU, 2002). In particular, the regulation (referred to here as Regulation 1774/2002) introduces stringent conditions throughout the food and feed chains including safe collection, transport, storage, handling, processing, use and disposal of animal by-products.

As a general principle, Regulation 1774/2002 describes a risk-based categorization system for animal by-products, and their possible uses. Three categories are defined.

- **Category 1**
  Regulation 1774/2002 requires the complete disposal, by incineration or landfill after appropriate heat treatment (133 °C /3 bars/20 minutes), of Category 1 materials. The raw material of Category 1 is defined as animal by-products presenting the highest risk for diseases such as BSE or other TSEs (e.g. scrapie). It also includes fallen stock from ruminants and from animals not intended for human consumption (e.g. companion animals, research animals).
- **Category 2**
  Category 2 material can be used in the same way as animal by-products of Category 1. In addition, it may be recycled for technical uses after proper heat treatment (e.g. biogas, composting, oleochemical products). The use for animal feeding is prohibited. The raw material of Category 2 includes animal by-products that have been rejected by ante/post mortem inspection in the slaughterhouse. They also present a risk of contamination with animal diseases other than BSE.
- **Category 3**
  Regulation 1774/2002 states that only Category 3 materials may be used in the production of animal feeds (including pet foods) following appropriate treatment in approved processing plants. The raw material of Category 3 is defined as animal by-products derived from healthy animals slaughtered for human consumption.

Thus, under Regulation 1774/2002, only materials derived from animals declared fit for human consumption following veterinary inspection may potentially be used for the production of livestock feeds. The regulation also requires the exclusion of dead animals and other condemned materials from the feed chain, the complete separation during collection, transport, storage, handling and processing of animal by-products not intended for animal feed or human food, and the complete separation of rendering plants dedicated to feed production from rendering plants processing animal by-products destined for destruction (i.e. by category of raw material processed).

It also sets out clear rules on what can and must be done with the excluded animal materials, including imposing a strict identification and traceability system and requiring certain products such as MBM and fats destined for destruction to be permanently marked to avoid possible fraud and risk of diversion of unauthorized products into food and feed. The control of movements of SRM by a record-keeping system and accompanying documents or health certificates is also required. Regulation 1774/2002 also prohibits any intra-species recycling of processed proteins (feeding material derived from a species back to the same species).

However, Regulation 1774/2002 does not in any way change or affect the current EU total ban on the feeding of MBM to farmed animals, which is a separate issue and remains in force without any termination date set. Regulation 1774/2002 only establishes clear safety rules for the production of MBM in case it is ever reauthorized for inclusion in feed for certain non-ruminant species, e.g. poultry or pigs.

With the adoption of Regulation 1774/2002, the environmental and economic repercussions of the feed ban can potentially be reduced in the future, as only two million tonnes of material derived from animals unfit for human consumption (compared to the 16 million tonnes of animal by-products in case of a total ban) would need to be disposed of.

6. THE FUTURE USE OF MEAT AND BONE MEAL

It is possible that, in the future, MBM could again be used in feed for farmed animals in countries where it is presently banned (including countries in Europe) if appropriate legislation and controls are implemented and enforced. However, its use will most probably always be restricted to non-ruminant animals. If SRM is removed, fallen stock are excluded, and the process ensures heat treatment at 133 °C at 3 bar pressure for 20 minutes it is assumed that the risk of infectivity of a TSE agent that might be present is markedly reduced. In addition, better classification and separation of different materials would further reduce the risk from the rendered products. However, caution is required as this step must only be taken if these measures could be effectively implemented.

Irrespective of any scientific justifications, however, the BSE outbreak has seriously affected the public perception, at least in Europe, regarding the rendering industry, the feeding of animal proteins to naturally herbivorous animals, and the feeding of proteins derived from one species back to the same species (which may be seen as cannibalism). These perceptions will undoubtedly influence the acceptance of the use of MBM or animal-derived products in animal feeds in the future.

7. SUMMARY OF TSE-RELEVANT CONCEPTS

- Rendering processes and parameters vary substantially among countries throughout the world.
- Appropriate rendering can reduce TSE infectivity in animal by-products, although infectivity is not entirely inactivated.
- Incineration by some method is currently the most effective method of disposal of TSE risk material (raw and processed animal by-products).
- Although there is limited capacity for alternate disposal of the large amount of rendered material that is now prohibited in feed, it must be assured that this material is properly disposed of so that it does not illegally re-enter the food or feed chain.
- Separation of by-products by risk category could eventually allow the use of some animal products in livestock feeds.

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MANUFACTURING OF COMPOUND FEEDS FOR LIVESTOCK[1]

1. GENERAL CONCEPTS

Although production of compound feeds for livestock has levelled off or is declining in many parts of Europe and North America (Figures 1a and b), it still maintains an important economic position. For example in Europe, compound feed production ranks third after meat and dairy production, accounting for 7% of the total sales of CHF750 billion in this sector. Feed manufacturing will likely maintain this role in the foreseeable future, although growth may shift geographically.

Compound feeds are manufactured primarily from organic compounds of vegetable or animal origin. The small inorganic percentage is primarily of mineral origin. Because of the historical importance of including ingredients of animal origin, manufacturing of compound feeds throughout the world has been affected by the appearance of TSEs. In many countries, feed products have had to undergo reformulation to compensate for prohibited materials, and stricter controls of contamination and cross contamination have been implemented in feed-producing plants.

In this chapter, a modern feed manufacturing process, using the most technologically advanced equipment possible, is described. This chapter does not contain a summary of TSE-relevant concepts. Other aspects of feed production are presented in other chapters of this course manual. For example, aspects of quality assurance for the processing steps described here are presented in the "Quality assurance in feed-producing plants" chapter.

2. STRUCTURE OF A FEED PRODUCTION PLANT

A feed production plant can generally be divided into the main processing areas and secondary processing areas, and services and infrastructure. The services and infrastructure will not be described here, as they do not directly relate to the quality or safety of the feeds produced.

Main processing areas:

- Reception (intake) and cleaning
- Proportioning and weighing
- Grinding
- Mixing and homogenizing
- Pelleting
- Finished products

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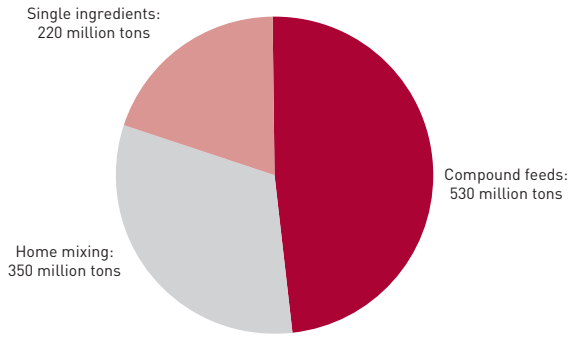
FIGURE 1A

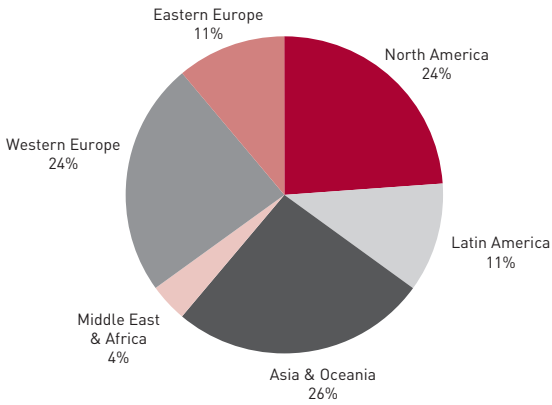
World feed production by feed type, 1994



Source: adapted from Nef (2002).

FIGURE 1B

Percent of world feed production by region, 1994



Source: adapted from Nef (2002).

Secondary processing areas:

- Crushing of grain
- Hulling of grain
- Flaking
- Extrusion
- Expansion
- Heat treatment

Services and infrastructure

- Exhaust systems
- Compressed air supply
- Steam supply
- Water supply
- Electric installation and control system

3. FEED PRODUCTION PROCESSES

3.1. Intake of raw ingredients

The raw ingredients to be processed may arrive at the feed plant via ship, rail, or road. Weight checks are usually performed at weighing bridges in the case of rail or road vehicles, and/or by intake scales in the various in-plant conveying systems.

Grain is normally supplied in bulk, and meals or meal-type ingredients may be supplied in bulk or in bags. Feed plants with a low capacity can use the same line for meal reception as for grain reception. Large capacity feed plants, on the other hand, must have separate receiving sections and conveying lines to accommodate the large volume of grain arriving per day.

Minerals as well as premixes may be supplied in plastic or paper bags, tin cans, or drums, or they may be supplied by bulk trucks (tankers) with attached blowers. For this type of supply, a pneumatic receiving line must be provided. Premixes may also be produced in-house.

Both fat and molasses are normally supplied in a heated and liquid form. They are discharged from the bulk trucks into the storage tanks directly by pumps.

The following machines and installations are used for receiving and conveying the raw ingredients:

- Ship unloading systems
- Chain conveyors
- Screw conveyors
- Belt conveyors
- Bucket elevators
- Pneumatic conveying systems
- Liquids pumps

3.2. Cleaning of raw ingredients

Depending on their origin and previous processing, raw ingredients may be heavily contaminated and contain foreign matter that must be removed. As a result, the cleaning system in a feed plant has two functions:

- to separate undesirable impurities such as stones, pieces of wood, strings, paper, straws, sand, and metal parts;
- to protect expensive feed plant equipment such as dischargers, hammer mills, mixers, pellet mills and others against damage.

The following equipment may be used for cleaning the raw ingredients:

- Drum sieves
- Centrifugal sieves
- Oscillating sieves
- Separators
- De-stoners
- Spout magnets
- Cascade magnets
- Drum type magnets

Depending on the particular machine, the output from the cleaning process includes the acceptable material, as well as course impurities, fine impurities, and dust.

3.3. Discharge and weighing of raw ingredients

Because of the importance of offering a balanced ration for a particular livestock species or class, compound feed products are usually formulated and continuously optimized by means of modern computer programs. Most of the raw ingredients are stored in bins. The individual percentages of the raw ingredients are discharged from the bins using a type of discharger appropriate to the particular material. The most common types are slide gate dischargers, rotary bin dischargers, and screw dischargers.

After discharge, raw materials are fed to the allocated scales. In order to guarantee the most precise input weighing possible, the machinery is capable of proportioning the material by fast and dribble flow.

To determine the number of bins and the scale size(s) needed, a product formula list is established showing the required quantities of the individual ingredients. The scale size and the number of scales are obtained from the lowest allowable ingredient weight and the total weight per scale to be weighed at one time. The lowest ingredient weight that can be weighed by a batch scale must be at least 4% of the scale capacity.

3.4. Grinding

Together with mixing and pelleting, grinding to reduce the particle size of raw ingredients is a key process in compound feed manufacturing. About 80% of all raw ingredients used for the production of compound feed require size reduction. Size reduction ranks second in feed plant energy consumption (after pelleting), which underscores the importance of correct sizing and optimized operation of grinding systems.

The purpose of grinding is:

- to achieve the required material fineness (texture) matched to the animal's nutritional requirements and digestive system;
- to achieve uniform particle size in order to obtain a homogeneous product during the subsequent mixing operation;
- to achieve the necessary fineness in order to obtain an acceptable pellet quality if the material is subsequently pelleted.

A basic distinction is made between pre-grinding and post-grinding, and both systems may also be designed for circulation or multi-stage grinding.

In *pre-grinding systems*, all the ingredients are first individually ground. The advantages of pre-grinding are that grinding is independent of mixing and can therefore be efficiently applied, night-time grinding at low power rates is possible, and the particle size distribution of the individual materials can be varied. The disadvantages are the high capital cost of grinding bins and conveyors for separate ingredients, the fact that with different particle size distributions there is danger of segregation (separation of raw ingredients) in the finished feed and that materials containing chaff are difficult to grind.

In *post-grinding systems*, all the ingredients of a mix are first proportioned, weighed, added together, and then ground. If possible, the fines should be sifted out before grinding. The advantages of post-grinding are that the end particle size distribution can be controlled and the product texture is uniform, hard to grind materials are easier to grind when mixed with other ingredients and building costs are lower because no separate grinding bins are needed. The disadvantages are that individual ingredients cannot be selectively reduced to a desired particle size and the mixing system is directly dependent upon the grinding capacity.

Depending on the required capacity, the particle size distribution requirements, and other developments in the marketplace, different grinding machines may be used.

A *toothed disk mill* can be used for breaking of grain ahead of flaking, for poultry feed production and for detaching and grinding of minerals. Its advantages are ease of operation and low percentage of fines, and its disadvantages are the low throughput (approximately 4 000 kg/hr) and a lack of ability to vary the particle size distribution.

A *roller mill* is used if a particle size distribution of maximum uniformity is required. Its advantages are a low power requirement, size reduction is accomplished gently and without a lot of heat generation and there is a narrow particle size distribution. Its disadvantages are a high capital investment, its unsuitability for fibre grinding, its expensive design and its limited input particle size.

A *conventional hammer mill* is considered the universal grinding machine in feed manufacturing and it allows size reduction of all dry feed ingredients. Its advantages are its universal applicability, high possible throughputs, easy variation of particle size distribution, simple design and easy operation. Its disadvantages are its wide particle size distribution and a high power requirement.

The development of the *vertical rotor (hammer) mill* has opened new possibilities in the grinding process. Compared to conventional hammer mills, its advantages are that no exhaust system is required, there is a lower burden on the environment, there is no loss of moisture, it has a low power requirement, a higher throughput and a lower noise level.

3.5. Conveying

Pneumatic suction systems, pneumatic pressure systems, or mechanical transport systems can be used for conveying materials after grinding. Because of their high operating costs, pneumatic conveying is generally applied for low-capacity plants only.

3.6. Mixing, addition of liquids, and homogenization

Mixers are the main machines in feed plants. Their function is to uniformly mix the individual raw ingredients, whose particle sizes and bulk density may vary considerably. Micro-ingredients may be gravimetrically added in the mixer using special dispensing units or by hand.

In the feed manufacturing industry, the mixers almost exclusively used are of the batch type, in which entire units (normally 500–5 000 kg, depending on the mixer size) are pre-weighed, ground and then mixed. This type of mixing system ensures a homogeneous product.

The mixer size, type (vertical or horizontal) and mixing time determine the capacity of a batch mixing system. Depending on the type of mixer used, the mixing times will vary considerably. For example using a vertical mixer with screw, the mixing time will be approximately 10-30 minutes, and if using a horizontal mixer with ribbon flight or paddles, the mixing time will be approximately one to four minutes. Generally, horizontal mixers with counter current mixing action on one shaft will achieve the required mixing quality within the shortest time. All mixers should meet the following standards:

- The mixing accuracy should be guaranteed at a dilution of 1:100 000.
- Homogeneity should be achieved within the shortest time possible.
- The material to be mixed should be handled gently as possible.
- When the mixer is emptied, residues should be at an absolute minimum.
- The mixer should be adaptable for addition of liquids.

In horizontal mixers, the dry ingredients are fed batch by batch into the mixer while the rotor is running, and are homogeneously mixed in the mixing chamber. The mixing time is generally two to four minutes. Horizontal batch mixers may be equipped with a paddle rotor or a ribbon flight rotor.

With ribbon flight rotors, loading is possible along the entire length of the mixer. However, partial batches must be loaded at a minimum of 50% capacity and ribbon flight changes are labour-intensive. The expected residue is about 0.2 %. With paddle rotors, loading is only possible in the centre but partial batches down to 20% are possible. The paddles are adjustable, and the materials are more gently treated. The expected residue is < 0.2%.

The use of horizontal speed mixers can contribute to the plant flexibility and to reduction of contamination. Compared to conventional horizontal mixers, speed mixers have:

- shorter mixing times (1.5 minutes);
- a throughput of up to 20 batches/hour;
- smaller mixer, scales and hoppers;
- fast and complete discharge;
- a residue level of only 0.05%
- a trough shape (length: depth ratio = 1:1);
- no requirement for an air replacement duct;
- no restriction regarding filling;
- easier maintenance.

The addition of liquid ingredients to feeds is becoming increasingly important. Liquids may be added in order to be able to use less expensive by-products from other production processes, to enrich energy, to control taste, smell and colour, to reduce dust generation and segregation, to increase moisture content and to improve preservation. Common liquids include water, fats/oils, molasses, propionic acid and flavouring agents. Because of their viscosity, fats, oils and molasses must be heated to a certain temperature before they can be added to feeds. The point of the process at which the liquid is added and the rate of addition depends on the type and quantity of liquid to be used.

A batch mixer is generally not suitable for the addition of liquids to the feed, however it may be possible if the addition rate is lower than 5%. Otherwise, problems may include lump formation, contamination of rotor and trough, increased power requirement and increased amount of residue.

In the feed industry, homogenization is the incorporation of liquids into dry solids in a continuous process. Homogenizers operate at fairly high rotary speeds and ensure thorough intermixing of the liquid with the solids. Homogenizers may be used ahead of or after the batch mixer, and can handle the simultaneous addition of up to three liquids.

3.7. Pelleting

The purpose of pelleting is to transform a loose, mealy bulk material into normally cylindrical pellets by compaction and shaping. Pelleted feed offers a number of advantages over fine and coarse meal-type feeds.

- Benefits to the feed producer:
  - less storage space and smaller conveyers required;
  - cost reduction (lower cost raw materials can be used);
  - lower risk of segregation and contamination.

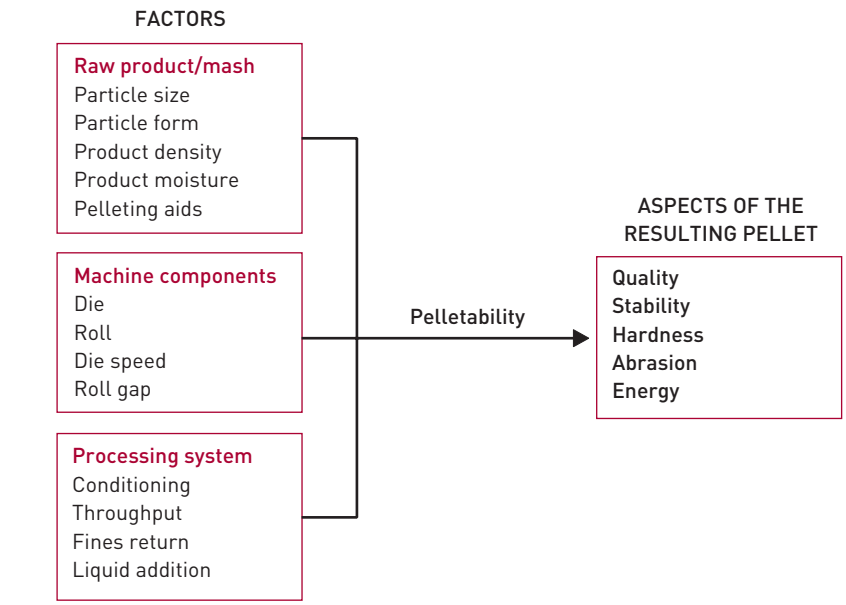
- Benefits in storage /handling:
  - simpler discharge from bins;
  - no segregation during bulk handling;
  - bridging (feed flow blockage during discharge) in bins is reduced;
  - less dust is generated.
- Benefits to the animal:
  - higher degree of nutrient assimilation;
  - reduction of bacterial and fungus counts in feeds;
  - all constituents of the mix are available in uniform proportions.

The pellet mill consists of three major components: (1) the screw feeder which allows a volumetric discharge of the mash from the pellet mill surge hopper, (2) the mixing and conditioning section which allows conditioning of the mash with addition of steam and liquids (usually molasses), and (3) the pellet mill which compacts the mash and shapes it into pellets. Within the pellet mill, the main pelleting components are the die and the rolls. After the mash has been distributed between the rolls and across the width of the die, the actual pelleting process starts at the moment the layer of mash touches the roll.

As a result of the ever-increasing quality and sanitation requirements that must be met, and because of the greater use of inexpensive raw materials with poor pelleting characteristics, it is often not possible using the conventional direct pelleting process to achieve the necessary binding forces for making high quality pellets (Figure 2). Therefore, a double pelleting system was developed that considerably improves the pellet quality and simultaneously allows a higher throughput.

FIGURE 2

Factors having a fundamental influence on the pelleting process and the final product



Source: adapted from Nef (2002).

3.8. Heat treatment and cooling

The demand for safe and hygienically produced feeds is increasing, not only in the domestic and international markets but also from consumers. End products must be as free as possible from *Salmonella* Spp. and other harmful bacteria.

Heat treatment is the most effective and economical method of destroying bacteria and other pathogens in livestock feeds and improving the quality of feeds. Heat treatment, through the process of expansion, also enhances the quality of energy, as the starch in the feed becomes more available (especially to non-ruminants). The two most common heat treatment methods are pelleting with heat shield and the application of expanders.

By equipping the pelleting mill with a double conditioner heat shield, the retention time in the mixer-conditioner section increases. The optimal temperature is maintained by means of electrical surface heating and addition of hot air.

Expanders may be applied as an independent process stage or in combination with a pellet mill. Typical applications of expanders include:

- salmonella control for broiler and laying hen feeds;
- production of crumbles for slurry feeding of pigs;
- starch gelatinization for young animals, especially piglets;
- production of low abrasion pellets;
- production of cattle feed with high liquid addition rates.

Pellets are discharged from the pellet mill at a temperature of approximately 80 °C. In addition, they are still moist and soft and therefore cannot be packed or stored before they have cooled to a temperature normally 5 to 10°C above the ambient temperature and have hardened. The most common cooler types are horizontal (belt type) coolers and counter current coolers.

Horizontal coolers can be used for all types and sizes of products, although they have a high specific air requirement and a relatively large space requirement. Counter current coolers can only be used for free flowing materials and pellets with a size of up to 10 mm but have a low specific air requirement and a relatively small space requirement.

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LIVESTOCK FEED REGULATIONS AND INDUSTRY GUIDELINES

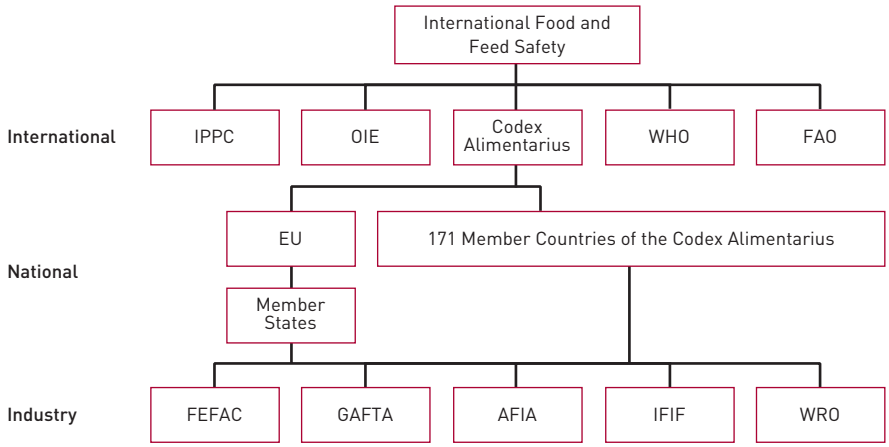
1. GENERAL CONCEPTS

The appearance of TSEs has presented new challenges to countries throughout the world. In order to protect both domestic public and animal health and maintain trade in animals and animal products, many countries have implemented some measures to control and prevent TSEs, and BSE in particular. These measures vary widely among countries, but generally include some form of feed ban in order to prevent ruminants from ingesting material derived from ruminants, as described in the "Overview: Implementation of TSE measures" chapter in this course manual.

International recommendations and regulations have been developed to improve public and animal health and facilitate fair trade through the standardization of BSE-related measures across countries and regions. National regulations, practice guidelines and codes of practice have been developed to help countries and individual agricultural operations effectively implement the measures in place. Many organizations at many levels participate in developing these documents. In Figure 1, the international, national and industry framework that contributes to the overall safety of animal feed in the

FIGURE 1

The network of players contributing to the goal of international food and feed safety



OIE: World Organisation for Animal Health
IPPC: International Plant Protection Convention
WHO: World Health Organization
FAO: Food and Agriculture Organization
EU: European Union

FEFAC: European Feed Manufacturers' Federation
GAFTA: Grain and Feed Trade Association
AFIA: American Feed Industry Association
IFIF: The International Feed Industry Federation
WRO: World Renderers' Organization

world, as well as with respect to BSE, is shown. It is clear that countries outside the Codex Alimentarius also contribute to and participate in this process.

2. INTERNATIONAL STANDARDS AND NATIONAL/REGIONAL REGULATIONS

WTO considers the standards set and recommendations made by two international bodies, the Codex Alimentarius Commission for feed and food issues and the OIE for animal health issues, to be the international standards. In addition, countries and regions adopt and implement legislation that should be in line with these international standards. Legislation of the EU and the USA are given here as examples.

2.1. The Codex Alimentarius

In 1963 the Codex Alimentarius Commission was created by the WHO and FAO to develop food standards, guidelines and related texts such as codes of practice. The main purposes are to protect the health of consumers, to ensure fair trade practices in the food industry, and to promote coordination of all food standards work undertaken by international governmental and non-governmental organizations. The output from the Codex Commission is called the *Codex Alimentarius,* which comprehensively describes basic principles of food hygiene, and is available at http://www.codexalimentarius.net.

At the 23rd Session of the Joint Codex Alimentarius Commission in 1999, an Ad Hoc Intergovernmental Codex Task Force on Animal Feeding (hosted by the Danish Government) was established to develop a draft code of practice on animal feeding. This code of practice was presented and approved by the Codex Commission in July 2004, and applies in addition to the basic principles already established in the Codex Alimentarius (Codex Alimentarius, 2004). It aims to establish a feed safety system for food-producing animals, and comprehensively covers the food and feed chains taking into account relevant aspects of animal health and the environment in order to minimize public health risks. The full code of practice covers:

- general principles and requirements with respect to feed ingredients;
- labelling;
- traceability/product tracing and record-keeping of feed and feed ingredients;
- inspection and control procedures;
- health hazards associated with animal feed;
- feed additives and veterinary drugs used in medicated feed;
- feed and feed ingredients;
- undesirable substances;
- production, processing, storage, transport and distribution of feed and feed ingredients;
- receiving, storage and transportation;
- personnel training;
- sanitation and pest control;
- equipment performance and maintenance;
- manufacturing controls;
- recalls;
- on-farm production and use of feed;
- good animal feeding practice;
- methods of sampling and analysis.

Although the code of practice does not specifically refer to the use of animal proteins in feed, it does cover regulation and control of animal feed manufacturing including labelling, traceability, inspection, production, processing, storage, transport, sampling and analysis, and training. These aspects ultimately contribute to assuring effective enforcement of BSE measures in place, including feed bans and prevention of cross contamination. The code of practice not only covers production at feed-producing plants, but also covers on-farm manufacture of feed. This is important to BSE control, as raw materials prohibited for use in certain livestock species may be used inappropriately under looser private control.

Further information on relevant aspects of control and safety that are not covered in the code of practice may be found in other Codex standards (e.g. General Principles, Food Labelling, Methods of Sampling and Analysis).

2.2. *Terrestrial Animal Health Code* of the World Organization for Animal Health

The OIE (http://www.oie.int/) is an intergovernmental organization representing 167 member countries. The OIE collects, analyses, and makes available the latest scientific information on animal diseases and disease control throughout the world. Scientific standards are then developed based on this information. The standards are prepared by elected specialist commissions and working groups comprising internationally-renowned scientists, most of whom are experts within the network of 156 OIE collaborating centres and reference laboratories. After adoption, the standards are made available as the *Terrestrial Animal Health Code* (OIE, 2005a) and the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE, 2005b). Similar standards are available for aquatic species.

Because the OIE sets standards for animal health issues, it does not provide specific guidance on feed production or feeding. However, it does provide specific information on BSE (OIE, 2005c), as well as recommendations on what products are safe to trade under what conditions (OIE, 2005a). The OIE code recommends that *Ruminant-derived meat and bone meal or greaves, or any commodities containing such products, which originate from a country, zone, or compartment* (with non-negligible BSE risk) *should not be traded between countries* (OIE, 2005d, Article 2.3.13.12), which clearly means that these ruminant-derived products from most countries should not be traded.

In addition, recommendations for TSE inactivation in the production of meat and bone meal (MBM) are given (OIE, 2005d). Other recommendations are made for trade in other products, according to a country's BSE status. The OIE recommendations for surveillance and diagnosis of BSE are described more fully in the "Introduction to TSEs and BSE" chapter in this course manual.

2.3. Regulations of the European Union

The EU comprises of 25 European states (countries) and was established by the Treaty on European Union in 1992 (although many elements of the Union have existed since the 1950s). The EU unites its states in many aspects, including a common single market consisting of a customs union and a single currency (adopted by 12 out of 25 member states), as well as a single agricultural policy.

Given that BSE was first identified in the UK and that initial spread was most evident within the EU, comprehensive regulations have been put in place to provide for the

control and eventual eradication of BSE. As additional countries look towards beginning the EU accession process, or wish to expand their opportunities for trade, the EU regulations must be considered. Thus, many other countries throughout the world are basing their new or revised regulations on those of the EU. Consequently, bans and other measures implemented by the EU continue to influence the world market in animals and animal products.

The chronology of adoption of the main EU regulations is given in Table 1, and specific regulations can be found on the EU Web site (EU, 2005). These regulations affect not only the member countries, but also other countries in their actions and trade with the EU. Individual EU states may have their own rules for implementation but all must ultimately comply with the EU regulations.

In 1994, all protein derived from mammals was formally prohibited for use in ruminant feed in the EU (EU, 1994), although some member states had implemented such a ban before that date. However, there was no prohibition against export of these materials at that time.

In 1996 and 1998, the EU formally prohibited export of mammalian MBM (and related products) from the UK and Portugal (respectively in these years) to the rest of the EU and third countries (EU, 1996 and 1998).

In 2001, the EU adopted a regulation laying down rules for the prevention, control and eradication of certain TSEs ("the TSE Regulation"; EU, 2001), which prohibits the feeding of mammalian protein (except gelatine from non-ruminants, milk and milk products, and eggs and egg products) to any farmed animal. This ban is often referred to as the "total feed ban". The regulation also sets out more restrictive regulations for member states or regions in the Geographic BSE Risk Assessment (GBR) category IV (i.e. currently UK and Portugal; a description of the GBR is given in section 6.2 of the "Introduction to TSEs and BSE" chapter in this course manual).

Currently in the EU, export of processed animal proteins derived from ruminants (and products containing such proteins) intended for feeding livestock is prohibited from the entire EU to third countries. However, fish meal and some blood products (but not blood meal) can still be used in feed intended for non-ruminants.

The EU regulations are continually being updated and it can be difficult to extract the most current and relevant information. Many specific decisions are no longer in force, and the relevant regulations have been incorporated into other current legislation. However, updated summaries of new information on all BSE topics can be found on the EU Web site (EU, 2006). Currently, the following two regulations directly apply to the feeding of livestock in the face of BSE.

Regulation 1774/2002. Most of the EU animal by-products legislation has been consolidated into the text of Regulation 1774/2002 (EU, 2002), which categorizes animal by-products (animal carcases, parts of animal carcases and products of animal origin which are not intended for human consumption) according to risk and controls their use and disposal. Regulation 1774/2002 also includes a fairly general prohibition on the feeding of a species with material derived from the same species. This regulation is discussed in detail in the "Rendering of animal by-products" chapter in this course manual.

Regulation 882/2004. In 2004, the EU adopted a new regulation laying down requirements for feed hygiene (EU, 2004), which details:

- compulsory registration of all feed business operators by the competent authority;

TABLE 1. Principle legislation on TSE regarding animal feed in the European Union, including legislation number, date, and title

Year	Livestock Feeds/TSE-relevant legislation number, date and title (or topic)
1989	D 89/469/EEC of 28 July 1989. Restrictions on the dispatch of certain live cattle from the UK
1994	D 94/381/EC of 27 June 1994. Ban on the use of proteins derived from mammalian tissues for feeding ruminants D 94/382/EC of 27 June 1994. Rendering systems for processing ruminant waste into MBM (inactivation of BSE agents) D 94/474/EC of 27 July 1994. Restrictions on the dispatch from the UK of live cattle and certain ruminant products - Destruction of specified bovine offal (Repeals D 89/469/EC and 90/200/EC)
1995	D 95/29/EC of 13 February 1995. Amendment of D 94/382/EC - Batch rendering systems D 95/60/EC of 6 March 1995. Amendment of D 94/381/EC - Derogation to the feed ban
1996	D 96/449/EC of 18 July 1996. Pressure cooking system for processing mammalian waste into MBM (inactivation of TSE agents)
1997	D 97/534/EC of 30 July 1997. Prohibition of the use of SRM (mainly brain, eyes and spinal cord) D 97/735/EC of 21 October 1997. Restrictions on trade in MBM
1999	D 1999/129/EC of 29 January 1999. Amendment of D 94/381/EC - Hydrolysed proteins D 1999/534/EC of 19 July 1999. Conditions for the production of MBM and tallow (Repeals D 96/449/EC) D 1999/881/EC of 14 December 1999. Postponement to 30 June 2000 of the date of application of D 97/534/EC (SRM)
2000	D 2000/418/EC of 29 June 2000. Prohibition of the use of SRM (Repeals D 97/534/EC) D 2000/766/EC of 4 December 2000. Temporary ban on use of MBM
2001	D 2001/2/EC of 27 December 2000. Amendment of D 2000/418/EC – Extension of the list of SRM (bovine intestines) D 2001/9/EC of 29 December 2000. Conditions for feeding certain animal proteins D 2001/25/EC of 27 December 2000. Prohibition of the use of dead animals in the production of animal feed D 2001/165/EC of 27 February 2001. Amendment of D 2001/9/EC – Hydrolysed proteins D 2001/233/EC of 14 March 2001. Amendment of D 2000/418/EC – Extension of the list of SRM (vertebral column) D 2001/270/EC of 29 March 2001. Amendment of D 2000/418/EC – Imports from third countries R 2001/999/EC of 22 May 2001. Rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies
2002	R 270/2002 of 14 February 2002. Amendment of R 999/2001 – SRM, surveillance, animal feeding and placing on the market of ovine and caprine animals and products thereof R 2002/248/EC of 27 March 2002. Amending Council D 2000/766/EC and Commission D 2001/9/EC with regard to the feeding of animal proteins R 2002/1774/EC. Laying down health rules concerning animal by-products not intended for human consumption
2003	R 1234/2003 of 10 July 2003. Amendment of R 999/2001 – introducing the current provisions of the feed ban into Regulation (EC) No 999/2001 without a fixed time schedule, thus ending the transitional nature of the feed ban
2004	D 2004/653/EC of 16 September 2004. Amendment of Commission Decision 2001/376/EC as regards the dispatch of meat-and-bone meal of mammalian origin and related products from Portugal
2005	R 1292/2005 of 5 August 2005. Amendment of R 999/2001 as regards animal nutrition.0

Note: R: Regulation, D: Decision.

Source: Derived from: http://ec.europa.eu/food/food/biosafety/bse/chronological_list_en.pdf accessed, Update of February 2006. Updates available through http://ec.europa.eu/food/food/biosafety/bse/legisl_en.htm.

- maintenance of the approval system for feed businesses dealing with sensitive substances;
- operation of all feed businesses in accordance with harmonized hygiene requirements;
- application of good hygiene practice at all levels of agriculture production and use of feed;
- introduction of Hazard Analysis Critical Control Point (HACCP) principles for feed business operators other than at the level of primary production;
- compulsory requirements for feed production at farm level;
- an EU framework for guides to good practice in feed production;
- provision that feed business operators are only permitted to import feed, including single feed materials, from countries outside the EU if the exporting country and the establishment comply with specific requirements and appear on a list. Feed from such establishments will need to comply with the requirements of the Feed Hygiene Regulation;
- an endorsement of the principle that feed business operators must provide a financial guarantee in order to cover the risks related to their businesses.

2.4. Rules of the Food and Drug Administration, United States of America

In 1997, the Food and Drug Administration (FDA) of the USA published a rule (hereinafter referred to as Rule 21; FDA, 1997) describing that mammalian protein for use in ruminant feed is considered a food additive. Thus, in the USA, the use of any material that contains mammalian protein is prohibited in ruminant feed, though in some cases pure porcine or pure equine materials from single-species slaughter facilities may be fed to ruminants.

According to the rule, renderers, protein blenders, feed manufacturers, and distributors that manufacture, blend, process, and distribute products that contain (or may contain) mammalian protein and that are intended for use in animal feed must properly label the materials with the words ''Do not feed to cattle or other ruminants'' and must maintain (and make available to the competent authority) records sufficient to track the materials throughout their receipt, processing and distribution. Moreover, renderers who obtain ruminant or non-pure porcine or equine materials must implement sufficient measures in order to prevent cross contamination of products that may be used for ruminants. They must also maintain written procedures specifying in these measures the procedures for separating products from the time of receipt until the time of shipment.

In addition, establishments and individuals that are responsible for the feeding of ruminant animals must maintain copies of purchase invoices and labelling for all feeds received that contain animal protein products.

Following the reporting of a clinical case of BSE in the USA in December 2003, an international review team was invited to consider the response to this finding. In July 2004, subsequent to this review, the FDA described potential measures related to animal feed (US FDA, 2004a). These measures, intended to reduce the risks throughout feed manufacturing and distribution and on the farm (due to misfeeding) and to decrease recycling of the agent (including an SRM ban for feed), were proposed as a rule in October of 2005 (US FDA, 2005).

In addition, FDA (US FDA, 2004b) measures prohibit the use of bovine materials that

could carry the BSE agent in cosmetics and human foods, including certain meat-based products and dietary supplements. These high-risk bovine materials include SRM (including brain, skull, eyes, and spinal cord of cattle 30 months of age or older, and small intestine and tonsils from all cattle), material from non-ambulatory disabled cattle, material from cattle not inspected and passed for human consumption, and mechanically separated beef.

Much as in the EU, individual states in the USA may have their own rules for implementation, but all states must ultimately comply with the FDA (and other federal) rules. Also as with the EU (and most countries), official rules and legislation are continually being updated as new information becomes available. The most recent legislation must always be complied with.

3. IMPLEMENTATION OF INTERNATIONAL AND NATIONAL STANDARDS AND REGULATIONS: INDUSTRY GUIDELINES

The various international, regional and national recommendations and regulations all include the banning of ruminant and/or all mammalian protein in animal feed and the identification, labelling and traceability of such materials through the feed chain. They also attempt to ensure that there is no cross contamination of feed ingredients all along the feed chain in order to prevent even extremely small quantities of infective material from being fed. Figure 2 shows the animal feed chain and the various steps and stakeholders that need to comply with these regulations.

The animal feed industry and various stakeholders in the feed chain have therefore developed codes and guidelines to facilitate compliance with the recommendations and regulations. Some of the major codes and guidelines are described below.

3.1. Grain and Feed Trade Association

The Grain and Feed Trade Association (GAFTA: http://www.gafta.com/) is an international trade association for grains and other feeds, with 930 members in 80 countries. It provides standard forms for contracts, training and professional development, a dispute resolution service, arbitration and mediation, schemes for superintendents and analysts, as well as information resources, notably the GAFTA Traders' Manual (GAFTA, 2004), which provides standards of best practice for all trade operations.

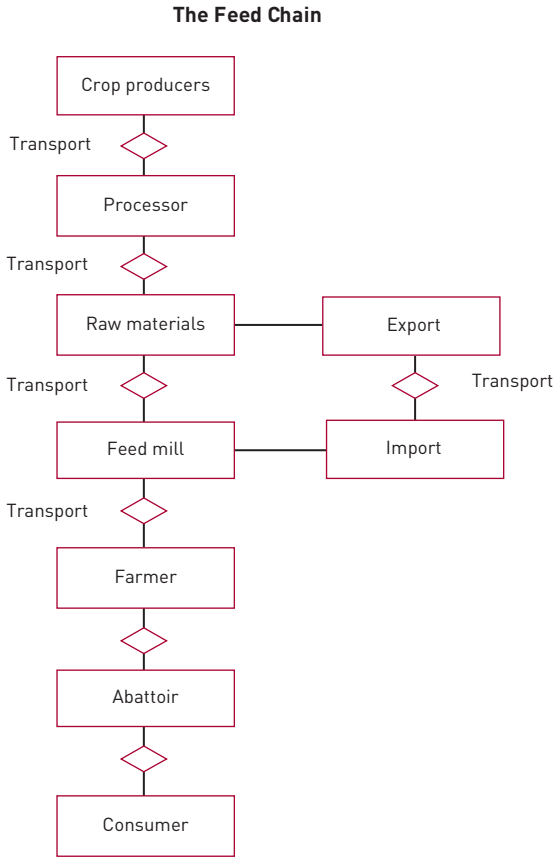
The GAFTA standard for the international grain and feed trade is an all-encompassing system to provide safe food and feed materials worldwide. Using a HACCP[1]-based approach, it links the "best practices" for transport, storage, loading, discharge, supervision and analysis from the farm onwards for all combinable crops and dry, moist and liquid animal feed materials. There are seven major sections:

- Storage - describing the storage of combinable crops, and dry, moist and liquid animal feeds from farm onwards.
- Loading, discharge, supervision and handling - describing each of these logistical operations with reference to the GAFTA weighing rules and the GAFTA Superintendents scheme.

1 Hazard Analysis and Critical Control Points (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).

FIGURE 2

Schematic of the livestock feed production chain and the areas to be considered in developing codes of practice.



- Analysis - describing analysis throughout the supply chain, with reference to all the GAFTA methods of analysis and the GAFTA analysts scheme.
- Transport - describing all transport operations from farm onwards, including road, rail, waterway and sea.
- Pest control and fumigation - describing minimization of losses and damage and an integrated pest programme.
- Introduction to HACCP - describing how the HACCP approach is taken to minimize losses, damage and contaminant risks. The basics of applying HACCP to the supply chain are explained.
- Audit/verification – because certain import markets require traders to have their supply operations independently audited/verified, this section describes how to gain GAFTA verification.

To ensure conformity, GAFTA requires verification of each trader's office annually for the first three years, then once every two years thereafter. Verifiers must be independent and are governed by their own GAFTA Code of Practice to ensure their operations constantly comply with the criteria and they must inform GAFTA of all verification results.

3.2. International Feed Industry Federation

The International Feed Industry Federation (IFIF; http://www.ifif.org/) is an organization that represents national and regional feed associations and federations and others involved in the production of compound animal feeds. Members of IFIF are mainly national associations, corporate/commercial members (suppliers to the feed trade), and other feed-related organizations. The IFIF has observer status in the Codex Alimentarius and is working closely with FAO in the practical implementation of the Codex code of practice on animal feeding, described in section 2.1 of this chapter.

3.3. American Feed Industry Association

The American Feed Industry Association (AFIA; http://www.afia.org/) is the feed industry association for the USA. It includes 690 member companies, representing nearly 75% of the commercial feed and pet food sold annually in the USA. The AFIA's members include manufacturers, ingredient suppliers, animal health companies, equipment manufacturers, large integrated livestock and poultry producers, and firms providing other goods and services to the commercial animal food industry. In addition, AFIA also includes more than 35 state, regional, national and international associations among its membership.

The AFIA publishes the *Feed Manufacturing Technology* manual, which is revised annually. The chapter on quality assurance describes controls aimed to promote the production of products that consistently meet predetermined quality standards. It comprehensively describes an in-plant quality commitment programme including policies, procedures, sampling, and testing relevant to feed production in the USA.

The AFIA also publishes a guide to help feed manufacturers comply with the FDA Rule 21 prohibiting mammalian protein in ruminant feed (AFIA, 2001). The specific requirements in the three principal areas (labelling, record-keeping, and equipment cleaning) are detailed. This guide is designed for plants that manufacture feed for more than one species, and that use MBM or other mammalian proteins. It emphasizes that specific written procedures must be developed and used for each individual facility.

It should be noted that, although meeting these guidelines will allow compliance with the FDA rules and BSE measures in the USA, these measures will not optimally prevent cross contamination with BSE infectivity, as described in the "Overview: Implementation of TSE measures for livestock feeds" chapter in this course manual.

3.4. European Feed Manufacturers' Federation

The European Feed Manufacturers' Federation (FEFAC; http://www.fefac.org/) consists of national associations from EU member states as full members, and many observer members from non-EU countries. As an independent organization, FEFAC represents the European feed industry in EU legislative negotiations, and holds observer status in the Codex Alimentarius.

Moreover, FEFAC develops professional rules and good manufacturing practices for improving the quality and safety of compound feed. It has published the *FEFAC guidelines for the implementation of a code of practice for the manufacture of animal feedingstuffs* (FEFAC, 2001) covering:

- quality management and quality control;
- risk analysis;
- undesirable substances and products, including bacteria;

- additives and medicaments;
- facilities and equipment;
- personnel;
- product conception and feed formulation;
- product safety;
- production (purchase, delivery, and intake, weighing, grinding and particle size, mixing, pelleting/heat treatment, cooling, storage);
- transport and storage;
- documents and records;
- registration of compound feedstuffs;
- complaints and product recall.

In an attempt to make all operations along the feed chain fully responsible for the products they deliver, this document includes the implementation of HACCP principles at all stages of production. Thus, it is stricter than the current EU legislation.

In addition, FEFAC lists codes of practice from associations recognized by FEFAC. These include codes of practice developed (or in development) for trade, and for the production of animal fats and meals, fish oils and meals and other products and by-products for use in feeds.

FEFAC also lists National Codes of Practice developed by FEFAC members, including

- Código de boas práticas para o fabrico de prémisturas e de alimentos para animais (IACA - Portugal)
- GMP-regeling diervoedersector (Productschap Diervoeder – The Netherlands)
- Code GMP general pour le secteur de l'alimentation animale (BEMEFA/APFACA – Belgium)
- Codice di buone pratiche per la produzione e la commercializzazione di alimenti composti per animali de reddito (ASSALZOO – Italy)
- Code de bonnes pratiques pour la fabrication d'aliments médicamenteux – Guide de mise à niveau pour l'agrément des établissements fabricants des aliments pour animaux (SNIA – France)
- Leitfaden für eine Gute Herstellungspraxis von Futtermitteln (DVT – Germany)
- UKASTA Feed Assurance Scheme (UFAS) - Code of Practice for the Manufacture of Safe Compound Animal Feedingstuffs (UKASTA - UK)
- Code of practice and general operating standard for poultry feed processing (DAKOFO - Denmark)
- Leitfaden für eine "Gute Herstellungspraxis von Futtermitteln", GHF (VSF - Switzerland).

4. SUMMARY OF TSE-RELEVANT CONCEPTS

- Most countries implementing BSE measures already have some sort of ban on feeding livestock with protein derived from animal by-products. Currently under discussion in these countries is an additional ban on SRM in all animal feeds, following the example of the EU.
- Internationally-recognized standards for control and prevention of BSE are available for general aspects of rendering, livestock feed manufacturing and livestock feeding in order to protect domestic public and animal health and maintain trade in animals and animal products.

- The regulations of the EU regarding implementation of international recommendations regarding BSEs are currently being adopted or otherwise incorporated into the legislation of many other countries.
- Other information, including several different codes of practice, is also available from governmental and private sources to provide additional details for effectively and consistently implementing the regulations.

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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 points[1] 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 HACCP[2] 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

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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 ºC/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 floatation 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 (tetrachlorehylene) 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.

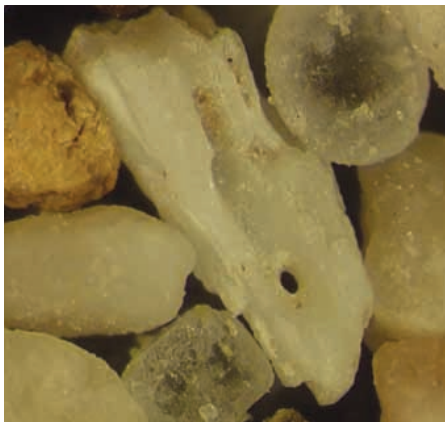


Plate 1

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

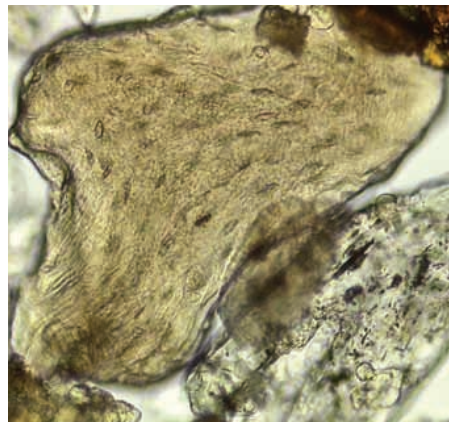


Plate 2

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

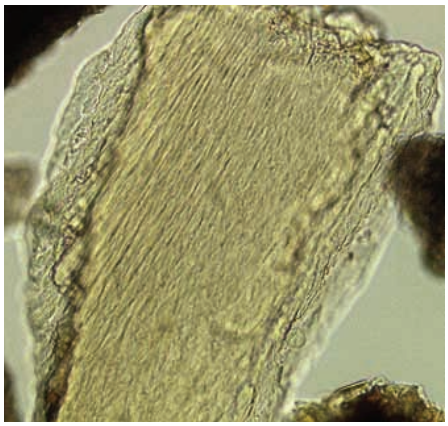


Plate 3

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

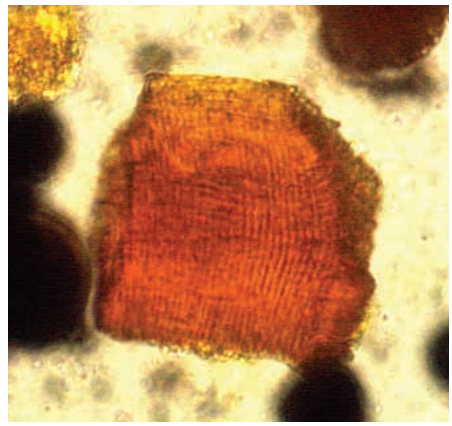


Plate 4

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

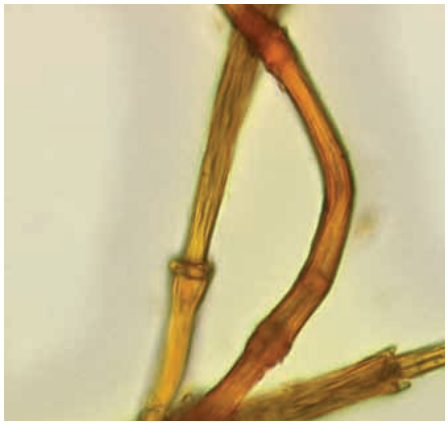


Plate 5

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

PHOTOS: COURTESY OF AGROSCOPE LIEBEFELD-POSIEUX, SWITZERLAND

2.2. Immunoassay

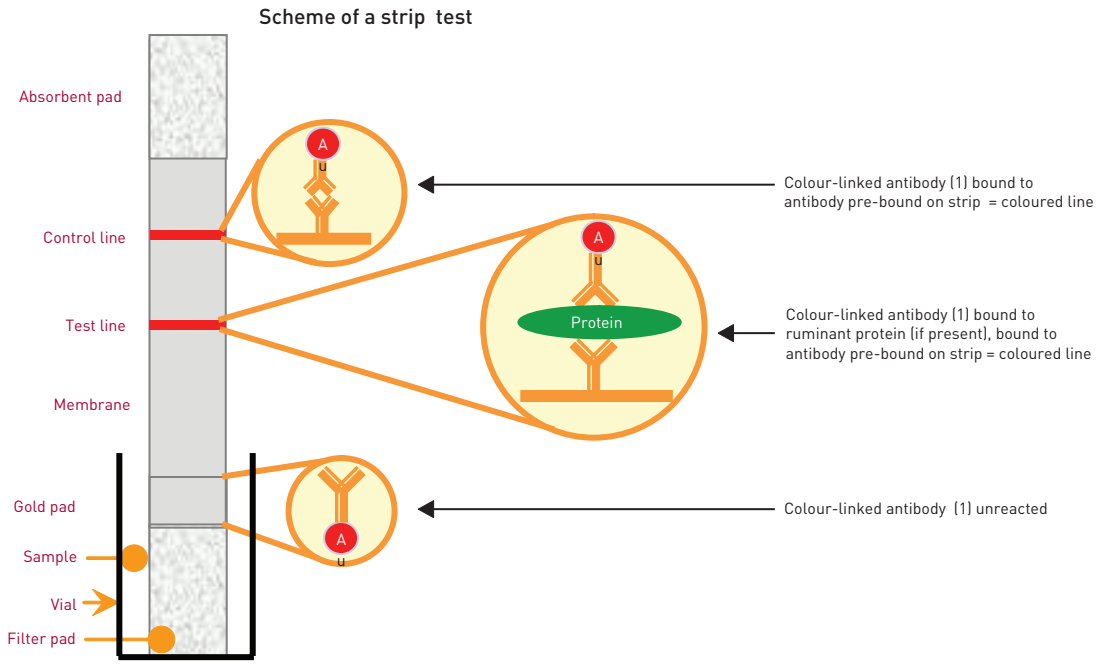
Immunoassay is a method to identify a specific protein in a sample. It involves extraction of the protein, reaction of the protein of interest with a specific antibody and then amplification and visualization of the response signal.

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	ReVeat®
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 ºC/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

	World Trade in Meat and Bone Meal (1996 – 1999) Importing Country										
Exporting 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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APPENDIX 1

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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.

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

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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/

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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

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

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

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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 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 *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

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.codexalimentarius.net/web/standard_list.do?lang=en

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
- 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.