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

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DIAGNOSTIC TECHNIQUES FOR TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

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

course manual

DIAGNOSTIC TECHNIQUES FOR TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

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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*, is the result of collaboration between the Food and Agriculture Organization of the United Nations (FAO), Safe Food Solutions Inc. (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 Agreement) 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 encephalopathy (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 are contributions 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 *Diagnostic techniques for transmissible spongiform encephalopathies*, which is given within the framework of the project. This practical course is targeted at veterinary diagnosticians who will contribute to the development and implementation of the national BSE surveillance and control programme, and to the BSE risk assessment for the partner countries.

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 *Diagnostic techniques for transmissible spongiform encephalopathies* 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 *Diagnostic techniques for transmissible spongiform encephalopathies*, of the project *Capacity Building for Surveillance and Prevention of BSE and Other Zoonotic Diseases,* the participants should:

- understand basic information on BSE and TSEs, including transmission, pathogenesis, risk variables and epidemiology;
- understand the concepts of testing for BSE, including limitations;
- be able to collect appropriate brain samples correctly from cattle heads;
- Be able to prepare brain samples correctly for histopathology, immunohistochemistry and rapid tests;
- be able to run rapid tests;
- be able to diagnose BSE correctly using immunohistochemistry and rapid tests.

INTRODUCTION TO TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

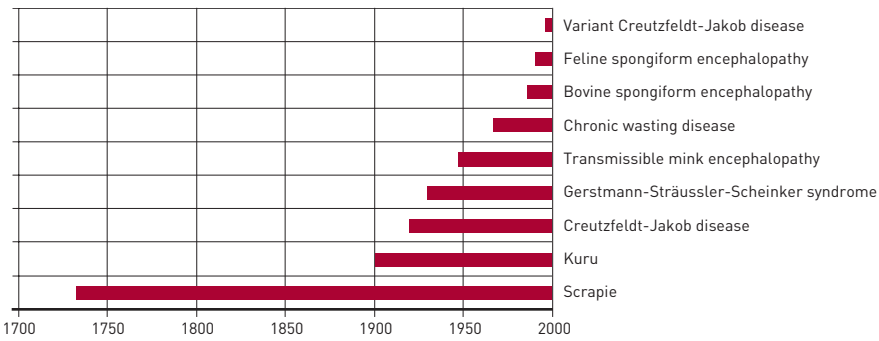
1. TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

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 Nothern 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.

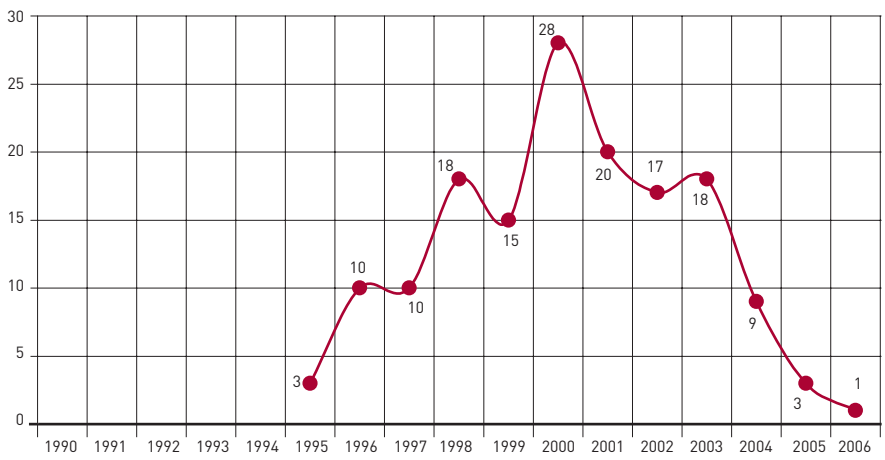
Several TSEs have been reported to occur in humans, including two forms of Creutzfeldt-Jakob disease (sporadic CJD and variant CJD [vCJD]), Kuru, Gerstmann-Sträussler-Scheinker syndrome, as well as fatal familial insomnia. Of these, only vCJD has been associated with BSE. Sporadic CJD was first identified 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 probable based on the current incidence of vCJD cases (Figure 2).

FIGURE 2

Number of vCJD cases in the UK over time



Source: Department of Health, UK (2006)

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

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, though 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 four and six 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 to 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

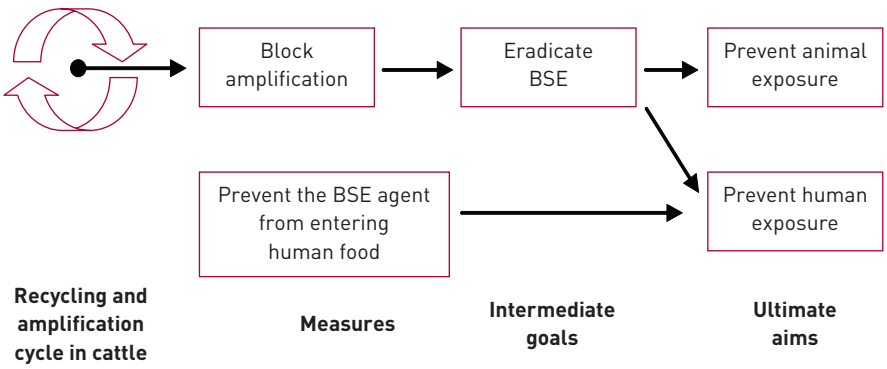
The ultimate aims of BSE control and prevention programmes are to reduce exposure risk both to cattle and to humans (Figure 3). Two levels of measures must therefore be considered:

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

Owing to 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 re-infection (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 the *Capacity Building for Surveillance and Prevention of BSE and Other Zoonotic Diseases* project course manual entitled *Management of transmissible spongiform encephalopathies in livestock feeds and feeding* (FAO, 2007a).

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 the *Capacity Building for Surveillance and Prevention of BSE and Other Zoonotic Diseases* project course manual entitled *Management of transmissible spongiform encephalopathies in livestock feeds and feeding* (FAO, 2007a).

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.

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

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 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 ordinary 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 and enforce effectively 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. Laboratory diagnostic testing is covered in depth in subsequent chapters in this manual.

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, 2007b).

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. Also, 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

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. SURVEILLANCE SYSTEMS

5.1. 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 World Organisation for Animal Health (OIE) *Terrestrial Animal Health Code* (OIE 2005b, c). These recommendations are considered by WTO and the international community as the international standards (WTO, 1994).

5.2. 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.

5.3. 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 over 30 months of age selectively 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 encephalophathies* (FAO, 2007c).

6. RISK ASSESSMENT

6.1. 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, 2005e,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

Surveillance for TSEs and other epidemiological investigations (especially surveillance for BSE conducted on the cattle population) should also 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 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

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

- 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

6.2. 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 Europan 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).

7. REFERENCES

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

DEFRA (Department for Environment Food and Rural Affairs, UK). 2004. BSE pages. http://www.defra.gov.uk/animalh/bse/index.html

Department of Health, UK. 2006. CJD-homepage. http://www.dh.gov.uk/PolicyAndGuidance/HealthAndSocialCareTopics/CJD/fs/en

Detwiler LA, Baylis M. 2003. The epidemiology of scrapie. *Rev sci tech Off int Epiz* 22(1), 121-143

Eloit M, Adjou K, Coulpier M, Fontaine JJ, Hamel R, Lilin T, Messiaen S, Andreoletti O, Baron T, Bencsik A, Biacabe AG, Beringue V, Laude H, Le Dur A, Vilotte JL, Comoy E, Deslys JP, Grassi J, Simon S, Lantier F, Sarradin P. 2005. BSE agent signatures in a goat. *Vet. Rec.* 156, 523-524

EC (European Commission). 2004. Food and feed safety: legislation concerning TSE. http://europa.eu.int/comm/food/food/biosafety/bse/legisl_en.htm

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

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

FAO. 2007c. *Epidemiology, surveillance and risk assessment for transmissible spongiform encephalophathies.* Course manual. Project *Capacity Building for Surveillance and Prevention of BSE and Other Zoonotic Diseases.* Rome

Heim D, Kihm U. 1999. Bovine spongiform encephalopathy in Switzerland - the past and the present. *Rev sci tech Off int Epiz* 18(1), 135-144

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

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

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

Heim D, Wilesmith J.W. 2000. Surveillance of BSE. *Arch Virol Suppl.* 16,127-133

Lasmezas CI. 2003. The transmissible spongiform encephalopathies. *Rev sci tech Off int Epiz* 22(1), 23-36

OIE (World Organisation for Animal Health). 2005a. Procedures for the reduction of infectivity of transmissible spongiform encephalopathy agents. *Terrestrial Animal Health Code,* Appendix 3.6.3. http://www.oie.int/eng/normes/mcode/en_chapitre_3.6.3.htm

OIE. 2005c, General guidelines for animal health surveillance. *Terrestrial Animal Health Code,* Appendix 3.8.1. http://www.oie.int/eng/normes/MCode/en_chapitre_3.8.1.htm

OIE. 2005c. Surveillance for bovine spongiform encephalopathy. *Terrestrial Animal Health Code,* Appendix 3.8.4. http://www.oie.int/eng/normes/MCode/en_chapitre_3.8.4.htm

OIE. 2005d. Bovine spongiform encephalopathy. *Terrestrial Animal Health Code*, Chapter 2.3.13. http://www.oie.int/eng/normes/MCode/en_chapitre_2.3.13.htm

OIE. 2005e. General considerations (risk analysis). *Terrestrial Animal Health Code,* Chapter 1.3.1. http://www.oie.int/eng/normes/MCode/en_chapitre_1.3.1.htm

OIE. 2005f. Import risk analysis. *Terrestrial Animal Health Code,* Chapter 1.3.2. http://www.oie.int/eng/normes/MCode/en_chapitre_1.3.2.htm

OIE. 2005g. Factors to consider in conducting the bovine spongiform encephalopathy risk assessment recommended in chapter 2.3.13. *Terrestrial Animal Health Code,* Appendix 3.8.5. http://www.oie.int/eng/normes/MCode/en_chapitre_3.8.5.htm

Prince MJ, Bailey JA, Barrowman PR, Bishop KJ, Campbell GR, Wood JM. 2003. Bovine spongiform encephalopathy. *Rev sci techn Off int Epiz* 22(1), 37-60

Schreuder BE, Somerville RA. 2003. Bovine spongiform encephalopathy in sheep? *Rev Sci tech Off int Epiz* 22(1),103-120

SSC (Scientific Steering Committee of the European Commission). 2000. Opinion of the Scientific Steering Committee on BSE-related culling in cattle. Adopted at the meeting of 14/15 September 2000. http://europa.eu.int/comm/food/fs/sc/ssc/out138_en.pdf

SSC. 2001a. Opinion on hypotheses on the origin and transmission of BSE. Adopted by the Scientific Steering Committee at its meeting of 29-30 November 2001. http://europa.eu.int/comm/food/fs/sc/ssc/out236_en.pdf

SSC. 2001c. Opinion on the six BARB BSE cases in the UK since 1 August 1996. http://europa.eu.int/comm/food/fs/sc/ssc/out237_en.pdf

SSC. 2001b. Safety of milk with regard to TSE: state of affairs. http://europa.eu.int/comm/food/fs/sc/ssc/out175_en.html

SSC. 2002a. The safety of bovine embryos. Amendment to the SSC opinion of 18-19 March 1999 on the possible vertical transmission of bovine spongiform encephalopathy (BSE). http://europa.eu.int/comm/food/fs/sc/ssc/out263_en.pdf

SSC. 2002b. Update of the opinion on TSE infectivity distribution in ruminant tissues. Initially adopted by the Scientific Steering Committee at its meeting of 10-11 January 2002 and amended at its meeting of 7-8 November 2002. http://europa.eu.int/comm/food/fs/sc/ssc/out296_en.pdf

SSC. 2002c. Opinion of the SSC on the additional safeguard provided by different culling schemes under the current conditions in the UK and DE. Adopted on 11 January 2002. http://europa.eu.int/comm/food/fs/sc/ssc/out242_en.pdf

TAFS (International Forum for TSE and Food Safety). 2004a. TAFS position paper on specified risk materials. http://www.tseandfoodsafety.org/position_papers/TAFS_POSITION_PAPER_SPECIFIED%20RISK%20MATERIALS.pdf

TAFS. 2004b. TAFS position paper on testing of cattle for BSE – Purpose and effectiveness. http://www.tseandfoodsafety.org/position_papers/TAFS_POSITION_PAPER_ON%20TESTING%20OF%20CATTLE.pdf

TAFS. 2007. TAFS position paper on the safety of bovine milk and bovine milk products". http://www.tseandfoodsafety.org/position_papers/TAFS_POSITION_PAPER_ON_MILK.pdf

Taylor DM, Woodgate SL. 1997. Bovine spongiform encephalopathy: the causal role of ruminant-derived protein in cattle diets. *Rev sci tech Off int Epiz* 16, 187–198

Taylor DM, Woodgate SL. 2003. Rendering practices and inactivation of transmissible spongiform encephalopathy agents. *Rev sci tech Off int Epiz* 22(1), 297-310

Taylor DM, Fraser H, McConnell I, Brown DA, Brown KL, Lamza KA, Smith GRA. 1994. Decontamination studies with the agents of bovine spongiform encephalopathy and scrapie. *Arch Virol* 139, 313–326

Wells GA. 2003. Pathogenesis of BSE. *Vet Res Commun.* Suppl 1, 25-28

Wells GA, Scott AC, Johnson CT, Gunning RF, Hancock RD, Jeffrey M, Dawson M, Bradley R. 1987. A novel progressive spongiform encephalopathy in cattle. *Vet Rec* 121(18), 419-420

Wells GAH, Dawson M, Hawkins SAC, Green RB, Dexter I, Francis ME, Simmons MM, Austin AR, Horigan MW. 1994. Infectivity in the ileum of cattle challenged orally with bovine spongiform encephalopathy. *Vet Rec* 135(2), 40-41

Wells GAH, Dawson M, Hawkins SAC, Austin AR, Green RB, Dexter I, Horigan MW, Simmons MM. 1996. Preliminary observations on the pathogenesis of experimental bovine spongiform encephalopathy. In: *Bovine spongiform encephalopathy: the BSE dilemma*. Ed. C.J. Gibbs, Serono Symposia, Norwell, USA Springer-Verlag, New York, Inc. pp. 28-44

Wells GA, Hawkins SA, Green RB, Austin AR, Dexter I, Spencer YI, Chaplin MJ, Stack MJ, Dawson M. 1998. Preliminary observations on the pathogenesis of experimental bovine spongiform encephalopathy (BSE): an update. *Vet Rec* 142(5), 103-106

Wells GA, Hawkins SA, Green RB, Spencer YI, Dexter I, Dawson M. 1999. Limited detection of sternal bone marrow infectivity in the clinical phase of experimental bovine spongiform encephalopathy (BSE). *Vet Rec* 144(11), 292-294

Wilesmith JW, Wells GA, Cranwell MP, Ryan JB. 1988. Bovine spongiform encephalopathy: epidemiological studies. *Vet Rec* 123(25), 638-644

Wilesmith JW, Ryan JB, Atkinson MJ. 1991. Bovine spongiform encephalopathy: epidemiological studies on the origin. *Vet Rec* 128(9), 199-203

Will RG, Ironside JW, Zeidler M, Cousens SN, Estibeiro K, Alperovitch A, Posers S, Pocchiari M, Hofman A, Smith PG. 1996. A new variant of Creutzfeldt-Jakob disease in the UK. *Lancet* 347, 921-925

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

Wrathall AE. 1997. Risks of transmitting scrapie and bovine spongiform encephalopathy by semen and embryos. *Rev sci Tech Off int Epiz* 16(1), 240-264

Wrathall AE, Brown KF, Sayers AR, Wells GA, Simmons MM, Farrelly SS, Bellerby P, Squirrell J, Spencer YI, Wells M, Stack MJ, Bastiman B, Pullar D, Scatcherd J, Heasman L, Parker J, Hannam DA, Helliwell DW, Chree A, Fraser H. 2002. Studies of embryo transfer from cattle clinically affected by bovine spongiform encephalopathy (BSE). *Vet Rec* 150(12), 365-37

LABORATORY-BASED SURVEILLANCE AND EPIDEMIOLOGY

Knowledge on the theory behind monitoring and surveillance for animal diseases, on diagnostic test characteristics and on sampling approaches is essential for the understanding and correct design of monitoring and surveillance systems (MOSS), and for the interpretation of results.

This chapter of the course manual addresses some fundamental concepts related to disease surveillance, measures of disease frequency (prevalence, incidence), sample size calculations for disease detection and prevalence estimation, diagnostic test evaluation (sensitivity, specificity, predictive values) and measures of association (relative risk, odds ratio). Additional information is available in the "Veterinary epidemiology – principles and concepts" and "Surveillance for BSE" chapters in the course manual entitled *Epidemiology, surveillance and risk assessment for transmissible spongiform encephalophathies* (FAO, 2007).

The exercises were designed for use with the free software package WinEpiscope v2; this software package can be downloaded from various Web sites (for example, EpiVetNet, 2006).

1. MONITORING AND SURVEILLANCE SYSTEMS

The expression "Surveillance" goes back to the time of the French Revolution (late eighteenth century) when this term described an activity of governmental forces "...to keep an eye on subversive subjects...", certainly with an intention to take action when deemed necessary.

In more recent documents produced by the World Organisation for Animal Health (OIE) and other international bodies, a clear distinction is being made between monitoring and surveillance:

Monitoring (to watch, follow, observe): a continuous (ongoing) process of data collection on the health status (health-related events) within animal populations over a defined period of time (could potentially be "forever").

Surveillance (monitor and control): extension of monitoring in which control or eradication action is taken once a predefined level of the health-related event ("disease") has been reached.

Unfortunately, this terminology has not been used consistently; quite frequently the term surveillance is used very globally to describe any activity related to detecting cases of disease within populations. One of the reasons could be that (in veterinary public health) basically all animal diseases that are monitored are also regulated by certain control programmes. There, the use of surveillance is indeed appropriate. If, as an example, the prevalence of Newcastle disease (ND) in wild birds is routinely assessed by testing hunted and found dead birds but no control measures are in place if the agent is found, then this would constitute a "simple" monitoring approach.

Reporting of clinically suspicious (sick) animals was introduced first regionally and then nationally during the nineteenth century in order to control rinderpest. The main

reason was that veterinary authorities realized that individual animal owners did not have the resources to prevent the spread of these diseases from heir livestock to other farms, thus resulting in large outbreaks with high economic losses. The concept of mandatory reporting of clinical suspects and subsequent movement restrictions, destruction and compensation for losses (by the authorities), once proven for that disease, was quickly adopted for other infectious (transmittable) animal diseases such as anthrax, rabies, foot-and-mouth disease (FMD), contagious bovine pleuropneumonia (CBP), sheep pox, glanders, dourine and scabies of sheep and horses. It has remained the core approach in order to control outbreaks of highly contagious animal diseases.

The list of diseases notifiable to the international authorities (OIE, 2005) are an extension of this earlier selection of reportable and controllable animal diseases. Legislation of the European Union as well as country-specific legislation might include additional diseases not listed by the OIE in order to account for regional differences.

1.1. Classifications of MOSS

Disease monitoring and surveillance systems can be classified based on different criteria (Doherr and Audigé, 2001):

- Reason for data collection (objectives, why a MOSS is implemented)
- Source of data and type of information
- Approach to data collection (passive, active etc.)
- Number of diseases included (one or several)
- Geographic region (local, national, international)
- Target population (clinical suspect cases, infected animals, potentially exposed animals, etc.)
- Approach to selection/sampling (whole population or defined sample)
- Control element (autonomous or integrated programme)

One of the most frequent reasons for the implementation of a MOSS is the documentation to others that the disease of interest is below a certain threshold level in order to support the trade of animals and animal products. Other reasons include the need to control a disease for its zoonotic potential, for the economic losses that it causes, for risk analysis and research purposes or for its historical importance. In times of limited resources, veterinary services should assess the existing MOSS programmes and make conscious decisions as to whether or not certain programmes need to be continued, and if additional programmes are needed.

There is a broad range of activities and institutions where information on the disease status of individual animals, groups of animals or the population is generated (Doherr and Audigé, 2001). Often, however, information from only one or two data sources is used to define the disease status of an animal population. One reason could be that the information is collected in different databases that are operated by different institutions, and that the exchange of information between the institutions (and therefore databases) does not exist. Other reasons include the lack of a common animal or farm identification (and tracing) system, making it impossible to link information reliably from different databases, and the difficulties in correctly weighing and pooling MOSS data from different sources into one estimate on the probability of a region or country being disease-free.

Once collected and analysed, there is a certain spectrum of "customers" interested in these MOSS data. This, to a varying degree, includes their own and foreign veterinary

services (trade, veterinary public health interventions), the respective industries, universities, the media, the general public and others. One has to be very careful in the way MOSS information is communicated to the different interest groups since their level of understanding and therefore correctly interpreting the conveyed information will vary substantially.

The most fundamental approach to collecting animal disease data from respective target populations is either through baseline (passive) monitoring or through targeted (active) sampling and testing. Sentinel networks form another approach to collecting health-related information from populations; this will not be addressed further here.

Baseline or passive "surveillance" is defined as the, often mandatory, reporting of clinical suspect cases to the veterinary authorities. In some countries, the legislation differentiates between immediate notification of a disease suspicion and routine reporting of past cases, often per month, quarter or year – depending on the disease. This system of "passive" reporting relies on the awareness of the animal owners and veterinary practitioners of the disease, and their willingness to report a suspicious case once they recognize one. This system has a long history, was successful for a certain range of diseases, uses an infrastructure (farmers, veterinarians) that is already in place (low cost for the individual disease), and can cover a broad range of diseases. However, it can only be used for diseases that present clear clinical signs, and works best for those diseases that are highly contagious and thus spread, and that have a short incubation period. Moreover, the approach often underestimates the true level of disease, and in some instances the disease can go undetected, or detected but not reported, for extended periods of time. Therefore, reported cases indicate that the disease is present at at least that level. No reporting of cases, however, cannot automatically be taken as the proof that a country or region is indeed free of the disease. In order to understand better the sequence of events that needs to take place before a clinically diseased animal is "processed" within such a system and identified as a "case", one can construct an event tree and assign probabilities of success to each step of that tree. An example is given in Doherr and Audigé (2001). For diseases with un-specific clinical symptoms and severe consequences (for the owner), the reporting and detection probabilities might be rather low.

Targeted (active) "surveillance" is defined as the ongoing (continuous) or periodic (once or repeated) scientifically based collection of samples/data on a certain disease from a predefined animal target population. It is a cost-intensive approach that needs a good scientifically based design. The results, however, should be representative for the target population, i.e. accepted as valid. This approach generally works well if a fast and inexpensive diagnostic test system is available to detect the condition of interest, and if a target population can reliably be identified in which the event of interest is likely to be higher when compared to the overall population. If no such target population can be identified, then a general population survey needs to be performed, resulting in higher costs.

1.2. Prevalence and incidence

The outcome of any MOSS can be expressed as a measure of disease frequency. The most common measures of disease frequency are:

- Prevalence
- Incidence count

- Incidence risk, cumulative incidence
- Incidence rate, incidence density

Prevalence is defined as the number of existing (measurable) events (cases) in a defined population at risk (of being a case) at a specific point in time (cross section). In Figure 1, this would be the number of cases (thick red horizontal lines) divided by the total number of animals (all horizontal lines) at a given point in time (cross sections at times A, B, C, ..., I). The lowest prevalence is measured at time D (0/16) while the highest prevalence values are measured at times F and G (both 6/22).

Incidence in general relates to the number of new cases observed in a population at risk over a defined period of time.

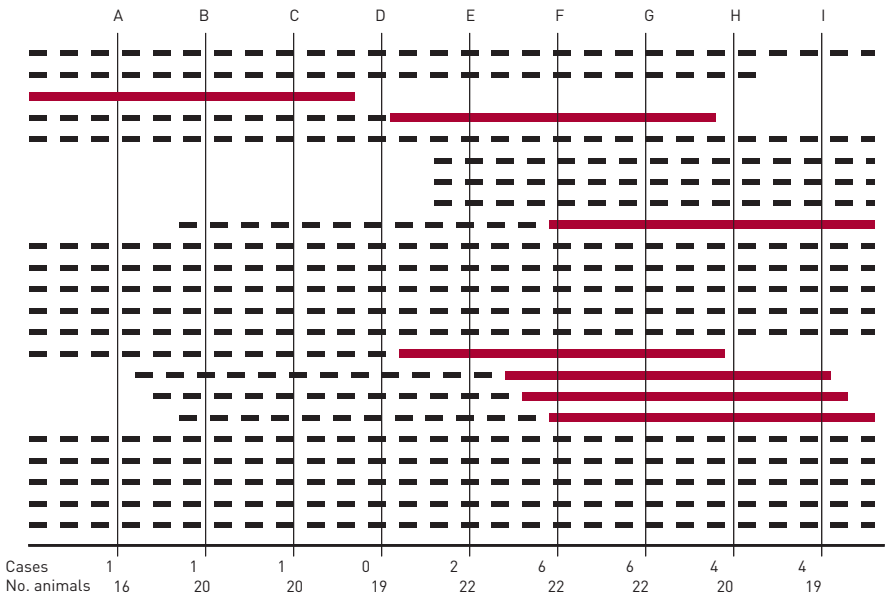
Incidence count is just the total number of cases over that time period not taking into account the number of animals at risk; in Figure 1, the incidence count for the time period A-I would be 6 (the first case was not new – it already existed at the beginning of the time period).

Cumulative incidence (risk), the most commonly expressed incidence, is the number of new cases over a specified time period (numerator) divided by the number of animals at risk of becoming a case during that time period (denominator). The new cases are counted as for the incidence count (A-I: 6). The difficulty lies with measuring the population at risk (denominator), especially in a dynamic population with exits and new entries. Frequent approaches are either to take the population at risk present at the beginning of the time interval (A: 16), the population at the midpoint of the interval (E: 22) or the average population during the interval ((A+I)/2: 17.5). The result is expressed as a proportion for the specified time period (month, year, etc.).

New cases for the incidence density (rate) are counted as before. The denominator, however, is now an accumulation of animal time at risk, and the resulting incidence rate

FIGURE 1

Schematic representation of prevalence and incidence (see text for explanation)



expresses the number of new cases per animal time (months) at risk in the given population. This measure is rarely used in veterinary medicine since exact data on animal time at risk are frequently not available.

2. DIAGNOSTIC TEST CHARACTERISTICS

In any MOSS approach, the characteristics of the diagnostic test or combination of tests, subsequently referred to as the diagnostic test system, is essential to the performance of that MOSS. Thus, the designers, operators and recipients of information should be aware of the properties and limitations of the system used to identity and to confirm diseased individuals.

In their field manual for veterinarians, Cannon and Roe (1982) give a good description of the most important diagnostic test characteristics (definition, calculation and interpretation); the equivalent chapter in the textbook by Thrusfield (1995) provides more technical details on this issue.

Initially, diagnostic test developers are primarily interested in the analytic test properties. These are defined as the analytic sensitivity, i.e. the lower detection limit or the smallest, still detectable, amount of the substance that the test is supposed to measure (antigen, antibody, chemical, protein, etc.), and the analytic specificity (cross reaction profile), i.e. the ability of the test not to react to or bind with rather similar (in structure, etc.) other substances.

When applying tests to populations, however, we need to know their operational properties, mainly their ability to classify correctly truly diseased and truly non-diseased individuals.

2.1. Diagnostic test sensitivity and specificity

The diagnostic test sensitivity (SE) is defined as the proportion of truly diseased ("gold standard" positive) individuals that the test correctly classifies as (test) positive. It can also be expressed as a conditional probability of a test-positive outcome (T+) given that the animal is diseased (D+):

$$SE = P(T+|\ D+)$$

The diagnostic test specificity (SP) is defined as the proportion of truly non-diseased ("gold standard" negative) individuals that the test correctly classifies as (test) negative. It can be expressed as the conditional probability of a test-negative outcome (T-) given that the animal is non-diseased (D-):

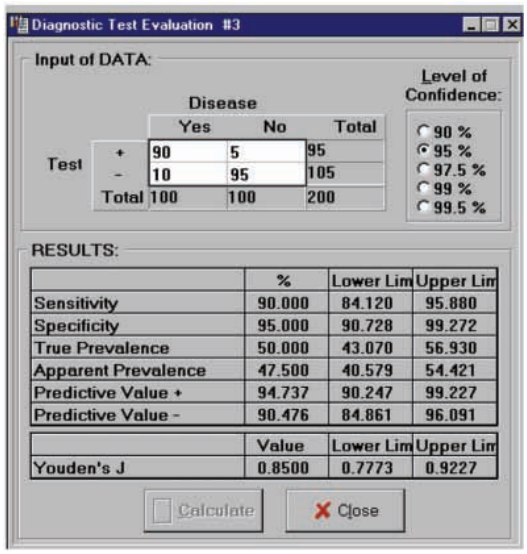
$$SP = P(T-|\ D-)$$

This information on the test results (pos/neg) in respective "gold standard" positive and negative groups of animals (samples) is very often presented in 2x2 tables. An example of such a 2x2 table from WinEpiscope v2 is presented in Figure 2. In this example, out of 100 truly diseased (gold standard positive) individuals, 90 were correctly classified as (test) positive, resulting in a sensitivity of 90% (95% confidence interval 84.1 – 95.9%). Of the 100 truly non-diseased (gold standard negative) individuals, 95 were correctly classified as (test) negative, resulting in a specificity of 95% (CI 90.7 – 99.3%).

A very important issue in diagnostic test evaluation is the definition of what consti-

FIGURE 2

Example of a 2x2 table (from WinEpiscope v2; see text for explanation)



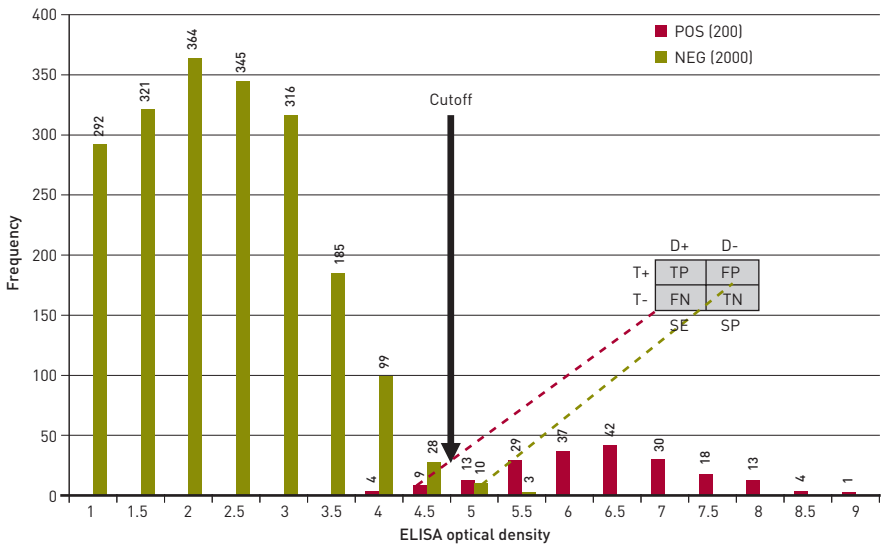
tutes the "gold standard (reference status)" classification for infected and non-infected individuals against which the new test is validated. The absolute (positive) gold standard is the demonstration of the infectious agent after (known) natural infection and clinical disease. This could be from clinically diseased animals in a natural disease outbreak from which the infectious agent was isolated by culture. Also possible as an absolute gold standard is the demonstration of clear and unique pathological lesions. Other indirect measures of disease (or exposure) such as the presence of antibodies in a different test system are defined as relative reference (gold standard) tests. Experimental infections and the use of animals from historically known negative populations are considered as alternative positive and negative gold standards, respectively.

One example is the validation of the first three rapid screening assays developed for BSE. The gold standard positive pool consisted of 300 brain samples of good quality from UK clinical BSE cases that were confirmed both by histology and immunohistochemistry (IHC). The gold standard negative pool consisted of 1000 good quality brain samples from a population assumed to be historically free of BSE that tested negative both in histology and IHC. These two groups clearly consist the extreme ends of the possible spectrum (with reference to levels of detectable "agent"), and were selected for good quality. It thus was of little surprise that the three tests correctly classified all samples within this trial. However, in the general population, agent levels and sample quality will show more variation, thereby reducing the overall test performance.

In the ongoing evaluation of new rapid tests for BSE, test developers have to document on a much larger number of field samples that the new tests are comparable (in performance) with the existing validated assays. For further details see the respective reports of the European Commission that are available through its Web site.

FIGURE 3

Frequency of ELISA optical density readings (see text for explanation)



2.2. Format of test results

In order to validate diagnostic tests using the traditional 2x2 table approach, test results need to be dichotomous (0/1, neg/pos, no/yes). For certain tests such as agglutination assays, immunohistochemistry, western blot, virus isolation and strip tests, the result is generated in such a way, and data can be used directly. For tests with a ordinal (dilution titer) or continuously measured outcome (temperature, optical density, chemino-luminescence) such as from an Indirect fluorescent antibody test (IFAT), ELISA, Red blood cell count (RBC) etc., a cutoff value is required to classify a test result as positive or negative. Only after classification is a transfer into a 2x2 table possible.

The selection of the cutoff value for ELISA, for example, will influence whether a non-perfect test will generate more false negative (FN) results (higher cutoff) or more false positive (FP) results (lower cutoff). This can be demonstrated by the histogram (Figure3) of 2000 negative (green bars) and 200 positive (red bars) samples.

Moving the cutoff value towards higher optical density (OD) values will reduce the number of false positive results and thus increase the test specificity. Reducing the cutoff value will result in fewer false negative test results and therefore higher test sensitivity. The Receiver-Operating-Characteristic (ROC) curve approach allows visual exploration of the possible sensitivity and specificity combinations over a range of selected cutoff (Greiner, 1996 a,b).

2.3. The diagnostic test users' view

Users of diagnostic tests have different questions that they should ask in relation to test performance.

True prevalence

If a diagnostic test was used to assess the proportion of test reactors within a sample, the result will be the apparent or test-positive prevalence (AP). The question is now what

the true prevalence (TP) of disease in the population is. If the test indeed is 100% sensitive and 100% specific, then AP and TP will be the same. If the test is not perfect, the Rogan-Gladen estimator using the AP, and knowledge on the test characteristics (SE, SP), can derive the TP, and the variance function can provide the necessary information to calculate 95% confidence intervals:

$$TP = \frac{AP + SP - 1}{SE + SP - 1} \qquad \sigma = \frac{AP * (1 - AP)}{n * (SE + SP - 1)^2}$$

Predictive value

If we see an individual test result (such as a positive pregnancy test strip), we will automatically try to assess how reliable that test result can be. This probability of a test to give the correct result is called the predictive value of an individual (positive or negative) test result. Predictive values are calculated separately for the test-positive and test-negative group.

The positive predictive value (PV+) is defined as the proportion of test-positive individuals that is truly diseased ("gold standard" positive). It can also be expressed as a conditional probability of having a truly diseased individual (D+) given that the individual is test positive (T+):

$$PV+ = P(D+ | T+)$$

The negative predictive value (PV-) is defined as the proportion of test-negative individuals that is truly non-diseased ("gold standard" negative). It can also be expressed as a conditional probability of having a truly non-diseased individual (D-) given that the individual is test negative (T-):

$$PV- = P(D- | T-)$$

Predictive values depend on the diagnostic test characteristics:

- high SE ➔ fewer FN test results ➔ higher PV-
- high SP ➔ fewer FP test results ➔ higher PV+

Predictive values, however, also depend on the true prevalence of the disease in the population where the (tested) individual came from:

- high prevalence ➔ higher overall probability that individual is diseased ➔ higher PV+
- low prevalence ➔ higher overall probability that individual is non-diseased ➔ higher PV-

Test characteristics SE and SP are assumed to be relatively stable across different populations and prevalence ranges, while predictive values vary with the population and their specific disease prevalence.

Serial and parallel testing

A combination of tests is often used in order to classify individual animals correctly. Tests can either be used in series (only test-positive reactors from the first test are examined in a second test), or in parallel (all samples are examined in two or more tests).

A simple example of a serial (sequential) combination of tests is the clinical suspect

reporting (screening test) and subsequent laboratory confirmation of those suspects. Another example is the use of a BSE rapid screening test on slaughtered cattle and the submission of test-positive samples to the reference laboratory for confirmation.

In order to maximize the overall performance of a serial test combination, the (first) screening test should have a very high sensitivity (>99%). This ensures that (almost) all positive individuals are captured in the screening, but it will also generate a certain proportion of false positive test results. The follow-up (confirmatory) test needs a high sensitivity but a very high (>99%) specificity to distinguish clearly between truly diseased and truly non-diseased (but screening test false positive) individuals.

If two or more tests are applied to the same sample in parallel, the decision rule will influence the overall test (combination) performance:

- positive if at least one of the tests is positive ➔ fewer false negatives ➔ higher sensitivity and lower specificity (more false positives)
- positive only if all tests are positive ➔ fewer false positives ➔ higher specificity but lower sensitivity (more false negatives)

Scenarios of expected results from combinations in series and in parallel can easily be explored using the WinEpiscope module "Tests/Multiple tests". This module, however, assumes that the test results of the two or more tests are independent from each other. If the (underlying biological) reason for two tests being wrong is the same, then the test outcomes are correlated, and using multiple tests loses its efficiency (higher costs without much diagnostic improvement).

The topic of herd-level testing (and diagnostic test characteristics) is not further explored in this brief introduction. For more information, other sources should be consulted.

2.4. Exercises

2A. Neospora study

A study of an ELISA to test for Neospora antibodies in cattle reported the following results:

		Neospora abortion	
		Yes	**No**
ELISA	Pos	41	2
	Neg	1	140

Use the WinEpiscope module Test/Evaluation to calculate the following:

1. Calculate the ELISA sensitivity and specificity with 95% confidence intervals.
2. Calculate the predictive value of a positive test result, as well as the predictive value of a negative test result.
3. Would you have the same predictive value of a positive and a negative test if you used the test in a population with only 5% of true prevalence (of Neospora-related abortions)?

Use the WinEpiscope module Test/Advanced Evaluation the calculate the following:

4. Calculate predictive values using a population of 500 cattle, the above calculated estimates of sensitivity/specificity and a true prevalence of 5% (Module Test/Advanced Evaluation).

2B. Testing for low prevalence diseases

You are working with a diagnostic test for disease X that has a SE of 99.5% and a SP of 98%. Apply that test in a population of 10 000 cattle with a prevalence of 0.1% (10 cases).

Use WinEpiscope Test/Advanced Evaluation to calculate the following:

1. How many true positive and how many false positive test results would you expect from this population? What is the positive predictive value of the test?
2. How many true negative and how many false negative test results would you expect from this population? What is the negative predictive value of the test?
3. If you apply the same test to a population with a disease prevalence of 0.01% (1 case), how many test positive samples would you expect?
4. Is the total number test positives from (3) very different from the test positive results in (1)?
5. Can this test be used to differentiate between the two prevalence levels?

2C. Combining tests

You use a screening test with a very high SE (99.8%) and a moderate specificity (95%) and follow up on all positive test results with a highly specific (99.9%) and rather sensitive (98%) confirmation test (serial testing). Test 100 000 samples from a population with a prevalence of disease of 0.1%.

Use the WinEpiscope module Test/Multiple Tests to calculate the following:

1. Calculate the expected frequencies of results for test 1 and test 2 when used independently, as well as when they are used in parallel and in series.
2. How many initial reactive samples do you expect from test 1 in the serial approach? How many positive samples do you get after application of the second (confirmation) test?
3. What are the main differences between the serial and the parallel approach?

3. SAMPLING ISSUES

The objective of animal health surveys (as part of a MOSS) is to assess, with an accepted level of certainty, whether the disease is present in a given animal population and, if yes, at which level. The main questions we can ask are:

- "Detection of disease" – is the disease present at a given level?
- "Maximum number positives" – what is the maximum number of positive animals in the population given that a random sample of size n was tested negative?
- "Prevalence estimation" - what is the likely prevalence of disease (with specified level of precision/error) in the target population?

In order to answer questions 1 and 3 one could simply examine all individuals in the target population. However, we are often limited by resources, and want to get the same answer from examining "just" a sample, i.e. a subset of the target population. In order to do that, the subset (sample) needs to be "representative" of the target population. In theory this can be achieved by drawing a simple random sample from that population. Two descriptions of the meaning of random sampling are:

- The best way to draw a truly representative sample from a population is to have the subjects included "by chance".
- A sampling procedure that has a truly random component provides each subject

within the target population with the same probability (likelihood, chance) to be included into the sample.

There are various sampling approaches that theoretically all fulfil the requirement of being representative – except for the first one:

- Convenience sample ➔ not random, not representative
- Simple random sampling ➔ truly random selection of subjects
- Systematic sampling ➔ random component (starting point)
- Stratified random sampling ➔ random selection within strata (breed, age, etc.)
- Cluster sampling ➔ random selection of clusters (herds, regions)
- Multistage sampling ➔ combination, often cluster and simple random sampling

For further details on the advantages, disadvantages and requirements of these sampling techniques, the respective textbooks and manuals should be consulted. The further discussion of this topic within this course manual is restricted to truly representative simple random sampling as presented in the field manual by Cannon and Roe (1982) and in the respective modules in WinEpiscope v2.

3.1. Detection of disease

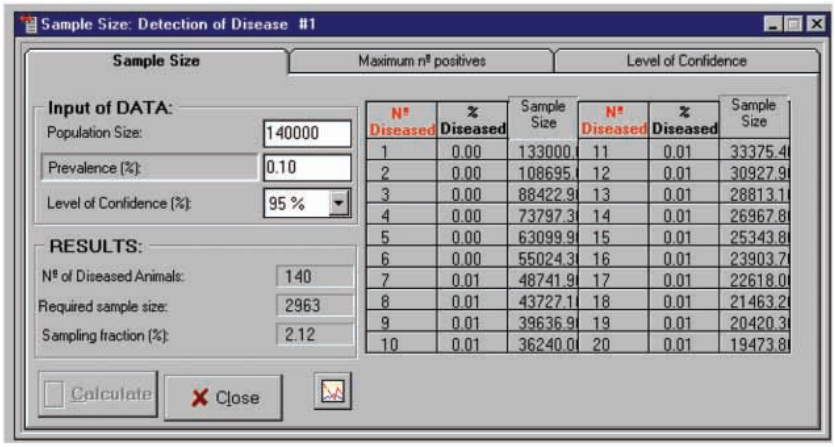
In order to assess whether a disease is present in a given target population (of 140 000 animals, for example) at a given (threshold) prevalence (0.1%) or not, a sample of a certain size needs to be randomly selected from the target population. The sample size calculation will ensure that – with a confidence of 95% – there is at least one infected animal in that sample IF the true population prevalence indeed is 0.1%. Figure 4 shows the calculation within WinEpiscope results in a required sample size of 2 963 animals.

3.2. Maximum number of positives

If, as an example, you have examined a sample of 14 000 randomly selected (representative) animals from a given target population of 14 0000 individuals, and all 14 000 were negative, the maximum number of diseased (positive) animals that theoretically could still be present in the target population can be calculated.

FIGURE 4

WinEpiscope results for sample size (see text for explanation)



Using the respective module in WinEpiscope, it can be determined that with 95% confidence the maximum number of positive animals in the population of 140 000 animals, given that the random sample of 14 000 animals was negative, is 29 (or 0.02% prevalence; Figure 5).

FIGURE 5

WinEpiscope results for maximum number of positives (see text for explanation)

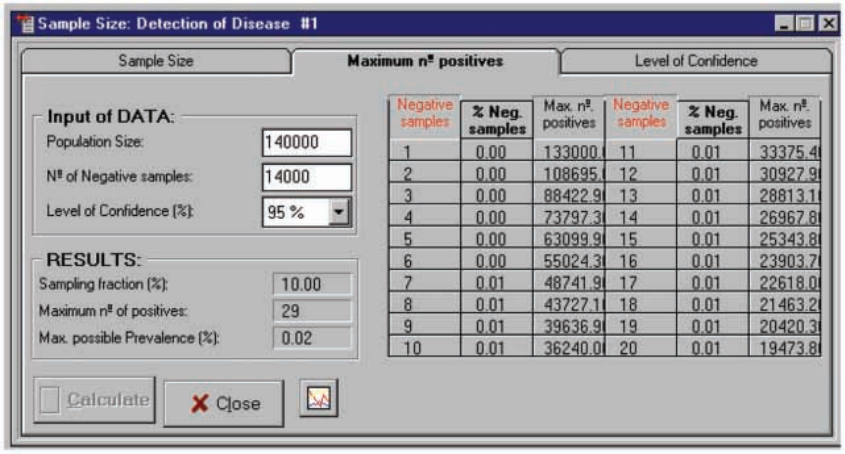
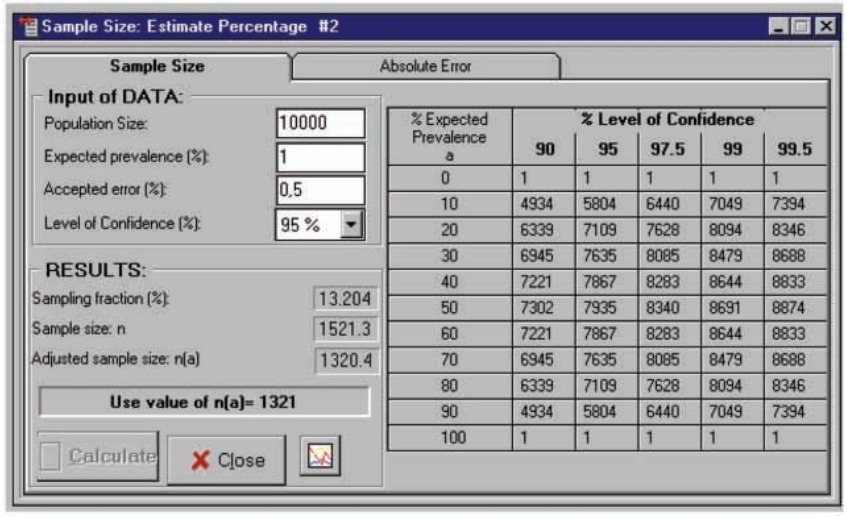


FIGURE 6

WinEpiscope results for prevalence (see text for explanation)



3.3. Prevalence estimation

The disease prevalence – with a sample – in a given target population can be estimated with a predetermined level of precision (accepted error). We need to provide our best guess of that prevalence in order to do the sample size calculation.

In this example with a population size of 10 000, an expected prevalence 1%, an accepted (absolute) error of +/- 0.5% and a level of confidence of 95%, the required sample size is 1 321 (adjusted sample size) (Figure 6).

In all these calculations an additional assumption besides the sampling population being representative (random), is that the diagnostic test system used to detect the cases within the sample is perfect. Violations of either of the assumptions result in the need for a larger sample size!

3.4. Exercises

3A. Detection of disease

You want to calculate the sample size required to detect at least one positive individual in your sample (with 95% confidence) when the prevalence is assumed to be x%.

Use the WinEpiscope module Samples/Detection of Disease to calculate the following:

1. Calculate the required sample sizes for a population of 10 000 individuals and prevalence values of 20, 10, 1 and 0.1% (for the lowest prevalence, enter 10 infected animals instead).
2. Calculate the sample sizes for the same prevalence values as in (1), but use a population size of 100 000 individuals (for the lowest prevalence, enter 100 infected animals instead).
3. Compare and discuss the results of (1) and (2) .

3B. Prevalence estimation

You are asked to specify the number of gold standard positive samples needed to estimate the diagnostic test sensitivity of a new test. The test developer assumes that the true test SE will be 99%.

Use the WinEpiscope module Samples/Estimate percentage to calculate the following:

1. How many gold standard positive samples have to be tested (out of an assumed large population of 10 000) in order to estimate that proportion (true SE) with an maximal accepted error of +/- 1% (and 95% confidence)?
2. Use the WinEpiscope Help Menu to look up the meaning of the adjusted (corrected) sample size.

4. MEASURES OF ASSOCIATION

Epidemiology is defined as the study of the distribution (occurrence) and determinants (risk factors) of health-related events (diseases) in populations. This section of the chapter addresses one of the core areas of epidemiology: the measurements used to describe the association between disease and potential risk factors.

One of the necessary steps in identifying potential causes (risk factors) for a disease is to show that this specific risk factor indeed has a statistically significant (numerical) association with the disease (outcome). Depending on the measurement scale, this

could be either a statistically significant difference in the mean values of a continuously measured variable (potential risk factor) between two or more distinct outcomes (often disease status), or a significantly higher frequency (proportion) of one level of a categorical variable (potential risk factor) in the outcome groups. The third alternative is the significant (linear) correlation between a continuously measured risk factor and a continuously measured outcome variable.

In the context of this course manual the discussion is limited to that of a categorical (binary) risk factor and a categorical (binary) outcome, i.e. disease status.

4.1. The basics of hypothesis testing

In statistical hypothesis testing, one assumes that in the overall population a null hypothesis (H_0) of no difference in the frequency of an event (risk factor) between two groups (diseased and healthy) exists. The alternative hypothesis H_A, or study hypothesis, that is tested against the null hypothesis – either one sided (a > b; a < b) or two sided (a<> b) – states that there is a significant difference between the two outcome categories. The test statistic calculates the expected range of outcome values assuming that H_0 is true, and compares these values and their related probabilities with the observed study values. If the study values are very extreme in comparison to the values expected under H_0, i.e. the probability of observing exactly the study values – given H_0 is true – is <5% or p<0.05, then one concludes that H_0 can be rejected in favour of H_A. This value of p<0.05 is the generally-accepted level of statistical significance.

4.2. Errors and p-value

The value of α defines the probability of deciding that there is a significant association between potential risk factor and disease while there truly is no association (Type I error). The p-value of 0.05 or 5% defines the proportion of times that making such an error is acceptable. Similarly, a Type II error is defined as the probability of missing a significant association in the study when one truly exists. The power of a study is the probability of a study (design) to find such an existing association (Figure 7).

FIGURE 7

Types of errors

	True Status	
Study decision	H_0	H_A
H_0	1 - α	β Type II error
H_A	α Type I error	1 - β Power

4.3. Example

The following discussion is restricted to the situation of a categorical (binary) risk factor and a categorical (binary) outcome variable – the easiest case. In Thrusfield (1995), Table 14.6 (page 211) displays the results of a study of urinary incontinence in a sample of spayed (castrated) and entire (non-spayed) canine females.

	Incont. (disease)	Normal	Total
Spayed	34 (a)	757 (b)	791 (a+b)
Entire	7 (c)	2427 (d)	2434 (c+d)
Total	41 (a+c)	3184 (b+d)	3225 (n)

The standard statistical test used to assess whether there is an association between the row (potential risk factor or exposure variable) and the column (disease) variable is the Chi-Square Test (χ^2 test). It compares the observed frequencies in a 2x2 table with the frequencies expected under the null hypothesis (that there is no association between the row and column variables). The respective formula is:

$$x^2 = \sum_{n_{ij}}^{n11} \frac{(O - E)^2}{E}$$

The degrees of freedom (df) for the test statistic are (rows – 1)*(columns – 1), i.e. in this example df = (2-1)*(2-1) = 1. The associated cutoff χ^2 value for statistical significance is 3.84, while the test statistic result for the urinary incontinence example is 76.01

Based on this value, it can be concluded that there is a significant association between spaying and urinary incontinence. However, this test statistic gives us neither a direction of the association (positive vs negative) nor a good estimation of its strength of it – it just says that there is one.

4.4. Relative risk and odds ratio

Two other epidemiological measures of association, the relative risk (RR) and the odds ratio (OR), provide more information both on the direction and strength of the association. Each measure has a possible range between zero and infinity. If both the (risk factor) exposed and non-exposed individuals have the same risk or odds of disease, then the ratio between the two risks or odds is 1, indicating no association (no influence of the risk factor on the disease).

The RR (depending on the situation also called prevalence ratio, rate ratio or risk ratio) is defined as the Risk(D|E)/Risk(D|NE) and can be calculated by the function:

$$RR = [a/(a+b)]/[c/(c+d)]$$

In the urinary incontinence example, the RR = 14.95 (95% CI 6.65 – 33.58) (calculated in WinEpiscope v2). In words, this would mean that the individual risk of urinary incontinence in this study sample was 14.95 times higher for spayed dogs when compared to non-spayed dogs. The 95% confidence interval (extrapolation to large population) is 6.65 – 33.58.

The OR (also called relative odds or cross-product ratio) is defined as the Odds(E|D)/Odds(E|ND) or the Odds(D|E)/Odds(D|NE). It can be calculated by the function:

$$OR = [a/c]/[b/d] = [a/b]/[c/d] = a*d/b*c$$

In the urinary incontinence example, the OR = 15.57 (95% CI 6.88 – 35.27) (calculated in WinEpiscope v2). In words this would mean that the individual odds (chance) of urinary incontinence in this study sample was 14.95 times higher for spayed dogs when compared to non-spayed dogs. The 95% confidence interval (after extrapolation to a large population) is 6.88 – 35.27.

The RR and OR become relatively similar in their absolute values when the prevalence of the outcome (disease) is <5%. They will always point in the same direction. Both measures are not statistically significant when the 95% confidence interval includes the value of 1, and the RR is not a valid measure for case-control studies. For further details on study design and the appropriate measures please consult the respective epidemiological textbooks should be consulted.

4.5. Exercises

4A. Association between neonatal deaths or culls in calves and the serum gamma globulin level

The gamma globulin level was measured in peripheral blood samples that were collected within the first 24 hours after birth. The outcome (whether they survived the first seven days post partum or not) was subsequently recorded.

The results are cross-classified in the following table:

	Died or culled	**Survived**	**Total**
gglob < 6.2%	12	61	73
gglob > 6.2%	6	214	220
Total	18	275	293

Use the WinEpiscope module Analysis/Cross-sectional to calculate the following:

1. Derive the appropriate measures of association (RR/OR) with 95% confidence intervals of neonatal death for the "exposed" group (gglob < 6.2%) when compared to the "non-exposed" group (gglob > 6.2%). ATTENTION: The table set-up in WinEpiscope is different from the way the data are presented above so the columns and rows need to be reversed.
   Interpret these results in words.

2. What is the value of "no association" for both the RR and the OR? Asked in another way: What would be the RR or OR that we expect if there is no difference in the risk (or odds) between the groups?
3. We do not get a p-value from WinEpiscope for the calculated RR and OR. Which other information provided by the package tells us whether the observed RR or OR are statistically significant?

5. REFERENCES

Cannon RM, Roe RT. 1982. *Livestock disease surveys: a field manual for veterinarians.* pp. 1-35. Department of Primary Industries & Bureau of Rural Science, Australian Government Publishing Service, Canberra

Doherr MG, Audigé L. 2001. Monitoring and surveillance for rare health-related events - a review from the veterinary perspective . *Phil Trans R Soc Lond B* 356, 1097-1106

EpiVetNet. 2006. Epidemiological analysis. http://www.vetschools.co.uk/EpiVetNet/Epidemiological_analysis_software.htm

FAO. 2007. *Epidemiology, surveillance and risk assessment for transmissible spongiform encephalophathies.* Course manual, Project. *Capacity Building for Surveillance and Prevention of BSE and Other Zoonotic Diseases.* Rome

Greiner M. 1996a. Two-graph receiver operating characteristic (TG-ROC): a Microsoft-EXCEL template for the selection of cut-off values in diagnostic tests. *J Immunol Methods* 185, 145-146

Greiner M. 1996b. Two-graph receiver operating characteristic (TG-ROC): update version supports optimisation of cut-off values that minimise overall misclassification costs. *J Immunol Methods* 191, 93-94

Greiner M, Gardner IA. 2000. Application of diagnostic tests in veterinary epidemiologic studies. *Prev Vet Med* 45[1-2], 43-59

OIE (World Organisation for Animal Health). 2005. Diseases notifiable to the OIE. http://www.oie.int/eng/maladies/en_classification.htm

Thrusfield M. 1995. *Veterinary epidemiology.* Second Edition. Blackwell Publishing, Oxford, UK

6. SUGGESTED SOFTWARE (CAN BE DOWNLOADED FROM WWW.VETSCHOOLS.CO.UK/EPIVETNET/)

WinEpiscope v.2 http://www.vetschools.co.uk/EpiVetNet/Sampling_software.htm

Survey Toolbox (with manual) http://www.vetschools.co.uk/EpiVetNet/Sampling_software.htm

7. SOLUTIONS FOR EXERCISES

2A.1: SE = 97.6% (93 – 100%); SP = 98.6% (96.7 – 100%)

2A.2: PV+ = 95.3% (89.1 – 100%); PV- = 99.3% (97.9 – 100%)

2A.3: No, PV+ and PV- depend on test characteristics and on prevalence. In a population with a lower prevalence one would have a lower PV+ and a higher PV-.

2A.4: PV+ = 78.6% ; PV- = 99.9%

2B.1: TP = 10; FP = 200; PV+ = 2.1%

2B.2: TN = 9,790, FN = 0; PV1 = 100%

2B.3: Test positives = 201 - compared to 210 in B.1.

2B.4: Difference is 9 test-positive animals, rather small.
2B.5: No, the test SE and SP are still not high enough to differentiate the prevalences.
2C.1: Look at the outcome of the different calculations, compare SE's, SP's and PV's!
2C.2: 5 095 initial positives (test 1), 200 confirmed positives (combined tests)
2C.3: Serial approach: only test 1 positives are tested in test 2, while in the parallel approach all samples are tested in both tests. Overall SE is higher in the parallel approach while overall SP is higher in the serial approach. This, however, will always depend on the test characteristics of the selected tests.

3A.1: N = 10 000 prevalence = 20%/n = 14, 10%/29, 1%/294, 0.1%/2 588
3A.2: N = 100 000 prevalence = 20%/n = 14, 10%/29, 1%/298, 0.1%/2 950
3A.3: Sample sizes here are almost independent of population size, but increase with decreasing prevalence values.
3B.1: If the true SE is 99% then a (adjusted) sample size = 381 gold standard positive animals will be needed to estimate that SE with an error of +/- 1%.
3B.2: WinEpiscope HELP/INDEX/2.3 -> look for sampling fraction/corrected sample size.

4A.1: OR = 7.02 (2.5 – 19.5); RR (Prevalence Ratio) = 6.03 (2.4 – 15.5)
In words, this would mean that the individual odds (chance) of neonatal death in this study sample was 7.02 times higher for calves with low gglob levels when compared to calves with high levels. The 95% confidence interval (extrapolation to large population) is 2.5 – 19.5. Similarly, the individual risk (probability) of neonatal death in this study sample was 6.03 times higher for calves with low gglob levels when compared with calves with high levels. The 95% confidence interval (extrapolation to large population) is 2.4 – 15.5.
4A.2: The value of 1 (unity).
4A.3: If the calculated 95% confidence interval does NOT include the value of no association (i.e. 1) then we consider the measure of association as statistically significant. This does NOT automatically mean that this association is also biologically important – that needs to be assessed through biological thinking and other indications of true (disease) causality!