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

course manual

EPIDEMIOLOGY, SURVEILLANCE AND RISK ASSESSMENT FOR TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

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EPIDEMIOLOGY, SURVEILLANCE AND RISK ASSESSMENT 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 *Epidemiology, surveillance and risk assessment for transmissible spongiform encephalopathies*, which is given within the framework of the project. This practical course is targeted at governmental epidemiologists 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 *Epidemiology, surveillance and risk assessment 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 *Epidemiology, surveillance and risk assessment for transmissible spongiform encephalopathies*, of the project *Capacity Building for Surveillance and Prevention of BSE and Other Zoonotic Diseases,* the participants should:

- understand basic principles of epidemiology, surveillance and risk assessment for animal diseases in general and BSE and TSEs in particular;
- be able to apply the acquired knowledge practically in their daily job activities.

Specifically, these principles include:

- basics of BSE and TSEs, including transmission, pathogenesis and risk factors;
- measurements of disease occurrence in animal populations (incidence, prevalence, ratio, proportion and rate) and summary statistics;
- design and implementation of appropriate national surveys and surveillance systems generally, and specifically those satisfying national/international requirements for TSEs/BSE;
- basic principles of risk analysis, measures of disease risk and generation of inferences regarding risk;
- national and import risk analysis for BSE based on the recommendations of the World Organisation for Animal Health (OIE), including data required and application.

INTRODUCTION TO TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

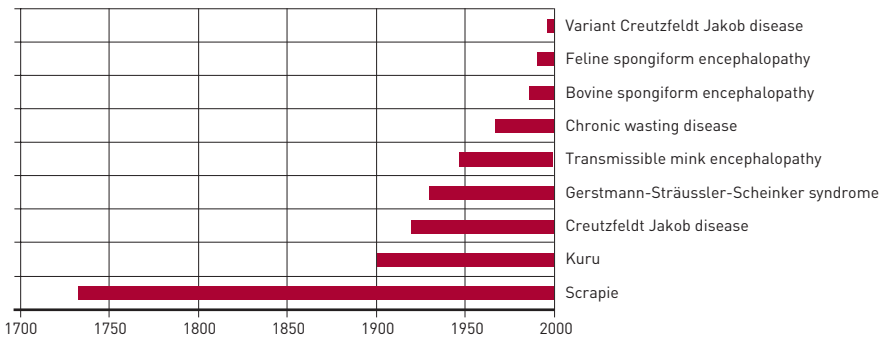
1. TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

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.

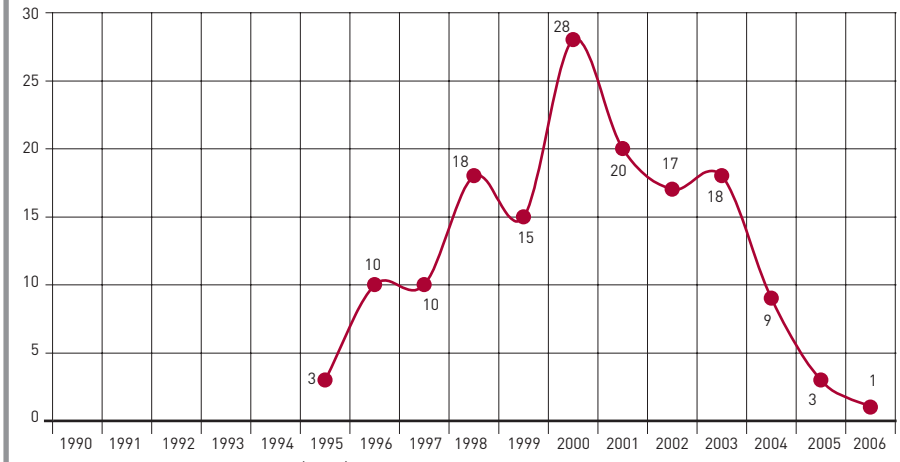
Several TSEs have been reported to occur in humans, including two forms of Creutzfeldt-Jakob disease (sporadic CJD and variant CJD /[vCJD]), Kuru, Gerstmann-Sträussler-Scheinker syndrome, as well as fatal familial insomnia. Of these, only vCJD has been associated with BSE. Sporadic CJD was first identified 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: UK Department of Health (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

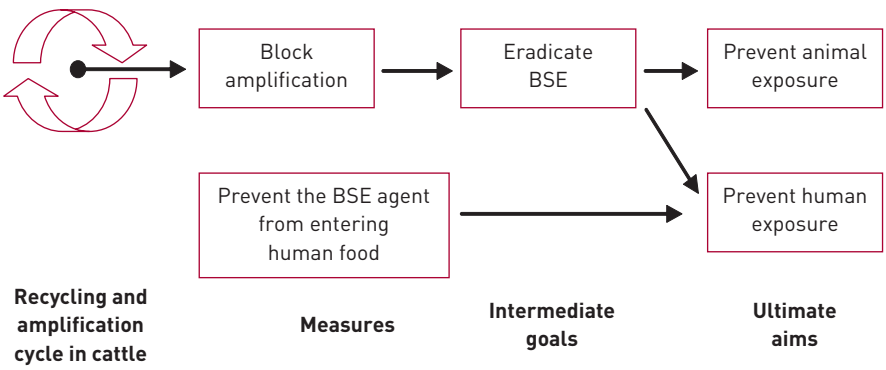
The ultimate aims of BSE control and prevention programmes are to reduce exposure risk both to cattle and to humans (Figure 3). Two levels of measures must therefore be considered:

- 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.

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, 2007).

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. DIAGNOSIS OF BSE

5.1. Biosafety

Microorganisms are classified by the World Health Organization (WHO) according to their pathogenicity for humans and animals. According to this classification, precautions must be taken when handling these agents primarily to protect the people handling them, and also to protect the general human population and livestock from accidental exposure. Depending on the classification of the microorganism, precautions must also be taken to protect laboratory workers and the community from possible exposure and infection. Thus, WHO has defined four biosafety level (BL) categories for laboratories. These categories correlate somewhat with the WHO risk group categories, but also reflect what is being done with the microorganism in the laboratory.

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

In 2000, the EU published a directive based on the WHO guidelines, which defines a new risk group for BSE and related animal TSEs based on BSE agent characteristics (e.g. limited risk for laboratory personnel and the community, inability to exclude aerosol transmission). This new risk group is called 3**, which means risk group 3 with some alleviations. Scrapie, on the other hand, is still classified as risk group 2.

According to the Swiss Expert Committee for Biosafety, different biosafety levels are required when handling BSE materials, depending on the type of material (Swiss Expert Committee for Biosafety, 2006). For example, histology and Immunohistochemistry (IHC) on formic acid-inactivated BSE material can be performed in a BL 1 laboratory, and routine BSE diagnostics can be performed in a BL 2 laboratory with some additional measures. A reference laboratory for TSE must be BL 3, but some modifications are allowed. Attention should be paid to the fact that BSE laboratory requirements often differ among countries.

5.2. Sample collection

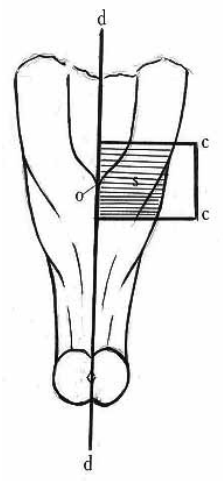
Because both the highest concentration of PrP^{Sc} and the most prominent related lesions tend to be located in the area of the obex region of the brainstem (Figure 4), sampling this region optimizes sensitivity, regardless of the diagnostic test method used. If this region is not sampled correctly, false negative results may be obtained. This requires that individuals collecting samples are familiar with the anatomy of this region.

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

In cases of emergency slaughter, fallen stock or routine screening, only the caudal brainstem (medulla oblongata) is generally removed for testing, without opening the skull. The caudal end of the brainstem should be visible through the foramen magnum after separation of the head, and a specially designed spoon can be used to remove the brainstem (including the obex region) through the foramen. The brainstem is then split

FIGURE 4

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



longitudinally, and one half fixed in formalin for histopathology and IHC while the other half is reserved and sampled for rapid tests. The fresh tissue remaining after sampling for rapid tests is then frozen at -20 °C or -80 °C.

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

5.3. Neuropathology and immunohistochemistry

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

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

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

However, because the normal PrP protein (PrP^{C}) present in the brain cells has the same amino acid sequence as PrP^{Sc}, antibodies normally used in IHC detect both PrP^{Sc} and PrP^{C}. Therefore, in order to be able to determine if there is any PrP^{Sc} present, the

two proteins must first be differentiated. Proteinase K is an enzyme that causes total proteolysis of normal PrP^{C}, although PrP^{Sc} is resistant to proteolysis by proteinase K to a large extent. Only small parts at the beginning and at the end of PrP^{Sc} are digested and the remaining part, generally referred to as the core fragment or PrP^{Res}, is still detected by the antibodies. Therefore, proteinase K is used in IHC to digest totally the PrP^{C} present in the sample, ensuring that any PrP detected will be PrP^{Sc}. Without this step, samples could yield a false positive result owing to the detection of normal PrP^{C}. Similarly, incomplete digestion could lead to false positive results.

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

5.4. Rapid BSE tests

Tests are available to analyse BSE suspect materials rapidly (OIE, 2005b). Which rapid tests are licensed and approved in various countries throughout the world is variable and lists are constantly being updated (EFSA, 2006).

All currently licensed BSE rapid tests have several things in common. First, they use material from the brainstem, i.e. they are post mortem tests. Second, current rapid tests are based on the same principles of homogenization, proteinase K digestion (with the exception of the IDEXX HerdChek BSE Antigen EIA) and detection. Although the principles of these steps are similar among tests, there are significant differences in the execution. The materials and procedures are specific to each test system and test performance is validated under these specific conditions, thus protocols cannot be modified or interchanged among tests.

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

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

5.5. New developments

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

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

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

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VETERINARY EPIDEMIOLOGY - PRINCIPLES AND CONCEPTS*

1. BACKGROUND - WHAT IS EPIDEMIOLOGY AND HOW IS IT USED?

Training in epidemiology is needed to provide the requisite knowledge, skills, and abilities for an animal health authority or a practicing professional veterinarian to complete the job requirements that involve planning, coordinating, adapting, and modifying control strategies including detection of cases for BSE and other TSEs in animal populations.

Definition

Epidemiology is the study of a disease pattern in a population in order to determine prevention and control strategies.

Veterinary epidemiology is concerned with studying disease patterns in animal populations.

Classifications of Epidemiology in Veterinary Medicine

- Descriptive Epidemiology - Clinical Epidemiology
- Analytical Epidemiology - Quantitative Epidemiology
- Experimental Epidemiology - Clinical Trials and Modelling
- Micro vs. Macro Epidemiology:
  - Micro-epidemiology is the study or investigation of disease patterns on farm/herd level. This approach is the traditional way of investigating a disease.
  - Macro-epidemiology is the study/investigation of disease patterns on the state/national level. Usually, governmental agencies are engaged in disease investigations on this level.

Who are the Veterinary Epidemiologists?

- In general, Veterinary Epidemiologists work within the branch of veterinary science that deals with the *incidence, distribution, and control of disease* in an animal population
- Specifically, Field Epidemiologists are engaged in activities to obtain *accurate and reliable field observations* and to collect the information needed for the decision making process
- Government epidemiologists usually apply epidemiological methods on a national or international scale (staff support activities)
- University Epidemiologists are engaged in theory and methods research, the education of future professionals, and the application of methods.

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Benefits of a field epidemiologic approach:

- Direct effects on the quality and success of disease eradication efforts
- Support of the decision making process
- Assessment of the efficiency and reliability of animal health programs

What are the roles of a successful field epidemiological operation?

- Support the implementation of animal health plans.
- Discuss animal health problems with the epidemiologists.
- Participate in disease investigations by:
  - searching files, records, documents,
  - collecting specimens,
  - visiting farms.

What are the requirements for a successful epidemiology in animal health arena?

- Should be patient and open-minded in his/her approach
- Should be willing to listen
- Should be a creative thinker
- Should have an inquisitive mind with analytical ability
- Should have the ability to use the quantitative and scientific approach to solve a disease problem
- Should have clinical experience in the field
- Should have the ability to seek and accept new knowledge
- Should enjoy working with the public

2. BASIC EPIDEMIOLOGICAL CONCEPTS AND MEDICAL ECOLOGY

The Disease Process in Populations

Factors important in the establishment and transmission of disease can be classified as agent, host and environmental factors. We sometimes look at these factors as discrete and independent entities but usually several factors will contribute to the occurrence of disease. In other words, most diseases are multifactorial. Figure 1 is one way to conceptualize the interaction of these factors.

Agents of disease

In veterinary medicine we are accustomed to thinking mainly of agents of infectious disease, but there are many other disease agents. Many epidemiological techniques were originally developed for the study of infectious diseases, but are also suited for noninfectious disease.

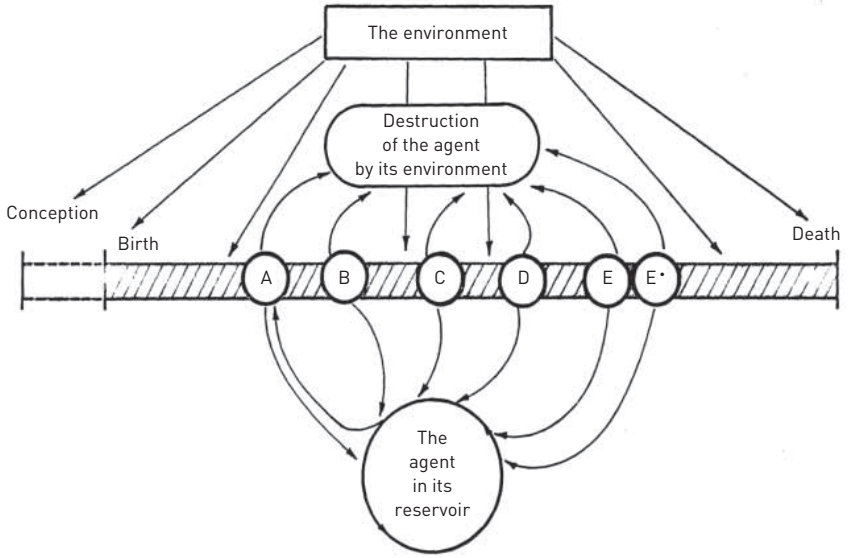
Nutritive elements (excesses and deficiencies): cholesterol, selenium, vitamins
Chemical agents: Poisons – toxic plants, Allergens – farmer's lung
Physical agents: sunlight, mechanical injuries
Infectious agents: parasites, bacteria, fungi, rickettsia, viruses

Disease Determinants

Disease determinants are divided into agent, host and environmental factors. The following is a partial list of factors.

FIGURE 1

The Natural History of an Infectious Disease



Legend:
A. Agent contacts host
B. Agent infects host
C. Point of detectable abnormality
D. Point of clinical disease
E. Recovery or death
E•. Carrier state

Agent Factors

Host range	The broader the range of hosts in which the agent can survive the better chance of survival.
Infectivity	The ability to enter, multiply and produce a change in the host (exception-helminthes may develop instead of multiply).
Infectious dose	The quantity of an agent necessary for transmission and infection.
Contamination	The presence of infectious agents on the exterior surface of the body (or on bandages, water, milk, food, etc.). In some circumstances, contamination may be internal.
Pollution	The presence of offensive, but not necessarily infectious, matter in the environment.
Pathogenicity	The ability to produce clinical disease.
Virulence	The measure of severity of disease.
Immunogenicity	The ability of an agent to stimulate an immune response. The likelihood of repeated infections is reduced if the agent is highly immunogenic.
Antigenic stability	The probability that the genome governing antigenic structure of an agent will undergo antigenic change.
Viability	The ability of an agent to withstand environmental stress.

Host Factors (Intrinsic Factors)

Host factors influence exposure, susceptibility and/or response to agents. These factors may enhance or limit disease. The following are common host factors.

- Age
- Sex
- Immune Status
- Breed and Genetic Make Up
- "Occupation"

Environmental Factors (Extrinsic Factors)

These factors include the physical environment (i.e. geography and weather) and the biological climate (i.e. management, nutrition, housing, etc.). These factors can also be categorized by the terms micro- and microenvironment. The macroenvironment is the physical environment in general. The microenvironment can be considered the immediate surroundings such as barns, pastures, kennels, etc. The micro- and macroenvironment of a host can effect the pattern of disease.

- Macroenvironmental factors
  - Geography
  - Air quality
- Microenvironmental factors
  - Nutrition
  - Housing
  - Management

Comparison of a natural and a man-made ecosystem

Natural ecosystem	Man-made ecosystem
wandering herds grazing extensive areas	herds are permanently housed (zero grazing)
intermingled species so that mixed	mixed herds have become
grazing occurs	monocultures
different species destroy the parasites of others	excreted pathogens are available to others of the same species
in the open air, expiratory droplet infections are of little importance	animals are crowded on limited land
natural avoidance distances minimize direct contact	crowding allows closer contact
predators remove diseases animals early in the course of the disease	predators are eliminated; sick are helped to survive while excreting pathogens
hosts and parasites reach a balance so that both live with little harm	balance is upset as new niches are created
epidemics occur only when populations increase past a certain point.	increased risk of disease

Natural herds often have a low rate of reproduction and production. Humans have domesticated animals so as to assure a more regular, safe and convenient food supply. The object of husbandry is to reach a natural balance between the host and its parasites while still promoting efficient and economical production. Any increase in production must be matched with a refining of management and disease control strategies.

Association of Factors

The presence of a factor in conjunction with a given situation does not necessarily mean a cause-effect relationship. Factors may be causative or associated (but not causative).

Summary

Agent factors and host factors are influenced by environmental factors. These interact to determine whether an individual animal or population experiences health or disease.

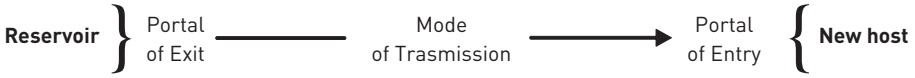
The objectives of epidemiology are to:

- identify major factors that contribute to disease and health.
- develop control measures against those factors that contribute to diseases and promote those factors contributing to health.
- prevent disease and promote health.

Disease Transmission

Disease transmission can be studied at various points. The factors below represent the major points along a dynamic continuum of transmission.

A. Reservoir
B. Portal of exit
C. Mode of transfer
D. Portal of entry
E. Susceptible host



A. Reservoir

Reservoir – the living organisms or inanimate matter (e.g. soil) in which an infectious agent normally lives and multiplies (where it maintains and perpetuates itself) and from which it can be transmitted.

If the reservoir is an animal, it is called a maintenance host. How widespread the agent is in a reservoir determines to a large extent the probability of exposure.

The reservoir may not always be obvious. Isolation of an agent from a host does not mean it is the reservoir. It could be an incidental host where infection is infrequent or where it is difficult to escape from the host.

Nidus – a localized reservoir that persists over a long period of time.

Source – the place from which the etiological agent passes directly to a susceptible host. The source may be the same as reservoir or it may be different.

Carrier – an infected animal that harbors a specific infectious agent in the absence of discernible clinical disease and serves as a potential source of infection for other animals. The carrier state may be in apparent throughout the infection (healthy or asymptomatic carrier) or may occur during the incubation period or convalescence of an animal with clinically recognizable disease (incubationary or convalescent carrier).

Incubationary carriers – shedding the agent prior to the appearance of clinical signs.

Convalescent carriers – shedding the agent for short periods after clinical signs have abated.

Intermittent shedders – intermittent shedding of the agent for moderate periods of time after recovery from disease.

Chronic carriers – shedding of the agent for extended periods of time after recovery from disease.

Healthy (asymptomatic) carriers – shedding of the agent by individuals who have never had clinical signs or symptoms.

B. Portal of Exit – Escape from reservoir

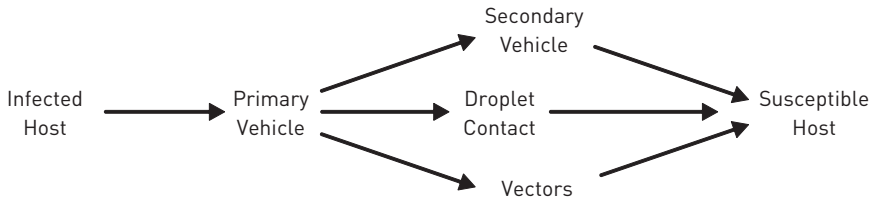
Ease of escape determines the importance of a reservoir.

Portals – usually one/agent and suggested by clinical signs

a. Respiratory – most common
b. Alimentary – high dosage of agents
c. Urogenital – important in animals
d. Percutaneous – depends on vector abundance
e. Multiple exits – diseases with more than one portal of exit complicate control procedures (Q-fever and streptococci)

C. Mode of Transmission

Diagrammatic Summary

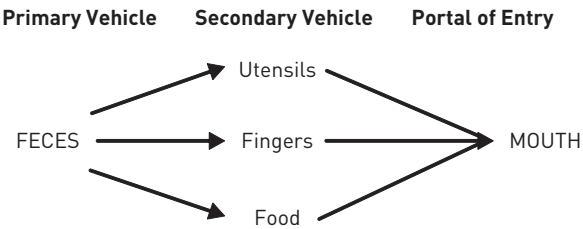


Primary vehicle – secretions, excretions or other body fluids or tissues of an infected host (saliva, urine, feces, blood, etc.)

Secondary vehicle – an inanimate object that might become contaminated with the primary vehicle (water, food, grass, etc.)

Vectors – invertebrate animals responsible for the transmission of an infectious agent (fleas, ticks, flies, mosquitoes, snails)

Example: Salmonellosis



Types of Transmission

Direct Transmission	**Indirect Transmission**
Contact	Vehicles and fomites
Venereal	Airborne
In utero	Ingestion
	Vectors

Direct Transmission (Contagious diseases, contagion = to touch)

- Physical contact
  - Fragile agents generally have to be transmitted directly.
  - Usually diseases of sporadic occurrence and dissemination
- *In utero* infections - Can result in congenital disease.
- Skin-Air (body environment)

Indirect Transmission

Vehicles and Fomites – inanimate objects that are contaminated with agents and consequently transfer an agent to a new host. Generally not very important, except in such diseases as *Salmonella* spp., equine infectious anemia (needles), ringworm (combs), foot and mouth disease (farm equipment). Historically, fomites were considered very important, but the discovery of carriers and subclinical infections has decreased their actual importance.

Airborne Transmission (a type of indirect)

Dust – caused by the grinding up of particles as in milling of food, animals scrabbling around in litter or bedding, drying up of fluids from a discharge. Dust always contains bacteria and fungi (i.e., spoilage organisms). Particles vary in size from 10 to several 100 µm. Dust is generally trapped in mucus of upper respiratory tract and can cause localized infections. Dust-borne transmission requires highly viability microbes.

Expiratory droplets – respiratory droplets that result from deep, energetic breathing, coughing and sneezing. Droplets are formed by the atomization of respiratory fluids that are projected violently from the nose and mouth. They are about 100 µm and usually travel no more than 3-4 feet (in humans). They may grow larger due to water condensation and are the most proficient disseminators of upper respiratory infections. Expiratory droplets require close proximity of individuals and, consequently, exhibit some of the same patterns as diseases transmitted by direct contact (e.g. propogative epidemics). They may become dust-borne agents if the agent is viable and falls to the floor.

Droplet nuclei – small particles (2-10 µm) that result from the rapid evaporation of small expiratory droplets into a dry environment. Upon inhalation into the respiratory tract, the nuclei encounter a saturated atmosphere. In addition, the cross sectional area decreases from the trachea to lung alveoli, which results in a decrease in air velocity. The droplet nuclei rehydrate, settle out and "stick" to the surface of alveoli. These nuclei can be spread over long distances. Droplet nuclei infections are best controlled by good ventilation, ultra-violet light sources and increasing resistance of susceptible hosts (vaccination).

Vapors and gases – size is irrelevant but proximity is important. Consequently, vapors and gases cause disease outbreaks with patterns similar to direct transmission.

Ingestion – a form of indirect contact that can be affected by airborne particles. Many diseases are transmitted this way and it often involves one of the resistant or adaptive organisms.

Fecal-Oral – usually a closed-type transfer cycle (e.g., from ingestion of fresh feces through fecal splash-droplets or coprophagous activity).

- Food – meat from sick animals (e.g., tuberculosis, brucellosis) or predator chain transmission of leptospirosis (rodent ➔ skunks) and rabies (bat ➔ foxes). Food items can produce point source, common vehicle outbreaks that can be very

explosive. These require either a highly viable agent or food that is a good growth media. In human food-borne disease outbreaks, most pathogens are introduced during processing and followed by temperature abuse of food. Animal feed-borne disease outbreaks occur from the use of unhealthy tissue (e.g., anthrax carcasses) or contamination during processing (e.g., *Salmonella* spp.).

- Milk – good bacterial growth media, but dilution of milk affects attack rate in humans.
- Water – can result in common source vehicle outbreaks which are widespread and have varying disease frequency. Municipal water distribution systems expose more people than any other water source. Animals are most often exposed through surface water.

Vectors (a type of indirect)

A vector is a living invertebrate carrier of a disease causing agent. Vectors may be mechanical or biological.

Mechanical Vector – the agent does not undergo any change while associated with the vector. The transmission interval is usually short and depends on the survival time of the agent on the body or mouthparts of the vector. Mechanical vectors may be external or internal carriers of the agent.

Biological Vector – the agent undergoes some change in the vector such a multiplication, maturation, sexual reproduction, or some combination such as maturation and multiplication. Biological vectors cannot transmit the agent immediately after becoming infected. A prepatent or extrinsic incubation period is required.

Some agents are very well adapted to their vectors. Examples of these types of relationships occur when transovarian and transstadial transmission within the vectors occur.

Transovarian Transmission – the agent is transmitted from the female vector to the eggs.

Transstadial Transmission – the agent survives through various stages of larva, nymph, and adult development.

Epidemiologically a distinction is made between flying and non-flying vectors.

Flying vectors can actively seek out their vertebrate hosts. The flight range of the vector and its biting patterns may determine the extent and rapidity of spread of an infection.

Non-flying vectors are dependent on passive contact with the host. To overcome this disadvantage many have developed transovarial and transstadial transmission abilities.

Water-inhabiting vectors may release infectious organisms into the fluid medium enabling them to b passively disseminated. In such cases infection of the vertebrate can occur remotely from the vector.

Communicability – A measure of dissemination; the ease and speed with which an infectious agent is transmitted in a population of susceptibles (also called transmissibility, infectiousness).

D. Portal of Entry

The portal of entry generally uses the same "system" and corresponds to clinical signs.

i. Respiratory – viral pneumonias
ii. Gastro-intestinal – enteroviruses

iii. Conjunctiva – leptospires
iv. Percutaneous – arboviruses
v. Reproductive tract – venereal diseases

The portal of entry is associated with the incubation period. If the portal of exit and entry are the same, there is usually a short incubation period. If the portal of entry and exit are far apart, the incubation period can be long. If the portal of entry is close to target cells, the incubation period is usually short.

Multiple portals of entry can affect pathogenesis and clinical signs.

E. Susceptible Host

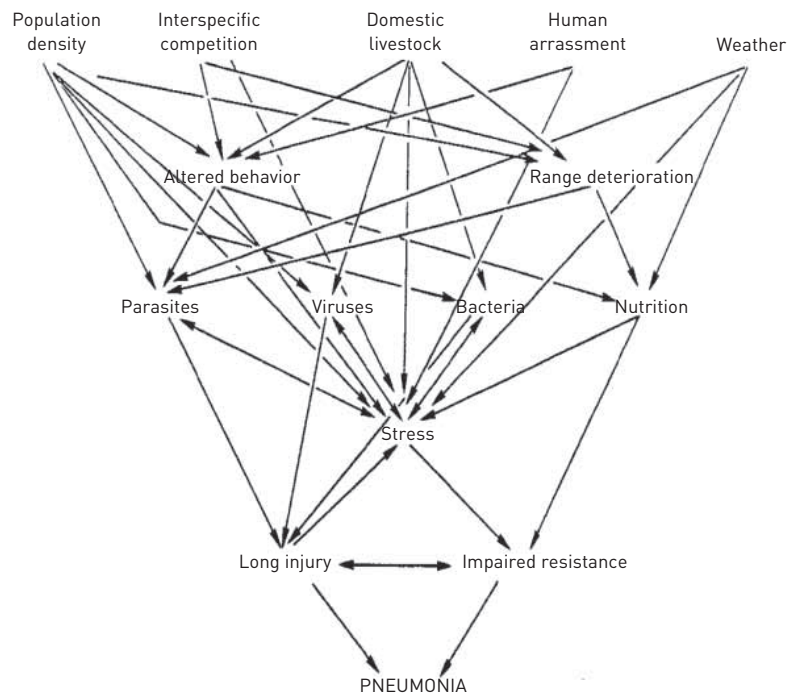
Individual susceptibility – all of the previous transmission processes generally influence the host's exposure to an agent. An epidemic generally results from a significant increase in either the exposure to a new agent or the increase in susceptibility of a population to an endemic agent.

Web of Causation

The essence of this concept is that effects never depend on single isolated causes, but rather develop as the result of chains of causation in which each link itself is the result of "a complex genealogy of antecedents". The large number of antecedents creates a condition which may appropriately be conceptualized as a "web".

FIGURE 2

Web of Causation



Source: Wobeser G. *Investigation and Management of Disease in Wild Animals*

3. MEASURING AND QUANTIFYING DISEASES IN AN ANIMAL POPULATION

A fundamental aspect of epidemiology is to quantify or measure the **occurrence of illness** in a population. Obtaining a measure of the disease occurrence or impact is one of the first steps in understanding the disease being studied. Measurement of disease occurrence is an integral part of determining the impact of the disease in the population, the possible mechanisms of the spread of the disease in the population, and the possible implementation and effectiveness of any control programs.

Ratios, Proportions and Rates

There are three types of descriptive mathematical statistics or calculations that are used to describe or quantify disease occurrence: ratios, proportions and rates.

A. Ratios

A ratio is expressed as $\frac{a}{b}$, ("a" is not part of "b")

where a and b are two mutually exclusive frequencies, that is to say the numerator (= a, the number on top of the expression) is not included in the denominator (= b, the number on the bottom of the expression).

Examples:

i) The ratio of rams to ewes in a sheep herd was 15/300 or 1:20. Note that the two quantities are mutually exclusive - rams cannot be included as ewes. The observed frequencies in a ratio are often re-expressed by dividing the smaller quantity into the larger one. Thus dividing 15 into 300 re-expresses the ratio in terms of 1 ram for every 20 ewes (i.e., 1:20).

ii) The feed conversion ratio for a particular hybrid of broiler chickens is 2.8:1. For every 2.8 units of feed consumed the chickens grow 1 unit.

iii) The ratio of abortions to live births in a sheep flock was 12/156 or 1:13. Again note the exclusiveness of the two frequencies - abortions cannot be included as live births.

B. Proportion

A proportion expresses a fraction in which the numerator (the frequency of a disease or condition) is included in the denominator (population). A proportion is dimensionless and can never take on a value less than 0 or greater than 1. Fractions may be multiplied by 100 to give a percentage.

Proportion = $\frac{a}{b}$ ("a" is included in "b")

Percentage = $\frac{a}{b} \times 100 = \%$

Examples:

i) The proportion of rams in a flock of sheep was 15/315 or 0.048.

ii) Of 116 confirmed pregnancies on a dairy farm, 98 resulted in a live calf. The percentage of confirmed pregnancies which resulted in a live birth was 84.5% (98/116 x 100).

iii) Of 168 ewes that were confirmed pregnant by ultrasound examination, 12 ewes did not produce live lambs, thus the proportion of abortions among these ewes was 12/168 or 0.071.

Note that some ratio measures can be converted to proportions (e.g., the ram to ewe ratio) whereas others cannot (e.g., feed conversion ratio).

C. Rates

Rates are special types of proportions that express the relationship between an event (e.g., disease) and a defined population-at-risk evaluated over a specified time period. The numerator is the number of affected individuals in a given time period, while the denominator is the population-at-risk over the same time period.

Rate = $\frac{a}{b}$ ("a" is included in "b")
("b" represents population time)

The essential elements of any rate are two fold: the definition of both a population-at-risk and a specific time period. As discussed below there are two types of rates commonly used as epidemiologic measures: the cumulative incidence rate and the incidence density rate.

Prevalence and Incidence

Measures of disease occurrence either describe the situation at one point in time (prevalence) or describe what is happening during a period of time (incidence). Prevalence measures describe what proportion of the population has the disease at one specific point in time. Incidence measures, on the other hand, describe the frequency of new cases that occur during a specific point in time. Incidence measures describe the flow of individuals from the disease-free state to the disease state.

Prevalence depends on both the disease incidence (the "flow") and the average duration of the disease state. A change in prevalence may be a result of a change in either factor or a change in the mortality associated with the disease.

In epidemiology, we are really interested in studying the flow of cases from the disease-free state to the disease state. The relevant measure of disease occurrence is therefore incidence. However, the choice between prevalence or incidence is sometimes made from a more practical point of view. In chronic diseases, where the "flow" between the disease-free and the disease state is very slow, prevalence measures are often employed. For acute diseases which change more rapidly, incidence measures are preferred. For example, the prevalence of pseudorabies positive swine herds in a certain county in Pennsylvania is 15%. In this case, because the disease status of the herds does not change rapidly it makes more sense to use the prevalence measure. On the other hand, if one wanted to study the rate of infection of a virus in individual pigs within a herd (a more dynamic situation), an incidence measure would be preferred.

There are three basic measures of disease frequency used in epidemiology: prevalence, cumulative incidence and incidence density. These measures are commonly confused, so understanding the differences between these measures is critical.

Prevalence

The prevalence of disease, also called point prevalence, is the proportion of the number of cases observed compared to the population at risk at a given point of time.

$$\text{Prevalence} = \frac{\text{Number of cases observed at time t}}{\text{Total number of individuals at risk at time t}}$$

Prevalence refers to all cases of disease observed at a given moment within the population at risk, whereas incidence, with which it is often confused, refers to new cases that have occurred during a specific time period for the population at risk.

Example: Calculation of the prevalence of neonatal diarrhea on a dairy farm

You are asked to investigate a neonatal diarrhea problem on a large dairy farm. On the day you visit the farm, you find 86 calves alive that are less than or equal to 4 weeks of age. Of these you find that 8 are exhibiting signs of diarrhea. The prevalence of neonatal diarrhea at this particular time is therefore 8/86 = 0.092 or 9.2%.

Other examples:

i) The prevalence of pregnancy toxemia in Mr. Smith's ewes on March 24th, 1990 was 4/168 = 0.024.

ii) During my routine monthly herd health visit to Mr. Jones' dairy on March 25th, I found that 12 of his 60 cows had cystic ovaries. The prevalence of cystic ovaries was therefore 12/60 = 0.20 or 20%.

Prevalence is a function of both the incidence rate (see below for definition of incidence rate) and the mean duration of the disease in the population:

$$\text{Prevalence} = \text{Incidence} \times \text{Duration}$$

For a given incidence rate, the prevalence will be higher if the duration of the disease is longer. The prevalence will also be affected by the mortality rate of the disease, as discussed earlier. A lower prevalence would result if the disease was often fatal. Incidence rates rather than prevalence are much preferred in epidemiologic studies because prevalence does not convey the true magnitude of the disease in the study population.

Cumulative Incidence Rate

The cumulative incidence rate (CIR), usually referred to as Incidence rate, is defined as the proportion of a fixed population that becomes diseased in a stated period of time. Simply stated, the CIR is the proportion of healthy individuals who get the disease during a certain period. Cumulative incidence incorporates the notions of population-at-risk and a specific time period; hence it is regarded as a rate, even though it is calculated as a proportion.

$$\text{CIR} = \frac{\text{Number of newly diseased individuals for a specific time period}}{\text{Total number of individuals in population at risk for the sample period}}$$

The CIR has a range from 0 to 1 and must be accompanied by a specified time period to have any meaningful interpretation. The CIR is a measure of the average risk, that is, the probability that an individual would develop disease in a specified time period. The length of the observation period directly affects the cumulative incidence: the longer the period, the greater the cumulative incidence. For example, the lifetime CIR of death is 1 - everyone dies eventually!

Example: Estimating the Cumulative Incidence rate of neonatal diarrhea on a dairy farm

You would like to estimate the 12-month CIR rate of neonatal diarrhea on a large dairy farm operation consisting of 250 milking cows. During the 12-month period of interest there are 180 calves born alive on the farm. These 180 calves represent the population-at-risk. During the 12-month period you determine that 12 calves met your criteria of being diseased i.e., neonatal diarrhea. The 12-month CIR of neonatal diarrhea on this farm is then 12/180 = 0.066 or on a percentage basis 6.6%. This figure means that a calf from this population would have a 6.6% chance (or risk) of developing diarrhea during this 12-month period.

Other examples:

i) The CIR rate of pregnancy toxemia in Mr. Smith's ewes for the 1990 lambing season was 12/168 = 0.071.
ii) The CIR rate of cystic ovaries on Mr. Jones' dairy for 1990 was 18/120 = 0.15 or 15%.

Other important CIR rates:

A specific type of CIR is the case-fatality rate, which is the proportion of affected individuals that die from the disease. In our example, if 3 of the 12 affected neonates had died as a result of the diarrhea then the case-fatality rate would have been 3/12 = 0.25 or 25%. The case-fatality rate is usually associated with the seriousness and/or the virulence of the disease under study.

Another specific type of CIR is the attack rate which is used as a measure of morbidity (illness) in outbreak investigations. It is calculated simply as the number of animals affected divided by the number of animals exposed. For example, after 3 days of an outbreak of respiratory disease at a feedlot of 1200 head, 50 cases were identified. The attack rate was therefore 50/1200 = 0.042 or 4.2%.

Incidence Density Rate

The incidence density rate, also termed simply the incidence rate (IR), is a measure of the instantaneous force or speed of disease occurrence. The IR is defined as the number of newly diseased individuals divided by the sum of the time periods of all individuals in the population who remain disease free.

$$IR = \frac{\text{Number of newly diseased individual}}{\text{Sum of time periods for all disease - free individuals at risk}}$$

Whereas the CIR simply represents the proportion of individuals affected, the IR takes into account for each individual at risk the time elapsed before disease occurs. The denominator of the IR is termed "person-time" or "animal-time" or "population-time". It represents the sum of the disease-free time experience for all the individuals in the population. There are many ways in which a given population time experience can be obtained. For example, if we were to follow each of our 180 calves for the first 2 weeks of their life, our denominator would be 180 x 2 = 360 weeks. Alternatively, we could follow only 90 calves for 4 weeks and obtain the same time experience. By dividing the number of cases by the time at risk, the length of the observation period is taken into account

in a way that was not possible with the cumulative incidence. Even different observation periods that occur for example, when individuals migrate into or out of the population during the observation period are accounted for.

The dimension of the IR is per unit time while its magnitude ranges from 0 to infinity. The IR is not a proportion like the previous two measures, since the denominator is measured in units of time. The IR can be thought of as the speed, calculated at a certain point in time that the disease is occurring in a population. This is analogous to the speed with which a motor car is travelling. That is, miles per hour is an instantaneous rate which expresses the distance travelled in a given unit of time (one hour). An incidence rate of 25 cases per 100,000 population years expresses the instantaneous speed which the disease is affecting the population. This measure is dynamic and can change freely just as the speed of a car.

The choice of "population time" to be used depends entirely on the context of the study and the disease. In human chronic disease studies, a standard measure is 100,000 person years. It is extremely unusual that such a large "population-time" experience can be assimilated in veterinary medicine.

Example: Estimating the incidence density rate of neonatal diarrhea on a dairy farm

In our example concerning calf diarrhea on a dairy farm, we can calculate the incidence rate of neonatal diarrhea in the following manner. First, the total disease-free time experience (in weeks) of all 180 neonatal calves on the farm is estimated. The 168 calves who did not develop diarrhea contribute 168 x 4 = 672 weeks of "population-time" by the end of their 4-week neonatal period. We also need to account for the "population time" of the 12 calves before they became sick. By checking the farm records we see that 3 of these calves developed diarrhea after 3 days, 3 after 10 days and 6 after 14 days. Therefore, the total "calf-time" experience before these calves got sick was (3 x 3/7 wk) + (3 x 10/7 wk) + (6 x 2 wk) = 17.5 weeks. Thus the total neonatal "population time" experience on the farm was 672 + 17.5 = 689.5 weeks. The neonatal diarrhea incidence rate was therefore 12/689.5 = 0.0174 cases per calf-week. This figure means, that on the average, the speed of occurrence of neonatal diarrhea on this farm is 0.0174 cases per calf-week or 0.069 (0.0174 x 4) cases per calf month.

Other examples:

i) The IR rate of pregnancy toxemia in Mr. Smith's ewes during the 3-month lambing season of 1990 was 0.023 cases per ewe-week.

ii) The IR rate of cystic ovaries on Mr. Jones's dairy farm is 0.16 cases per cow-year.

The IR shown in the above calf example has been calculated by summarizing the information obtained from the whole calving season. However, the IR could have been calculated using a much shorter time period e.g., the IR of neonatal diarrhea during the first 2 months of the calving season. In this case all the calves born alive during the March and April period would have contributed to the "population-time" estimate. In some situations, such as an outbreak of disease where it is important to monitor the progress of the disease, it is often useful to re-calculate the incidence rates at frequent intervals e.g., every week. In our example, if the IR of neonatal diarrhea was monitored at weekly intervals, the rate obtained would undoubtedly fluctuate as new cases occur, as new calves are born and enter the pool of neonates at risk, and as calves leave the population at risk. Calves can leave the population either because they become older

than 4 weeks of age, they become affected with diarrhea, they die from causes unrelated to diarrhea, or they leave the farm for some other reason.

Alternative methods of estimating population-time

The calculation of the "population-time" by summing up each individuals time contribution is obviously laborious and often impractical. To overcome this problem, an estimate of the population-time can be obtained by either counting the population midway through the time period or taking the average of the population at the beginning and end of the time period.

For example, to estimate the neonatal diarrhea incidence rate for the 1990 calving season on our dairy farm, the population-time can be approximated by counting the number of neonatal calves present on the farm half way through the year (i.e., on July 1st). The estimated neonatal calf population-time in weeks for 1990 is then calculated by multiplying this figure by 4 (i.e., the 4 week neonatal period). For example, if there were 25 healthy calves less than 4 weeks old on the farm on July 1st, the population-time estimate would be 100 calf weeks. In this instance, this estimate is grossly inaccurate compared to the figure of 689.5 weeks calculated by summing the individual time experiences for each calf born during the whole year. For this method to be accurate, the number of calves being born into the population pool and the number of calves leaving the population pool over the year must be approximately evenly balanced. Because the timing of the calvings on this farm are not evenly distributed over the 12 month period, this estimate is very inaccurate. However, for more stable populations this method usually works quite well.

Other important IRs:

A specific type of incidence rate is the mortality (death) rate, defined as the incidence rate of death per "population-time". It is calculated by:

$$\text{Mortality rate} = \frac{\text{Number of deaths during time period t}}{\text{Total population-time at risk during time t}}$$

The denominator for the mortality rate is population-time. The mortality rate measures the speed of death due to a specific disease in a population at risk. It should be clearly distinguished from the case-fatality rate described earlier. The denominator for the case-fatality rate is the number of affected individuals, not the population-time at risk. The use of these two terms is often confused.

Crude vs specific IRs:

Rates are often referred to as either crude or specific. Crude rates reflect the total number of observed cases and the total population-at-risk time experience. They are easy to compute and to explain but they have the disadvantage in that they ignore potentially important influences such as host or management factors. Specific rates characterize the frequency of disease for specific subpopulations or groups e.g., age-specific or sex-specific rates. Cause-specific rates limit the numerator to the specific cause of interest. The mortality rate of mucosal disease is an example of a cause-specific rate. Specific rates have several advantages. More accurate comparisons, for example between herds or regions, can be made using specific rates and they often illuminate

important trends which are lost when only crude rates are used. For example, the crude death (mortality) rate on a Kansas beef ranch was 39.5 per 1000 cattle years, while it was 120 per 1000 cattle years on a nearby Holstein breeding operation. However, when age-specific death rates (using 1 year intervals) were calculated for both farms, the beef ranch had higher age-specific death rates for every age category:

Crude mortality rates (per 1000 cattle years)

	Beef Ranch	Breeding Farm
All animals	39.5	120

Age-specific mortality rates (per 1000 cattle years)

Age Group (yrs)	Beef Ranch	Breeding Farm
1 - 2	6.56	3.73
6 - 7	227.0	153.1

In this instance the crude rates are misleading by suggesting that life on the breeding farm was dramatically riskier than on the beef ranch; a conclusion that the age-specific rates clearly contradict. The misleading crude rates can be explained by noting that a greater proportion of the animals on the breeding farm were in the older age categories where the mortality rates are much higher. The beef ranch consists of mostly young animals where the age-specific rates are low.

As mentioned earlier, the terms CIR and IR are frequently confused and carelessly used in the literature when disease occurrence is being discussed. The term risk is also often used to describe disease incidence without any explicit definition of whether CIR or IR is being considered. Finally, the use of the term incidence is frequently abused by medical professionals to describe their frequent experience of a particular condition; for example, "we see a high incidence of milk fever in our dairy practice." Seldom has the practitioner defined a population-at-risk and accounted for the population-time experience to calculate a true incidence rate. All that can be stated in such instances is that a particular disease entity is "common".

4. INVESTIGATING DISEASE IN AN ANIMAL POPULATION

An outbreak (or short-term epidemic) is a series of events clustered in time and in space. The events usually are new cases of a disease occurring at higher frequency (rate) than what is normally expected. Outbreak investigation is a systematic procedure to identify causes and sources of epidemics. It should answer the following questions:

- What is the problem?
- What immediate steps can be taken to deal with the problem?
- Can future occurrences be prevented?

Outbreak investigation relies on the premise that cases of a disease are not distributed randomly in a population but rather occur in certain patterns. The investigator attempts to discover this pattern. Once identified, the pattern will lead the investigator to hypothesize on its determinants (causes). Three major types of patterns can be distinguished: temporal patterns, spatial patterns, and animal patterns.

Temporal patterns. One can represent the temporal pattern of an outbreak in graph form. The frequency of cases on the ordinate (y-axis) is plotted against time on the abscissa (x-axis). Such a graph is termed and epidemic curve; its shape may reveal infor-

mation about the nature of the epidemic, e.g. point epidemic vs. propagated epidemic.

Spatial patterns. These are represented by various types of maps. Identification of spatial patterns also helps in determining the nature of the disease. Some of the most common types of maps used in epidemiology are spot maps and transparent overlay maps.

Animal patterns. These patterns are often confounded with either the time pattern, the spatial pattern, or both. It exists mainly because of a natural or artificial susceptibility or resistance of groups of animals. In outbreaks it is usually some artificial resistance that protects the animals that remain well during the outbreak, e.g. these animals did not get exposed because they arrived "too late" or were in different units (pens). It is convenient to use these animals as a comparison group in constructing the attack rate table. Age, breed, sex, strain and genetics are the most frequently described host attributes.

Temporal Descriptions of Disease

Timing Disease Events

It is unlikely that causes of a disease occur at random intervals in a population of animals. The timing of onset of cases rather follows one of three patterns.

Cases may occur sporadic – they do not seem to be associated with any other identifiable factor, or with each other.

Cases may occur regularly, or at an endemic level. In this situation, one would attempt to explain the pattern in light of other events happening in a similarly regular fashion.

Cases may occur in clusters, a pattern typical of outbreaks or epidemics. A useful means to represent the temporal distribution pattern of disease events is to construct an epidemic curve which can illustrate both the magnitude of the problem, i.e. the number of new cases occurring, and the rapidity with which the epidemic progresses.

The endemic level is the expected level of disease. An epidemic is said to prevail when the frequency of cases (or outbreaks) in a population clearly exceeds the normally expected level of a given areas and/or season. If an epidemic takes international proportions, it is termed a pandemic.

The Epidemic Curve

The epidemic curve refers to the graphing of new cases (the vertical or Y axis) over time (the horizontal or X axis). The slope of the ascending limb or branch of the epidemic curve can reveal something about the type of exposure or about the mode of transmission of the disease agent. If transmission is fast and effective, the slope of the ascending branch is likely to be steeper than if transmission is slow or if the incubation period is long. Exposure of a large number of animals to an agent at once or within a short period of time, e.g. through exposure to a common source, results in a point epidemic curve, typically a feed or waterborne disease. The ascending branch of the corresponding curve could be almost vertical before reaching its peak.

When the disease agent is transmitted via contact or vectors the resulting curve is typical of a propagated epidemic curve. Here the slope of the curve also depends on some agent characteristics such as its ability to survive outside the host; on some host factors such as contact rates, population density, etc. The extent of the plateau and the slope of the descending branch are mainly a function of the availability of susceptible animals which in turn may be a function of such tings as the composition of the population at risk with respect to their immune status—a concept referred to as herd immunity—or some intervention, e.g. vaccination or treatment.

A secondary peak may occur which is usually due to: a) introduction of susceptible animals into the previously epidemic area, or b) movement of infected animals from the epidemic area and contact with susceptible animals. The main peak of the curve is at times preceded by a smaller peak which could represent the index case, i.e. the first case to occur. The interval between this first peak and the beginning of the next or main peak could indicate the incubation period.

If the epidemic curve extends over a relatively long period of time and is based on frequent observations at short intervals, it may be examined for such patterns as seasonal variations, cyclic fluctuations, or secular trends.

A seasonal variation is said to exist when the ups and downs occur at periodic intervals which coincide with "seasons", where seasons can be periods of time other than the classical four seasons of the year. A season can be as short as a week or as long as a year, depending on what biological phenomena one is measuring.

Cyclical fluctuations are said to exist when the variations occur at rather regular intervals – these intervals are usually longer than seasons.

Secular trends are long term changes where, in addition to short term ups and downs, the curve either climbs or declines more or less steadily over an extended period of time, usually years.

5. OUTLINE OF OUTBREAK INVESTIGATION PROCEDURES

The following outline is a checklist of items to consider when investigating an outbreak. Not all steps are necessarily followed in each outbreak, nor do they always follow this sequence; at times several activities are initiated simultaneously.

1. Verify the diagnosis

When a diagnosis (tentative or final) has already been established, it should be verified by the investigator, who usually does this by verifying records and/or initiating a clinical-pathological work-up and by collecting specimens.

2. Define a "case"

Even when the goal of the investigation is to find a diagnosis, the cases at hand still have to be defined in clinical terms and included into a syndrome so as to exclude cases of diseases other than the one(s) under investigation.

3. Determine the magnitude of the problem

At this point one should ask, "Is there an epidemic?" To answer this, one computes the attack rate (AR) and compares it to the normal or expected occurrence of disease (or deaths). The attack rate is a measure of incidence and can be computed according to the following formula:

$$AR = \frac{\text{\# new cases (and/or deaths) since the beginning of outbreak X 100}}{\text{Total No. of animals at risk at the beginning of the outbreak}}$$

4. Temporal pattern

To examine the temporal distribution of new cases, one should draw one or more epidemic curves using various time intervals (on the x-axis) that might be appropriate for the disease under study, e.g. hours, days, weeks.

5. Spatial pattern

In order to examine the special distribution of the cases, one now sketches a topographic map of the area and the cases within, or of the layout of pens and the cases within. Next, one inspects the drawing for interrelationships among cases, and between the location of cases and other physical features.

6. Animal pattern

Age, sex, breed an strain patterns are noted.

7. Analysis of data

One now computes factor-specific attack rates for such factors as age, sex, breed, feed, pen, etc., using the formula given for AR but counting only animals associated with the factor. An important application of this is the construction of the attack rate table.

In the attack rate table, one compares factor-specific (e.g. feed-specific) attack rates among animals exposed to a given factor with those among animals not so exposed.

8. Working hypothesis

Based on the above information, one should arrive at one or more hypothesis as to:

a) the kind of epidemic: point epidemic vs. propagated epidemic;
b) the source of the epidemic: common source, multiple exposure;
c) the possible mode of spread: contact, vehicle, vector.

One needs to check that the hypothesis fits all the factors, i.e., that it is compatible with all observations if it does not fit, revise the hypothesis. At this point, one should also be able to **make recommendations for corrective action** (e.g. change feed, move the animals, etc.) and for prevention of future cases.

9. Intensive follow up (clinical and epidemiological)

This includes clinical, pathological, microbiological, and toxicological examination of tissues, feeds, objects, etc. It includes making detailed diagrams, flow charts of preparation of feed and of movement of animals. It involves epidemiological follow up such as the search for additional cases on other premises or outbreaks of a similar nature in other locations. It may call for conducting of a clinical trial on susceptible animals so as to prove the implication of a postulated determinant (cause).

10. Report

"No job is complete till the paperwork is done." It is important that the findings of an outbreak investigation be reported accurately, precisely, and that the presentation is done in a professional style.

6. ASSESSING HERD IMMUNITY AND CHOOSING A DISEASE PREVENTION STRATEGY

Host resistance and *Herd Immunity*

- Host Resistance
  - Present or absent
  - Two types:
    - Circumstantial Resistance - lack of contact
    - Biological Resistance - host immunity

- Herd Immunity
  - The amount of the resistance of a group to an attack by a disease.
  - It is a likelihood statement.
  - The term "herd immunity" first appeared in the 1920s.
  - Herd immunity as a phenomenon that leads to the reduction of transmission of an infection in a population.
  - It indicates the presence of sufficient immune individuals in a population (above a specified threshold) which leads to the "die off " of the infection.
  - It reflects the development of the concept of herd immunity in the context of research on disease elimination through intervention programs.
  - "High" herd immunity in a population = less likelihood of a disease individual in this population to transmit the disease to an average susceptible individual.
  - Herd Immunity Depends on:
    - Composition of the population:
      susceptible and infected groups
      Contact rate
- Mass Action Principle
  - It is a function of the number susceptible individuals in the population:
  - f (S_t)= C_{t+1}/C_t, or
  - $C_{t+1} = S_t C_t r$
  - Mass Action Principle = Law of mass action (velocity of a chemical reaction is a function of the concentrations of the initial reagents)
  - The "mass action principle" is actually the theoretical basis of the phenomenon of herd immunity
- The basic reproductive rate (R_0)
  - It is the average number of cases directly infected by an infectious case during the entire infectious period for a case.
  - It is comprehensible measure of the transmissibility or spreading potential of an infection in a population
  - R_0 is an abstraction: no population actually experiences spread of an infection at this rate
  - R_0 is independent of the number (or proportion) of individuals susceptible or immune in a given population
  - R_0 is determined by basic biological features of the microorganism and the susceptible population
  - R_0 is specific to a microorganism and a population
  - In real life, populations are not "totally susceptible"; usually immune individuals are also around
  - Therefore, an infection is actually reproduced at a rate which is equal to the basic
  - R_0 reduced by the fraction of susceptible individuals in the host population ("effective" or "actual" R)
  - This can be expressed as: $R = R_0 (S/N)$
  - if R=1, in the next time period, there will be one new case per infected person, which is a state of equilibrium (endemic)
  - if R>1, in the next time period of the spread of the infection, there will be more than one new case per infected person, and therefore (if this continues) there will be an epidemic

- if R<1, in the next time period, there will be less than one new case per infected person, and eventually (if R continues to be <1) the microorganism will disappear
- $R = R_0 (S/N) = 1$
  or
- $R = R_0 [1 - (I/N)] = 1$
- Thus, $R_0 = 1 / [1 - (I/N)]$
- $(I/N) = 1 - (1/R_0)$
- Eradication of the microorganism (PC) Therefore, $PC = 1 - (1/ R_0)$

Methods for Directed Action Against Diseases

- Prevention
- Control
- Eradication

Prevention, Control or Eradication?

- Exclusion of a disease from a population of unaffected people/animals = Prevention
- How do we accomplish this type of exclusion?
- Quarantine of potentially sick/exposed individuals
- Requires a prior knowledge about the disease agent and its incubation period
- A type of exclusion of a disease
- Requirement:
  - Availability of test(s) for screening and diagnosis
  - Confidence in the negative test results

Methods of Protection

- Immunization
- Chemoprophylaxis
- Environmental sanitation/vector control
- Genetic Engineering and selective breeding
- Public awareness and education

Immunization

- The most common method of protection
- Requires good knowledge about the characteristics of the disease agent
- Not all disease agents are suitable for this method
- Vaccine should be evaluated carefully for both its safety and efficacy
- A potential for interference in the detection of diseased individuals
- Mass immunization vs. targeted immunization

Chemoprophylaxis

- Routine medication as a prophylactic measure to protect individuals from the disease
- Requires available effective and safe treatment that can reduce the agent's infectivity
- Must be cost effective
- Routine medication vs. mass treatment
- Microbial evolution and its relationship to this method of disease protection

Environmental sanitation

- Less controversial issues
- Historically known to be effective
- Does not require an extensive knowledge about the disease agent
- Protection from several disease agents

Genetic Engineering and selective breeding

- Contradictive approach
- Limited in its use
- Potential for long term environmental negative impact

Public awareness and education

- Emotional response vs. scientific facts
- Require a careful evaluation of the message(s)
- Effective methods when the disease is a zoonotic
- Understanding of the epidemiology of the disease

Disease Control

- All measures used to reduce the frequency of disease in a population (sick and healthy)
- Prevention and control programs represent continuing efforts
- All preventive measures are part of control approaches
- Prevention
- Decrease communicability and contacts
- Increase resistance
- Early detection

Decrease communicability and contacts

- Increase herd immunity through a reduction of contacts
- Segregation of sick animals is an example of this approach
- Closed herds/all in all out practices
- Hard to apply in dynamic populations (e.g. animal shelters)
- Easy to apply in homogeneous and steady populations

Increase resistance

- Increase herd immunity through a reduction in susceptibility
- Immunization is part of increasing resistance but it is not the only method
- Early exposure to the agent, competitiveness, genetic selection, and feed ingredients are part of this concept

Early Detection

- Most applicable in contagious diseases
- Efficient when the mode of transmission is effective
- Detection of reservoir(s) can be part of this method
- Requires an active searching of cases = surveillance
- A plan of action

- Availability of screening test(s)
- Availability of diagnostic test(s)
- Overall confidence in the positive results

Eradication

- Elimination of the agent from specific geographical region/premises as well as selected host species
- It is the ultimate action against a disease
- Difficult to accomplish
- Difficult to maintain
- The focus should be on specific region/premises/shelter/home

Methods of Eradication

- Mass treatment
- Mass immunization
- Quarantine = Maintaining the disease outside of the region/premises
- Selective Slaughter
- Depopulation
- Mass treatment regardless of the disease status of individuals in the population (e.g. Anthrax)
- Potential for serious side effect
- No requirement for identifying diseased animals
- Availability of an effective treatment that can work for both clinical and subclinical cases
- Not suitable for several disease agents that need to be eradicated

Mass immunization

- Immunization regardless of the disease status
- No requirement for screening of the disease
- Effective if the immunization does not interfere with the disease detection (e.g. Pseudorabies)

Quarantine

- Maintaining the disease outside of the region/premises
- Scientific justification is required
- Hard to be effective due to our lifestyle

Selective Slaughter

- Test and slaughter those positive
- Its application is limited to selected animal species
- Requires several tests
- Requires an overall confidence in a positive test result
- It is a costly approach
- Animal welfare and social pressure
- Proves to be an effective approach in some diseases

Depopulation

- Elimination of animals that are exposed to a disease
- Elimination of animals that show symptoms of a disease
- Most likely done on a herd basis
- Difficult to sell to the public
- Impact on the environment
- Sacrificing of false positive animals/premises

Conclusion

- No single action against a disease is sufficient
- Actions against a disease require prior knowledge about the epidemiology of the disease
- In addition to scientific facts, other factors related to social, political, and environmental conditions should be considered before a set of actions is recommended
- Local action (premises, home, etc) should be the priority prior to implementing a regional one
- Epidemiological tools should be considered in the evaluation of the effectiveness of an action

7. PROPERTIES OF DIAGNOSTIC TESTS

Diagnostic Tests, Screening Tests, and Prevalence Surveys

Prevalence Surveys

The type of survey most commonly done in epidemiology is the prevalence survey to determine the frequency and distribution of some infectious agent by measuring the occurrence of antibody (produced to the agent) in the serum of the individuals. The result of such a test is classified as either positive or negative. A positive result does not necessarily mean that the individual has been recently infected with the agent in question. A positive result can mean an infected, incubating, or recovering inidivdual. A positive result may also indicate a prior vaccination to the agent or passive antibody transfer. A test may also be positive at times due to lab or sample handling; errors. Occasionally, antibodies to another agent that has infected the individual will cross react in tests used to determine exposure to another agent.

Similarly, a test may be negative when the individual is actually infected. An individual may have been infected recently that it has not had enough time to develop an antibody response at the time of the test. In addition, a test may not be finely tuned enough to detect small quantities of antibody to an agent. As with a positive test, a negative result could also be due to lab or sample handling errors.

Screening Tests

Screening is the presumptive identification of unrecognized disease by application of simple tests to sort out apparently healthy individuals whom may have the disease from those that probably do not have the disease. Therefore, screening tests, be they serological or other (e.g. metabolic profiles, physical measurements, etc.) are applied to apparently healthy individuals in search of disease. Unlike prevalence surveys, done to measure the amount of the disease, screening is done with the objective of early detection of disease.

As a general rule, screening tests are applied to a large number of individuals and are often followed by a *diagnostic test* on those indviduals found to be positive. When a screening test is applied to a "high risk group" of individuals, it is thought of as *case finding*. High risk means that individuals are suspected or known to have a higher prevalence of the disease compared to the total population. They may be higher risk because of factors such as age, location, use or an increased exposure to disease agents.

Diagnostic Tests

The aim of a diagnostic test is to confirm the presence of infection in individuals. Serological tests are also valuable for use as *diagnostic tests.* To be of use as a diagnostic test, two serum samples must be obtained at an interval of two weeks. (Usually one is taken at the initial exam and the second is taken two weeks later.) If the antibody titer (concentration) is considerably higher in the second sample (usually must be a four fold increase), the individual is considered to have an active infection.

The *aim* of serological testing is **early detection**, which leads to prevention, early treatment and disease control.

Properties of Tests

Accuracy of a Test

Most tests are not generally 100% accurate in their ability to correctly identify infected or non-infected individuals. This is a problem of *misclassification.* The accuracy of a test can be measured and expressed by its ability to correctly classify these individuals according to their disease status. These measures are termed *sensitivity (Se)* and *specificity (Sp).*

Sensitivity

is the probability that a test correctly identifies those individuals that are infected.

Specificity

is the probability that a test correctly identifies those individuals that are not infected.

To establish these two test attributes, the test must be conducted on the sera from a number of individuals for which the disease status is known. The results can be tabulated in a 2-by-2 table from which sensitivity and specificity can be calculated.

For example, in Figure 3 is shown a 2-by-2 table for a generic disease.

The following information can be obtained from the table:

- The total positive (T+) to the screening test is 33.
- The total negative (T-) to the screening test is 116.
- The total number of diseased individuals (D+) is 56.
- The total number of non-diseased individuals (D-) is 93.

Those individuals that are misclassified by the screening test are:

I. *False positive (FP):* those individuals that are not diseased but are positive to the screening test. (15)
II. *False negative (FN):* those individuals that are diseased but are negative to the screening test. (38)

FIGURE 3

Gold Standard: disease status

		Disead (D+)	**Not Disead (D-)**	
Screening Test: Diseas- o-check	positive	18 TP	15 FP	33
	negative	38 FN	78 TN	116
		56	93	149

The Gold Standard is the definitive diagnosis of the diseas (the disease status) and usually is determined by a standard method (i.e. virus isolation).

Those individuals correctly identified (accuracy of the test) are:

I. *True Positive (TP):* those individuals that have the disease and are positive on the screening test. (18)
II. *True Negative (TN):* those individuals that do not have the disease and are negative to the screening test. (78)

For most screening tests, Se and Sp are not known and the consequences of misclassification must be understood. Interpretation of misclassified results depends upon the purpose which the test is given and upon the person who does the interpretation.

In the context of a disease control program in which individuals positive to a screening test will be isolated (for instance), the consequence of a false positive result would not be good.

Calculating Properties of Screening Tests

I. True vs. Apparent Prevalence

A. True prevalence

the proportion of individuals tested with the disease condition of interest (by the Gold Standard).

$$\text{true prevalence} = \frac{D+}{N \text{ (total \# of individuals tested)}}$$

For our generic disease, true prevalence would be 56/149.

B. Apparent prevalence

the proportion of individuals that are positive to the screening test. (This is also known as the test positive rate.)

$$\text{apparent prevalence} = \frac{T+}{N}$$

For our generic disease, the apparent prevalence would be 33/149.

Note: the calculations for the generic disease will be shown beside the equation from this point on.

II. Accuracy vs. Misclassification:

A. Accuracy

the proportion of those individuals correctly identified by the test.

$$\text{accuracy} = \frac{TP + TN}{N \text{ (total \# of individuals tested)}} \qquad (96/149)$$

B. Misclassification

the proportion of those individuals not correctly identified by the test.

$$\text{misclassification} = \frac{FP + FN}{N} \qquad (53/149)$$

III. Proportions of False Positives and False Negatives

A. False positive proportion

the proportion of the truly non-diseased (D-) that the test identifies to be positive.

$$\text{False positive proportion} = \frac{FP}{(TN + FP) \text{ [those individuals without disease]}}$$

The false positive proportion for the generic disease is 15/93 or 16%. This means that 16 out of 100 non-diseased individuals will have a positive test.

B. False negative proportion

the proportion of the truly diseased population (D+) that the test identifies as negative.

$$\text{False negative proportion} = \frac{FN}{(TP + FN) \text{ [those with the disease]}}$$

The false negative proportion for the generic disease is 38/56 or 68%. This means 68 out of 100 infected indviduals (D+) will have a negative test (T-).

IV. Sensitivity (Se) vs. Specificity (Sp):

A. Se:

the proportion of truly diseased individuals (D+) that the test correctly identifies (those among the diseased population that test positive).

$$Se = \frac{TP}{D+} = \frac{TP}{TP + FN} \qquad (18/56 \text{ or } 32\%)$$

This means that for the generic disease, that out of 100 infected dogs 32 will be + on the screening test.

B. Sp:

the proportion of the truly non-diseased individuals (D-) that the test correctly identifies (those that tested negative among the non-diseased population).

$$Sp = \frac{TN}{D-} = \frac{TN}{(TN+FP)} \qquad (78/93 \text{ or } 84\%)$$

This means that out of 100 non-diseased individuals, 84 will be negative on the screening test.

If the Se and Sp are known, True Prevalence can be calculated:

$$\text{True prevalence} = \frac{\text{apparent prevalence} + Sp - 1}{Sp + Se - 1}$$

V. Predictive Values:

A. Predictive value + (PV+)

this is an important property of a screening test. It indicates what proportion of the T+ are really infected (D+). It is the probability that a positive test result is correct.

$$PV+ = \frac{TP}{T+} = \frac{TP}{TP + FP} \qquad (18/33 \text{ or } 54\%)$$

This means that there is a 54% chance that the individual is infected if it has a positive test result.

A. Predictive value of a negative (PV-)

this is the probability that a negative test result is correct.

$$PV- = \frac{TN}{T-} = \frac{TN}{TN+FN} \qquad (78/116 \text{ or } 67\%)$$

This means that there is a 67% chance that an individual is not infected if it has a negative test result.

PV+ is closely related to specificity and PV- is closely related to sensitivity. PVs indicate the test accuracy given the test result is known. PVs depend on the prevalence of the disease in the population and the Sp and Se of the test used.

For example, another way to calculate PVs is:

$$PV+ = \frac{Se \times \text{Prevalence of diseased}}{[(Se \times \text{Prevalence}) + (FP \text{ proportion} \times \text{Prevalence of nondiseased})]}$$

$$PV- = \frac{Sp \times \text{Prevalence of non-diseased}}{[(Sp \times \text{Prevalence of non-diseased}) + (FN \text{ proportion} \times \text{Prevalence of diseased})]}$$

The PV of a positive test result varies directly with the prevalence of disease when the Sp and Se are held constant. The following table illustrates:

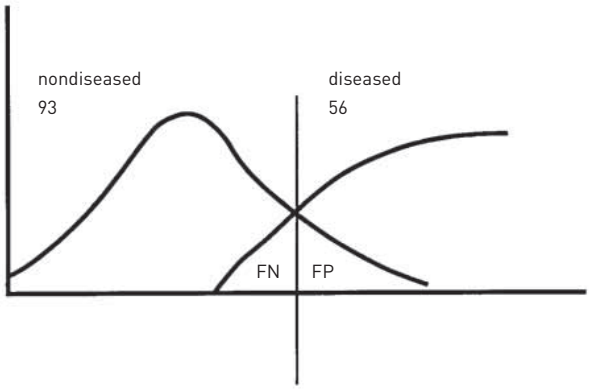
Effects of Prevalence on Positive Test Results with Se and Sp = .95 (95%)

Prevalence (%)	.1	1.0	2.0	5.0	50.0
PV (%)	1.9	16.1	27.9	50.0	95.0

Evaluating the Usefulness of a Test

The *sensitivity* of a test is directly related to the amount of *false positives* and conversely, the *specificity* is related to the amount of *false negatives*.

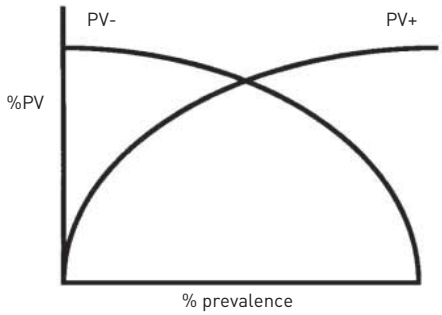
Given a generic graph of a screening test done by the ELISA:



If we want to *increase* Sp for the above screening test, the cut off point must be moved to the right which will decrease false positives and increase false negatives.

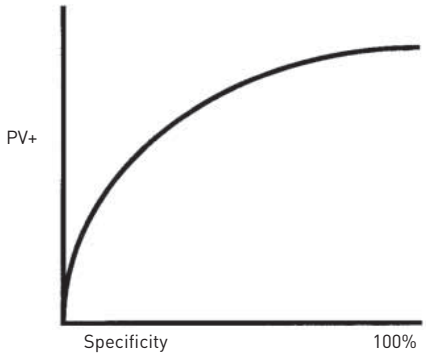
As prevalence of the disease increases, the PV+ increases and the PV- decreases.

This means, as more individuals become infected, the probability of an individual being infected it tests positive increases.



Specificity and the Positive Predictive Value of a Test

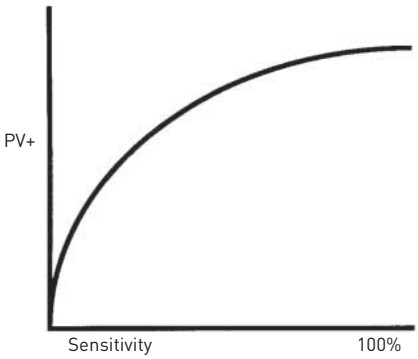
Sp is related to PV+ in the following way:



As the specificity increase, the PV+ increases and this indicates that the probability of a positive result being correct increases. Specificity allows more confidence in a positive test.

Sensitivity and the Negative Predictive Value of a Test

Se is related to PV- in the following way:



As the sensitivity increases, the PV- increases and this indicates that the probability of a negative result being correct increases. Sensitivity allows more confidence in a negative test.

Choosing Between Tests

I. Use a test with a high Se and high PV- when:

A. it is advantageous to "rule out" a diagnosis in the early stages of a diagnostic workup to decrease the possible number of individuals to treat. This would build a trust in the negative test result and would have a confidence in that those not treated (because they tested negative) will not spread the disease.
B. a FN is dangerous. For example, a FN of an individual entering this country with a exotic disease would have serious consequences.

II. Use a test with high Sp and high PV+ when:

A. it is advantageous to confirm a diagnosis. Since there is more confidence in a + test, this allows those that should be treated to be confidently determined.
B. a FP is dangerous. For example, if realization is the measure taken following a positive result, the cost of too many FPs could be quite high.

Since diagnostic tests are done with the purpose of identifying cases and bringing them to treatment, it is desirable for the test to have high PV+, otherwise a large proportion of individuals are treated or realized unnecessarily. If a test aims at finding potential cases of one specified disease (i.e. in the form of a diagnosis), the PV of a positive test can be termed its *diagnosability.* It is also highly desirable for screening tests used in the early stages of a control program to be highly sensitive (so that there are few FN) and the test used in the latter stages to be highly specific (to decrease FP). This is especially true when prevalence is low (2%), when most of the individuals are free of the disease and the results of even a highly sensitive and specific test will include a large number of false positives. Most diagnostic tests, therefore, are aimed directly at high risk groups. With high risk groups, the prevalence and diagnosability is increased.

Testing in Series and in Parallel

I. Testing in series

The results of every test run must be +, otherwise the individual is considered negative for disease. For testing in series, it is desired to have tests with high Sp and PV+ so that there is confidence in the + result. With each positive result, the next test is performed.

II. Testing in parallel

The results of every test must be negative, otherwise the individual is considered positive for the disease. It is desirable to have tests with high Se and PV- so that there is confidence in the negative result.

III. Test Batteries

This is running all the available tests and panels for disease. The more tests administered, the greater the probability of a false positive. This practice can be good if any probability of disease is dangerous.

8. THE ROLE OF STATISTICS IN VETERINARY EPIDEMIOLOGY

Definitions

I. *Census* a collection of information on every individual in the population or all the members of a group with certain specified attributes.

II. *Sample* collection of information upon a subset of individuals in which the results are inferred to be representative of a larger population.

III. *Survey:* an investigation in which information is systematically collected to estimate the occurrence of an event in the population, **but in which the experimental method is NOT used.** This means no intervention or manipulation of the population is used to obtain this information.

IV. *Surveillance:* an ongoing scrutiny, generally using methods distinguished by their practicality, uniformity, and frequently their rapidity rather than complete accuracy. In essence, it is a monitoring of certain events that is used to detect a change in trend or distribution in order to initiate investigative or control measures. A survey is not a surveillance, but could become one if it is continued to monitor the population initially investigated. For example, a surveillance can be used to measure a change in infection rate between seasons, geographical regions, etc.

Two examples of surveillance organizations are:

A. *NAHMS (National Animal Health Monitoring System):*

- supervised by the USDA
- monitors disease prevalence and cost of production

B. *MCI (Market Cattle Identification):*

- also supervised by the USDA
- collects serum samples from every adult cow slaughtered and tests for antibodies to *Brucella*. The surveillance is followed back to the herd of origin. This system requires extremely competent animal identification methods.

Types of Sampling

1. Probability sampling: is a random access to every individual. Every individual in the population has a **known chance** of being sampled (i.e. 1/10, 1/1000, etc.). Inference of the sample is applied to the rest of the population. The degree of bias depends on how the sample was taken and this will determine if the sample truly represents the rest of the population.

2. Non-probability sampling: this is done on the basis of convenience and the sample is usually not representative of the population under investigation. For example, if the investigation was to determine prevalence of a certain disease among deer and only those deer easily caught were sampled, this may not be representative because maybe those deer that are easily caught are that way because they are ill. Another example would be in a survey to measure prevalence of Heartworm in Colorado, the investigator would only ask those veterinarians that he knows. This may lead to a bias, for example if those veterinarians were located in the western slope area where heartworm prevalence is higher. There is no design to this method of sampling. The problem with this type of sampling comes when the results are applied to the entire population. This type of sampling may work and may actually be necessary at the beginning of an investigation because it may answer an initial question (e.g. Is there heartworm at all?).

Types of Probability Sampling

I. Simple random sampling: this is the ideal situation. Every individual will have an equal chance to appear in the sample. This type of sample can be done correctly in several ways.

For example:

A. Assign each individual a number and using tables (computer or book), select five numbers. This will not guarantee a representative sample, but it will decrease bias and give a better chance for a representative sample.
B. Pull names from a hat.

The disadvantage of this is that a list of every individual in the population is needed. This could prove to be a difficult task.

II. Simple stratified sampling: the population is divided into strata (subgroups) according to certain criteria that are important to the investigation. For example, in the Heartworm study dogs could be divided into large and small size because large dogs have a higher incidence of the disease. Then a random sample is performed among each strata. The problem of this method is that each stratum needs to be equal in size to the others and this is not likely to happen.

III. Proportional stratified sampling: this takes into account the problem of strata of unequal size. The sample among strata is obtained with regard to the contribution of the strata to the size of the total population. For example, if large dogs contributed 50%, medium dogs 30%, and small dogs 20%, a sample of 300 dogs would include: 150 large dogs, 90 medium dogs and 60 small dogs. Then a simple random sampling can be done among them. This kind of sampling is the most commonly used.

IV. Cluster sampling: the unit of sampling will be a group of individuals rather than a single individual. For example, if there are three dogs in a kennel cage, this would represent one dog unit. If any one of these dogs were positive for Heartworm, then the unit would be considered positive. Every animal in the unit must be surveyed.

V. Multistage sampling: this is when more than one of the above methods is incorporated into the investigation design. For example, in the heartworm survey:

A. a letter is sent to all vets asking whether or not they wish to participate.
B. those that respond are used in the survey.
C. the sample is clustered by clinic and every dog that comes in for a period of six months must be surveyed.
D. the clusters are stratified by size of dog and region.

The Table 1 indicates how these various methods could be used:

Concepts of Statistics Used in Veterinary Medicine

Statistical inference is the process whereby one draws conclusions regarding a population from the results observed in a sample taken from that population. There are two categories of statistical inference: estimation and hypothesis testing. Estimation is concerned with estimating the specific value of an unknown population parameter while hypothesis testing is concerned with making a decision about a hypothesized value of an unknown population parameter. In either case, we first need some background concerning something called the *standard error.*

TABLE 1. Population characteristics and sampling techniques appropriate for each population type

Population Characteristic	Population Type	Appropriate Sampling Technique
Population is generally a homogeneous mass of individual units.	Number of breeding bitches of a particular breed housed in a specific kennel from which random samples are selected for testing the presence or absence of a disease in the vaginal swab.	Simple Random
Population consists of definite strata, each of which is distinctly different, but the units within the stratum are as homogeneous as possible.	A particular bull breeding farm in which the total population consists of three breeds (strata), each with equal numbers of bulls. A sample is needed to evaluate the libido among bulls on the farm.	Simple Stratified
Population contains definite strata with differing characteristics. Each strata has a proportionate ratio in terms of number of members of every other strata.	A county in which the total dairy population consists of farms with three different size herds.	Proportional Stratified
Population consists of clusters whose characteristics are similar, yet whose unit characteristics are as heterogeneous as possible.	A survey of small animal wards in a teaching hospital to evaluate the presence or absence of antibiotic resistant bacterial spp. All wards are similar in atmosphere, purpose, design, etc. Yet the patients differ widely in individual characteristics: species, breed, sex, reason for hospitalization, and so forth.	Cluster Sampling

The standard deviation of the mean (standard error)

Suppose we defined a population to be all 100 dairy cows on a farm, and we took repeated random samples consisting of 20 cows from the herd and calculated the mean body weight of each sample. We would find that the estimated mean body weight of each 20 cow sample would vary around the true (unknown) population mean body weight of the whole herd. We would also note, that after consulting with a statistician, that these sample means follow a t distribution. A t distribution is like a standard normal distribution with slightly fatter tails and a lower center. The statistician would also tell us some other interesting facts about what we did.

First, if we had chosen a larger sample size, perhaps 30 cows, then the distribution of the sample means would be approximated by the standard normal distribution (Z). Secondly, our experiment was in fact demonstrating the principle of the Central Limit Theorem (CLT). The CLT states that whenever n is moderately large, the mean has approximately a normal distribution regardless of the distribution of the underlying variable. So even if the body weights of the 100 cows in the herd were not normally distributed, our sample means would be.

An estimate of the average variation or standard deviation of the sample means is called the standard error (SE). It is estimated as the sample standard deviation (S) divided by the square root of the number of observations in the sample (n):

$$SE = \frac{S}{\sqrt{n}}.$$

Example: the mean body weight of a sample of 20 dairy cows was 650 kg with a standard deviation of 40 kg. The standard error of the mean estimate is $40/\sqrt{20}$ = 40/4.472 = 8.94. Therefore the best estimate of the mean body weight of this dairy herd is 640 kg with a standard error of 8.94 kg.

As the number of observations in the sample increases the variability of the mean decreases i.e., the standard error gets smaller. The SE provides a measure of how far from the true population value the estimate is likely to be. Most often the estimate will be within one standard error of the mean and is unlikely to be more than 2 SE's away from it.

The standard error and standard deviation are commonly confused, which is understandable given that the standard error is the standard deviation of the sample means. The standard error is used to describe the *preciseness* of our estimate, while the standard deviation is used to describe the *variability* of the population or distribution.

The standard error of a proportion

Binomial or dichotomous data are often viewed in terms of proportions - for example, the proportion of individuals who have a particular condition in a given population. An estimate (p) of the true population proportion can be obtained simply by counting the number of events in a sample:

$$p = r/n$$

where r = the number of events
n = the number of observations in the sample

The standard error of this proportion is given by:

$$SE(\hat{p}) = \sqrt{\frac{pq}{n}}, \text{ where } q = 1 - p$$

For example, suppose we did not know the true first service conception rate in a particular dairy herd. We could estimate it by observing the number of pregnancies which result from breeding, the 20 recently freshened cows. Suppose 11 cows became pregnant. An estimate of the true herd first service conception rate (p) is therefore 11/20 = 0.55 (or 55%). The standard error of this estimate is

$$\sqrt{0.55 \times 0.45/20} = 0.11$$

Estimation

When we wish to estimate unknown population parameters such as the mean body weight of cows or the variance of the body weights of cows, we take a random sample of the population and calculate the sample mean ($\bar{\chi}$) and the sample variance (S^2). These estimates are called point estimates. They represent estimates of the true population parameters and, as such, have a certain degree of inherent variability associated with

them. After calculating a point estimate, we would like to know how good an approximation of the true population value this estimate is (i.e., what is the precision of the estimate?). A confidence interval (CI) is a way of quantifying the precision of the estimate. A CI consists of a lower and upper limit on either side of the point estimate. It is calculated using the following format:

$$\text{Point estimate of parameter} \pm \text{percentile of the distribution} \times \text{Standard error of the estimate}$$

Example: To calculate a 95% CI for our estimate of the body weight of a herd of cows, we first find that the appropriate percentile value of the t distribution [with 19 (n - 1) degrees of freedom] is 2.093. We know that the standard error of the mean is = 8.94. Thus the 95% CI for the mean body weight is 650 kg $\pm$ (2.093 x 8.94) = 650 kg $\pm$ 18.71 = 631.29, 668.71.

The interpretation of the CI is critical. This 95% CI means that in repeated sets of samples, 95% of such intervals would be expected to contain the true value of the population (herd) mean. So, if we were to repeat the sampling of the herd many times, there would be a 95% chance that the CI of 631.29 to 668.71 would include the true (unknown) value of the mean body weight of the herd. As we shall see below, calculation of CIs is also very useful when performing hypothesis testing.

The exact level of confidence is explicitly stated, for example, a 99% CI or a 95% CI. A 99% CI for this same estimate would be 650 kg $\pm$ (2.861 x 8.94) = 650 kg $\pm$ 25.58 = 624.42, 675.58 (the 99th percentile of the t distribution with 19 degrees of freedom is 2.861). This interval is wider that the 95% limit, as we would expect, since the mean is more likely to be included.

Hypothesis Testing - an example using the t test

Estimation using CIs and hypothesis testing are closely related. In estimating a CI, we use the sample data to estimate what we think is a likely set of values for the population parameter of interest. In hypothesis testing we use our sample data to test whether our estimated value for the parameter is different enough from a hypothesized value to conclude that a true difference exists. Hypothesis testing actually centers around rejecting or not rejecting the null hypothesis. The null hypothesis is a statement which you are trying to refute using your data. This is best explained by an example:

We are interested in determining whether BST (growth hormone) affects the body weight of adult dairy cows. We perform an experiment where we randomly assigned half our dairy herd to receive BST (growth hormone) and the other half to receive a placebo (e.g., saline).

To test the effect of BST we first formulate a null hypothesis that states that there is no difference in the mean body weight of the two groups of cows:

H_o = the mean body weights of the treatment and control groups do not differ.

If we reject this null hypothesis, then we accept our alternative hypothesis, which formally stated is:

H_a = the mean body1 weights of the treatment and control groups do differ.

Another important concept is the p value. The p value quantifies exactly how unusual the observed result from our experiment would be *if the null hypothesis were true*. The formal definition of a p value is:

The p value is the probability of obtaining a value of the test statistic at least as large as the one observed, *given that the null hypothesis is true.*

So, if the observed result is very unlikely, given that the null hypothesis is true, we would get a very small p value (e.g., a probability of 0.001) and we would reject the null hypothesis in favor of the alternative. In other words, if we find that the cows receiving BST gained a lot of weight, say an average of 50 kg, compared to the control (saline) group, our test would have a very small p value associated with it. This would say that if the mean body weights of the two groups of cows really did not differ, the probability of observing a difference of 50 kg is very unlikely. In this case, we would decide to reject the null hypothesis and conclude that the alternative hypothesis was correct i.e., that the mean body weights of the treated and control cows really do differ.

Prior to actually performing the test we need to define a descriptive level of significance or an alpha value which forms the decision rule for rejecting or not rejecting our null hypothesis. Defining a descriptive level of significance or alpha level is simply deciding how unlikely our result has to be before we decide to reject the null hypothesis. Frequently an alpha level of < 0.05 is chosen, although if one wanted to be very stringent a level of < 0.01 could be specified.

Let us calculate a hypothesis (or significance) test for our experiment. We first set an alpha level of < 0.05. The results obtained from the experiment were as follows:

Treated group: n= 50, mean body weight ($\overline{\chi}_T$) = 700, S_T = 38

Control group: n= 50, mean body weight ($\overline{\chi}_C$) = 650, S_C = 42

This particular two-sample significance test is performed using a T statistic:

$$T = \frac{(\overline{x}_T - \overline{x}_c) - 0}{Sp\sqrt{\frac{1}{n_T} + \frac{1}{n_c}}}$$

where S_p refers to the pooled standard deviation of the two groups - in this case equal to 40.

The denominator of the T statistic is in fact the pooled standard error of the mean difference between the treatment and control groups, which in this example equals 8.

$$T = \frac{(700 - 650)}{8} = 6.25$$

By referring this value to a t table with n - 2 degrees of freedom (= 98) we find that the p value associated with this result is < 0.001. Thus a difference of 50 kg between the two groups, if the null hypothesis was true, is so unlikely that we reject the null hypothesis in favor of the alternative.

If we were to calculate a 95% CI around the observed treatment difference of 50 kg, we would obtain values of 34.12 and 64.12. Because this interval does not contain 0, we can conclude with 95% confidence that there is a significant change in body weight with BST treatment. Thus, calculating a 95% CI is equivalent to performing the above significance test at an alpha level of 0.05.

Hypothesis Testing - an example using the chi-square test

Frequently we want to perform a hypothesis test on data which are either qualitative or binomial (i.e., a proportion). In this situation the chi-square test is an appropriate method of testing whether a relationship or association exists between two variables.

For example, in our BST experiment on the 100 dairy cows, we were also interested in knowing whether fertility was affected by use of the hormone. During the 12-month period following treatment with either BST hormone or the placebo, the following data were collected concerning the subsequent fertility of the 100 cows.

Table of Observed frequencies

		Pregnancy Status		
		Yes	**No**	**Total**
Treatment	BST	40	10	50
	Placebo	30	20	50
	Total	70	30	100

The null and alternative hypotheses are defined as:

H_o = there is no association between BST treatment and subsequent fertility.

H_a = there is an association of some type between BST treatment and subsequent fertility.

To perform the test, we find for each cell in the table the frequency that we would expect to occur, if the null hypothesis were true. We use the row and column totals (called the marginal totals) to do this. The first row of the table represents the 50 cows that received BST. The probability of a cow being in the first row is therefore one half. If there was no association between BST and fertility, we would expect each column of the table to have the same proportion of its members (i.e., 1/2) in the first row. So, we would expect 35 of the 70 cows in the first column to be in the first row (i.e., 70 x 50/100) and 15 of the 30 cows in the second column to be in the first row (i.e., 30 x 50/100). The general formula used to calculate expected frequencies is therefore:

$$\text{Expected frequency} = \frac{\text{Column total x Row total}}{\text{Grand total}}$$

Under the null hypothesis of no association, the following table of expected frequencies was produced:

Table of Expected frequencies

		Pregnancy Status		
		Yes	No	Total
Treatment	BST	35	15	50
	Placebo	35	15	50
	Total	70	30	100

We now compare the observed frequencies with the expected frequencies using the chi-square test. If the two variables are not associated, the observed and expected frequencies in each cell should be close. The chi-square statistic is:

$$x^2 = \sum \frac{(\text{observed frequency} - \text{expected frequency})^2}{\text{expected frequency}}$$

$$x^2 = \sum \frac{(O - E)}{E}$$

For our BST experiment:

$$x^2 = \frac{(40-35)^2}{35} + \frac{(10-15)^2}{15} + \frac{(30-35)^2}{35} + \frac{(20-15)^2}{15}$$

$$x^2 = 0.714 + 1.667 + 0.714 + 1.667$$

$$x^2 = 4.762$$

When the null hypothesis is true the test statistic is distributed as a chi-square with degrees of freedom equal to:

(number of rows - 1) x (number of columns - 1)

So, the chi-square test in this example has one degree of freedom. Referring the value of 4.762 with one degree of freedom to a chi-square table, we find the p value to be < 0.05 (the critical value for $p < 0.05$, for a 1 degree chi-square test is 3.84). We therefore reject the null hypothesis and conclude that there is an association between BST treatment and subsequent fertility. In this instance, BST was associated with better fertility.

Power and Error rates

The following table summarizes the decisions that result in hypothesis testing:

Outcomes of hypothesis testing

		Truth	
		Different	Not Different
Decision	Difference (Rejected H_0)	Power ($1-\alpha$)	Type II error (α)
	No Difference (Accepted H_0)	Type II error (α)	Confidence ($1-\alpha$)

If the true state of nature is that the null hypothesis is really true and the decision is made to accept it, then a correct decision has been made. However, if the null hypothesis was rejected, we have made a false positive decision by accepting the alternative

hypothesis. This is called a *type I error* and occurs with a probability of alpha (α). The probability of correctly accepting the null hypothesis as true is therefore 1-α.

If the alternative hypothesis is really true, then we can either make a correct decision by rejecting the null hypothesis or a wrong decision by failing to reject the null hypothesis - a false negative result. A false negative result is called a *type II error* and occurs with a probability of beta (ß). The probability of correctly accepting the alternative hypothesis is (1 - ß) which is commonly called the power of the test. This is a measure of how likely your experiment is to find a real difference in your data, if a real difference actually exists.

For a fixed sample size, a and ß are inversely related. If one guards against making a type I error by choosing a small a or p value, then ß will be correspondingly large and the power of the test will be reduced (the probability of making a type II error increases). Conversely, if ß is reduced to avoid making a type II error, a is increased so the risk of a type I error is greater.

Ideally in research design we would like both a and ß to be small. Usually alpha is set by convention e.g., a = < 0.05. The resulting power of a test (1 - ß) for a given a, is dependent on the number of observations in the experiment. Sample size calculations can be made to determine the number of observations required to achieve a given level of power (again, refer to your local statistician for help).

9. DESIGN OF SURVEYS AND SURVEILLANCE SYSTEMS

Although many of the principles are the same for surveys and surveillance systems we will consider them separately.

Surveys (cross-sectional studies)

Determination from livestock owners by questionnaire and/or laboratory testing of animals about prevalence of disease, management factors, owner knowledge and attitudes, and the relationship between characteristics of herds and disease occurrence.

For many national disease surveys such as NAHMS, two-stage sampling (herds, and then animals within herds) is used for selection of study subjects. In the first stage, a random sample of herds is selected and then in the second stage, a sample of animals within a herd is selected. This latter sample might be random or might focus only on "high-risk" animals

1. Objectives – need to be clearly specified
2. Data type - prevalence only, useful to evaluate relationship between fixed factors (e.g. breed and sex and disease outcome)
3. Reference population and unit of interest (animals or herds or both)
4. Sample selection and sample size – this issue will be covered in more detail at the end of this section
5. Relevant observations and measurements
   - will they be collected by farm visit, telephone interview, or mail questionnaire?
   - how sensitive and specific are the tests that will be used?
   - If a questionnaire is to be used have all design and implementation recommendations been followed?
6. Data management – how will data be entered, cross checked and errors minimized?

7. Statistical analysis
   - estimates of prevalence (herd and animal prevalence)
   - odds ratios for risk factor studies
8. Organizational aspects
   - logistical including manpower availability
   - training of personnel
   - sample submission and handling

Surveillance systems

Surveillance is an ongoing system that collects, analyzes, and interprets data on disease frequency and distribution in the population with the purpose of initiating control measures or further investigative action. Surveillance often is considered **active** or **passive** according to the data collected (see descriptions later in the text). Surveillance data are used both to determine the need for public health action and to assess the effectiveness of control programs.

Surveillance differs from monitoring because the latter does not imply application of any disease control measure. For disease monitoring, owner's names and addresses may remain confidential to preclude such action.

Surveillance systems for BSE require a clearly defined set of objectives, and a description of actions that will result from the data. A BSE surveillance system has the potential to answer some or all of the following questions:

- Is BSE present in cattle?
- If BSE occurs, what is its prevalence? Note that estimation of BSE prevalence requires estimation of the number of cases of BSE, confirmed by a reference laboratory, divided by the number of animal at risk in the population or risk group. Often, the denominator for this calculation will be the number tested rather than the population at risk.
- If BSE has been present for several years, is the prevalence decreasing, remaining static or increasing? In this context, temporal trends in the data might need to be interpreted in context of the time interval since implementation of BSE control measures
- How are BSE cases spatially distributed? Is there evidence of freedom in some regions of a country?

The following data and testing aspects need to be considered:

- Which tests will be used to generate the data and will all tests positive results from screening tests be confirmed?
- How will the data be entered and stored in computer systems?
- What methods of analysis will be used and how frequently will analyses be done?
- How complete and valid are collected data?
- What reports will be generated from the data - how often will they be done, to whom and how will they be distributed?

In addition, resources (direct costs) needed to run the system should be defined and allocated. This includes personnel considerations for sample collection, laboratory testing, data handling and analysis and evaluation. Legislative support is required to allow implementation and enforcement of the system.

The adequacy of a surveillance system can only be determined once its goals are clearly defined. For example, a surveillance program to detect all potential and true cases of BSE might have different components to a program whose goal is to only estimate prevalence or a program whose goal is to provide evidence in support of claims of BSE freedom. The cost structure will vary according the level of surveillance, whether **passive** (defined as the mandatory reporting and investigation of BSE clinical suspects) or **active** (defined as the targeted sampling of one of more risk groups), the diagnostic strategy that is used, and the resources (personnel etc) required to run the system.

Any surveillance system should be designed to meet certain quality control criteria

- Usefulness (contribution to control of BSE in cattle)
- Simplicity (structure and ease of operation)
- Flexibility (ability to adapt to changing needs of end-users)
- Acceptability (willingness of farmers and other participants to provide requested data)
- Sensitivity (probability of detecting a true BSE infected animal). This will vary depending on whether cows are clinical or preclinical.
- Positive predictive value (proportion of test positive results that are confirmed as BSE cases). This is primarily dependent on the specificity of the test and prevalence of infection
- Timeliness (time between detection of a case and notification of the case to those who take action)
- Representativeness (if the population is sampled, tested samples should be representative of the geographic distribution of the population at risk and any changes that occur over time)
- Stability (whether the system can collect, manage and provide data without failure and be operational when needed)
- Documentation (all components of the surveillance system such as sampling, sample processing, data recording, diagnostic methods etc should be available in written format)
- Cost effectiveness (cost per case detected)
- Practicality (considerations include feasibility)

An effective surveillance system requires a system of individual animal identification that provides traceability to the herd of origin, and a method of verification of identification. Sample size issues for BSE surveillance will briefly be considered in the context of proof of freedom from BSE in section 10 of this chapter.

10. SAMPLE SIZE DETERMINATION

"How many do I need?" is one of the most common questions asked of an epidemiologist. The required sample size depends on the purpose of the study. More often than not the investigator has not precisely determined what question is to be answered. It is essential that this be done before sample size calculations can be preformed.

There are 5 common situations requiring sample size calculation for veterinary field studies:

1. Calculation of the minimum sample size needed to detect disease or a condition in a given population, at a specified level of significance given a certain disease prevalence or level of infection.

2. Finding the minimum sample size required to estimate the population proportion having a characteristic of interest at a specified level of significance and within desired limits of error.
3. Finding the minimum sample size required to estimate the population mean of a characteristic of interest at a specific level of significance and within desired limits of error.
4. Finding the minimum sample size required to detect the difference between two population proportions that one regards as important to detect, at a stated level of significance and desired power.
5. Finding the minimum sample size required to detect the difference between two population means that one regards as important at a specified level of significance and desired power.

Importance of Sample Size Calculations:

1. Forces specification of outcomes.
2. Leads to a stated recruitment goal.
3. Encourages development of appropriate timetables and budgets.
4. Discourages the conduct of small, inconclusive trials.

Common Mistakes Related to Sample Size:

1. No discussion of sample size.
2. Unrealistic assumptions (e.g. disease incidence or prevalence).
3. Failure to explore sample size for a range of values.
4. Failure to state power for a completed study with negative results.
5. Failure to account for attrition by increasing the sample size above calculated size. The size of the sample is what you need to end up with not what you start out with!

Factors contributing to inadequately-sized studies:

1. Failure to document sample size at all.
2. Use of sample size of convenience.
3. Lack of adequate financial support.
4. "Publish or perish" mentality.
5. Lack of rigorous editorial policy of journal.

Where to go for help in calculating sample sizes

1. Computer software (e.g. Epi Info)
2. Tables in books (Cannon and Roe, 1982).

Sampling and sample size consideration for surveys

In two-stage sampling, where the goal is to estimate the proportion of infected herds, two sample size calculations are necessary.

First, one needs to calculate the number to sample in each herd to correctly classify the herd (e.g. diseased or not diseased). Once the herd status is determined then the proportion of diseased herds is counted. This leads to the second calculation of the number of herds that need to be examined to estimate prevalence (proportion of infected herd) with a specified level of confidence.

1. Sample size to detect disease

The basic formula for the calculation is given in the paper by DiGiacomo and Koepsell (JAVMA 1986;189:22-23) and is shown by the equation:

$$n = \frac{\log (1 - C)}{\log (1 - P)}$$ where C = confidence level, P = prevalence of infection

This calculation can be readily done with hand calculators – note that log is $\log_{10}$ not the natural logarithm (ln).

The formula above is appropriate for infinite populations (or very large populations >1000) but numbers can be adjusted downwards for smaller populations using the finite population correction factor.

Often we are also interested in estimating the numbers needed to detect positive reactors (apparent rather than true prevalence). The formula can be modified to include test characteristics. Positive reactions can come from infected or non-infected individuals and hence the proportion of test positives is estimated as P*Se + (1 - P)*(1 - Sp) from the standard 2 x 2 table. If this is substituted in the denominator of the original equation we obtain the general formulation:

$$n = \frac{\log (1 - C)}{\log (P*(1 - Se) + Sp*(1 - P))}$$

Special cases

a) If Se = 1 and Sp = 1 then

$$n = \frac{\log (1 - C)}{\log (1 - P)}$$

b) If Se < 1 and Sp = 1 then

$$n = \frac{\log (1 - C)}{\log (1 - P*Se)}$$

c) If Se = 1 and Sp < 1 then

$$n = \frac{\log (1 - C)}{\log (Sp - P*Sp)}$$

Example

Assuming P = 0.1 and C = 0.95, we can estimate the impact of test characteristics on the required numbers to detect reactors.

Se	Sp	n
1	1	29
0.9	0.9	15
0.5	1	58
1	0.5	4

Note that the test with poor specificity (0.5) will "help" detect reactors but application of the test would result in many reactors in non-infected herds. Moreover, the predic-

tive value of a positive test would be low for the individual reactors that were detected in infected herds. This provides more evidence of the importance of using tests of high specificity for aggregate level interpretation.

2. Sample size to estimate prevalence

Since we are estimating a proportion we can use the familiar formula for generating a (1-α)% confidence interval for a proportion:

$$N = \frac{Z^2 * P(1 - P)}{e^2}$$

where: P = prevalence e.g. 0.3
e = error margin on the estimate e.g. ± 0.1
Z = value from normal tables corresponding to the desired level of confidence e.g. 1.96 for 95% confidence

For the values above $N = (1.96)^2*0.3*0.7/(0.1)^2 = 80.64 \approx 81$

If it is difficult to make an a priori "guess" about prevalence, use the worst case scenario i.e. P = 0.5

Note to obtain an unbiased estimate of prevalence, the sampling of herds must be random. Animals within the herd do not need to be randomly selected if the goal is just to determine whether the herd is infected or non-infected (sampling to detect disease) and not to estimate the prevalence within the herd.

Determination of sample size in comparative trials

Probabilities:

There are two kinds of errors one must guard against in designing a comparative study:

1. Type I error (referred to as α): Declaring that the difference in proportions being studied is real when in fact there is no difference.
2. Type II error (referred to as β): Failing to declare the two proportions significantly different when in fact they are different.

The power of a test, also to be considered, is defined as the probability of finding a difference between two proportions when in fact they are different.

For example, consider the hypothesis: $H_o: P_1 = P_2$
$H_a: P_1 \neq P_2$

α = P (Reject H_o | H_o true)
β = P (Fail to reject H_o | H_a true)
1- β = P (Reject H_o | H_a true) = Power of the test

Comment: In order to control for a Type II error, the investigator must be able to specify just what difference is of sufficient biological importance to be detected.

Finding the Minimum Sample Size Required to:

1. Estimate the population proportion P having a characteristic of interest at a specified level of significance (α), and within desired limits of error (e).

Let $\hat{p}$ = sample estimate of P
e = desired limits of error

Formula:

$$n' = \frac{\hat{p}(1-\hat{p})(Z_{1-\alpha/2})^2}{e^2}$$

In n'/N > 10%, then $n = n'/\{1 + [(n'-1)/N]\}$
< 10%, then $n = n'$

Example: An investigator wishes to estimate the percentage of cats in Colorado that are infected with Cryptosporidia spp. From a small pilot study, it is suspected that approximately 10% of the cats in Colorado are infected. It is decided that a random sample of cats can be obtained. The investigator will be content if her sample estimate is within ±5% of the true population proportion P, at a level of significance of 0.05. How large a sample of cats needs to be examined?

We know: $\hat{p} = 0.10$; $(1-\hat{p}) = 0.90$; $e = 0.05$; $\alpha = 0.05$; $Z_{1-\alpha/2} = 1.96$

$$n' = \frac{(0.10)(0.90)(1.96)^2}{(0.05)^2} = 138.30 = 138 \text{ cats}$$

Suppose there are 50,000 cats in Colorado; then 138/50,000 = 0.002. Since 0.2% is less than 10%, 138 cats is our final answer.

2. Detect the difference between two population proportions:
For consistency, let P_1 = hypothesized proportion of nonexposed group or control group having the factor
let P_2 = hypothesized proportion of exposed or case group having the factor
Power = $1-\beta$ = P (Accept H_A | H_a true)
α = P (Reject H_o | H_o true)

Formula:

$$n' = \frac{\left[Z_{1-\alpha/2}\sqrt{2\bar{P}\bar{Q}} - Z_{1-\beta}\sqrt{P_1Q_1 + P_2Q_2}\right]^2}{(P_2-P_1)^2}$$

= required sample size from each of two populations being compared before the continuity correction is employed.

where $\bar{P} = (P_1 + P_2)/2$
$\bar{Q} = 1 - \bar{P}$
$Z_{1-\alpha/2}$ = two tailed critical normal value associated with the distribution of P_1 (positive value)
$Z_{1-\beta}$ = one tailed critical normal value associated with the distribution of P_2 under H_a (negative value)

$$n = \frac{n'\left(1+\sqrt{1+4}\right)^2}{4n'\left|P_2 - P_1\right|}$$

$n = n' + \frac{2}{|P_2-P_1|}$ is a good approximation when $n'|P_2-P_1|>4$.

Example: an investigator wants to determine if the mortality rate in calves raised by farmer's wives differs from the mortality rate in calves raised by hired managers. He/she hypothesizes a calf mortality rate of:

$P_1 = 0.25$ for calves raised by farmer's wife
$P_2 = 0.40$ for calves raised by hired managers

The level of significance, α, is stated to be 0.01, and the desired power of the test is 0.95. How many calves should be included in the study?

H_o: $P_1 = P_2$
H_a: $P_1 \neq P_2$

$\alpha = 0.01$; $Z_{1-\alpha/2} = 2.576$; $1-\beta = 0.95$; $Z_{1-\beta} = -1.645$

$\overline{P} = (0.25+0.40)/2 = 0.325$
$\overline{Q} = (1-0.325) = 0.675$

$$n' = \frac{\left[2.576\sqrt{2(0.325)(0.675)} - (-1.645)\sqrt{(0.25)(0.75)+(0.4)(0.6)}\right]^2}{(0.40-0.25)^2} = 344$$

and

$$n = n' + \frac{2}{\left|P_2 - P_1\right|} = 344 + \frac{2}{0.15} = 357$$

The minimum required number of calves to be raised in each group to carry out this study at the stated level of significance and desired power is 357 calves per group.

Example: The case-fatality rate among cancer patients undergoing standard therapy is 0.90, and is 0.70 for cancer patients receiving a new treatment. Find the required sample size to test a hypothesis that the case-fatality rate differed between groups at the stated level of significance, $\alpha = 0.05$, and desired power of the test, 0.90.

For consistency, by using survival rates rather than case-fatality rates, P_2 will be larger than P_1.

$P_1 = 0.10$ = survival rate of cancer patients with standard treatment
$P_2 = 0.30$ = survival rate of cancer patients with new treatment
$\overline{P} = (0.10 + 0.30)/2 = 0.20$
$\overline{Q} = (1 - 0.20) = 0.80$
$Z_{1-\alpha/2} = 1.96$
$Z_{1-\beta} = -1.282$

$$n' = \frac{\left[1.96\sqrt{2(0.20)(0.80)} - (-1.282)\sqrt{(0.1)(0.9)+(0.3)(0.7)}\right]^2}{(0.30-0.1)^2} = 82$$

and

$$n' = 82 + \frac{2}{|0.3-0.1|} = 92 \text{ (patients/group)}$$

Calculating the Power of a Test with Given Sample Sizes:

Suppose you are limited to 20 patients in each group by cost considerations. With what power would you be working at?

Formula:

$$Z_{1-\beta} = \frac{Z_{1-\alpha/2}\sqrt{2\bar{P}\bar{Q}} - |P_2 - P_1|\sqrt{n - \frac{2}{P_2 - P_1}}}{\sqrt{P_1Q_1 + P_2Q_2}}$$

$$Z_{1-\beta} = \frac{\{1.96\sqrt{2}(.2)(.8) - (0.2)\sqrt{20-2}/(0.3-0.1)\}}{\sqrt{(.1)(.9) + (.3)(.7)}} = 0.8695$$

2. Formula for Unequal Sample Sizes:

$$m' = \frac{\left(Z_{1-\alpha/2}\sqrt{(r+1)\bar{P}\bar{Q}} - Z_{1-\beta}\sqrt{rP_1Q_1 + P_2Q_2}\right)^2}{r(P_2 - P_1)^2}$$

$$m = \frac{m'\left[1+\sqrt{1+2(r+1)}\right]^2}{4m'r|P_2 - P_1|}$$

or,

$$m = m' + \frac{r+1}{r|P_2 - P_1|}$$

where m = required sample size from first population
rm = required sample size from second population
$\bar{P} = (P_1 + rP_2)/(r+1)$
$\bar{Q} = (1-\bar{P})$
r is the ratio between the 2 samples and it is specified in advance

Determination of sample size requirements in cohort and case-control studies of disease based on the relative risk of disease that one regards as important to detect:

1. Cohort Study

The investigator needs to specify:

a) A hypothesized or known incidence of disease among the nonexposed, P_1.
b) The relative risk of disease, R, which one regards as important to detect.
c) The level of significance, α.
d) The desired power of the study, 1-β.

Formula: (Equivalent to the previous formula, with $R = P_2/P_1$).

$$n' = \frac{\left[Z_{1-\alpha/2}\sqrt{2P^{-}Q^{-}} - Z_{1-\beta}\sqrt{P_1\{1+R-P_1(1+R^2)\}}\right]^2}{\left[P_1(1-R)\right]^2}$$

where $P^{-} = P_1 (1+R)/2$; $Q^{-} = 1 - P^{-}$

$$n = \frac{n'\left(1+\sqrt{1+4}\right)}{4n'\left|P_1(R-1)\right|}$$

2. Case-Control Study

The investigator needs to specify:

a) The prevalence of exposure to the factor in the control group, f.
b) The relative risk of disease, R, which one regards as important to detect.
c) The level of significance, α.
d) The desired power of the study, 1-β.

Formula:

$$n' = \frac{\left(Z_{1-\alpha/2}\sqrt{2u(1-u)} - Z_{1-\beta}\sqrt{f(1-f)+P_3Q_3}\right)^2}{(f-P_3)^2}$$

where $u = (0.5)\, f(1+R/[1+f(4-1)])$, and
$P_3 = f\, R/[1+f(r-1)]$ = prevalence of exposure to factor in disease group.

Detecting the difference between 2 population means:

Example: From the results of a pilot study an investigator assumes that the gizzard weights of a certain strain of turkeys are normally distributed with mean μ=30 grams and a variance σ^2 = 23 grams. A study is being conducted to examine the effect of a new feed formula on gizzard weight. It is hypothesized that due to the new feed formula, treated turkeys have gizzard weights greater than 30 grams on the average. We wish to test the following null hypothesis at a 5% level of significance.

H_o: μ_0 = 30 grams
H_a: μ_1 > 30 grams

The investigator must choose the difference which is biologically important to detect. Suppose this difference is thought to be 2 grams (i.e. how many turkeys need to be chosen for the experimental and control groups in the feed trial in order to have a "high probability" of detecting a 2 gram difference in gizzard weights?)

H_o: μ_0 = 30 grams
H_a: μ_1 = 32 grams

$\alpha = 0.05$
$1-\beta = 0.90$ = Desired power of test
Assume $\sigma_0^2 = \sigma_1^2 = \sigma^2$

$$n = 2\ \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2\ \sigma^2}{(\mu_0 - \mu_1)^2}$$ Two tailed test (will give larger sample size - is conservative)

$$n = 2\ \frac{(Z_{1-\alpha} + Z_{1-\beta})^2\ \sigma^2}{(\mu_0 - \mu_1)^2}$$ One tailed test

For our example, choose one tailed test as most appropriate to test given hypothesis.

$$n = 2\ \frac{(1.645 + 1.282)^2\ (23)}{(30-32)^2} = 100$$

The required number of turkeys needed to have a high probability of detecting the hypothesized 2 gram difference in gizzard weights is:

100 turkeys on regular feed formula
100 turkeys on new feed formula
200 total number of turkeys needed

11. USING EPIDEMIOLOGICAL TOOLS IN ANIMAL HEALTH PROGRAMS

Definition: A factor X "causes" Y if a change imposed directly on X results in a change in Y. For many reasons, this is difficult to "prove" (except perhaps in a clinical/field trial), hence we make judgements based on 4 pieces of information:

1. the chance that the observed association occurred just because of random variation (the P value);
2. the possibility that the so-called cause and effect are related intrinsically in some non-causal fashion ("night and day go together");
3. the chance that there was bias [systematic or not random error] in the study design; and
4. the "Surgeon General's Criteria".

Lets consider these 4 aspects in more detail.

1. Random variation

Examples a) Biologic variation within and among animals
b) Imprecision in measuring devices/methods

Techniques to improving precision (reliability)

a) Selecting better measuring devices and standardization of the measurement methods
b) Sampling subset of population only
c) Repeating measurements - use mean of 2 or more measurements on a single animal/sample
d) Increasing sample sizes for estimation of mean response

Evaluation of random variation

a) Hypothesis testing - relate the observed difference between groups to the predicted or expected variation.
   Null hypothesis (H_o) vs alternate hypothesis (H_a)
   "P-value" is the probability (P for "probability") that there could be "this much or more observed relative difference" if the H_o were true. The smaller the P value, the less likely it is that the observed relative difference is just due to random variation.

b) Errors in making decisions (complete the table - possibilities are power, type II error, type I error, and confidence).

		Truth	
		Different (H_o false)	**Not Different (H_o true)**
Decision	Difference (Rejected H_o)		
	No Difference (Accepted H_o)		

2. Intrinsic non-causal relationships

Certain things just go together!

Examples a) Suntan lotion and drownings
b) Shaving under arms and breast cancer

3. Bias

"Bias" is a **systematic** error in the data. It is not a matter of random variation or imprecision. Bias is caused by flaws in the study design (sample selection, measurement, and failure to account for confounding). The term "validity" means lack of bias.

Concept of target shooting - difference between validity (lack of bias) and precision (lack of random error)

	Validity	
	High	Low
High	•	•
Precision (reliability)		
Low	•	•

The only thing worse than a small amount of bad (biased) data is a large amount of bad data - why is this true?

Impact of biases

1. Make a factor seem important when it is not
2. Make a factor seem unimportant when it really is
3. Under or overestimate the true incidence/prevalence

Three main categories of bias with examples

a) Selection bias . This bias is associated with sample selection or allocation
b) Information bias. This bias occurs during data gathering or measurement and is attributable to the imperfect sensitivity and specificity of the test that is used
c) Confounding bias . This bias is due to failure to account for a 3rd unknown variable in the design or analysis. To be a confounder, a variable must be a risk factor for disease, associated with the exposure of interest, and not on the causal pathway

Some strategies to reduce bias

1. Random selection or allocation
2. Standardized, clearly defined criteria
3. Accurate diagnostic tests
4. Blinding / masking
5. Objective vs subjective criteria
6. Statistical methods – stratified analysis or multivariable analysis to adjust for confounding

Adequate planning in the design phase of the study is most important. In most cases, adjustment for biases other than confounding after the study has been completed is difficult!

4. The Surgeon General's Criteria

Koch's postulates - developed for highly virulent infectious agents (agent both necessary and sufficient). Did not consider the influence of environmental and management factors nor were they applicable to non-infectious disease. The Surgeon General (or the USA) put together newer criteria on which to base decisions about disease causation.

These criteria which we will consider in some detail are:

a) Time sequence
b) Strength of association
c) Dose-response relationship
d) Consistency of findings
e) Biologic plausibility
f) Specificity
g) Analogy

Let's consider these ideas one by one:

a) Time sequence

A cause must always occur before its effect. Choice of study design influences the ability to determine this sequence.

List some reasons why it might be difficult to establish the temporal sequence in some studies:

b) Strength of Association

The larger the value, the more likely a factor is on average to be causal. The converse is not necessarily true, however.

Strength of association usually is measured by a statistic such as a correlation coefficient, a relative risk, odds ratio, or an attributable risk. Strength is **NOT** measured by the size of the P value (as long as the statistic is significant).

RR: The relative risk (also called "risk ratio") is the ratio of the incidence (IR) in the exposed group to the incidence in the unexposed group.

		Disease/outcome +	Disease/outcome -	
Risk factor	+	a	b	a+b
	-	c	d	c+d

IR exp = a / (a +b) IR non-exp = c / (c+d)
RR = IR exp / IR non-exp = a / (a+b) ÷ c / (c+d)

Example
Suppose that as part of evaluation of the risk of acquiring pseudorabies (PRV) infection in swine herds in an area, 147 herds (73 confinement and 74 non-confinement) that were initially PRV negative were followed over 3 years for the occurrence of infection (herd classified as positive or negative). Infection was determined by routine serologic testing and clinical evaluation of pigs in the herd. The following data were obtained:

		Infection +	Infection -	
Confinement	+	12	61	73
	-	1	73	74

Incidence in confinement group = = %
Incidence in non-confinement group = = %
Relative risk (RR) =
Specifically, what does this RR value mean?
What would the following RRs mean?
RR = l
RR = 2
RR = 0.33

Evaluation of significance of the RR
1. Statistical test - usually a chi-square test to test whether the calculated value (12.2) in this example differs significantly from 1

Steps in a chi-square test
a. Hypotheses: Null (H_o): RR = 1
Alternate (H_a): RR ≠ 1
b. Assumptions: Independence, random sampling
c. Select level of significance for test e.g. P = 0.05
d. Calculate χ^2 statistic
i) Calculate expected values for each cell

cell	obs	exp	obs-exp	$\frac{(obs-exp)^2}{exp}$
a	12	6.5	5.5	4.65
b	61	66.5	-5.5	0.45
c	1	6.5	-5.5	4.65
d	73	67.5	5.5	0.45
ii) Sum 4 values in RHS column			=	10.2

e. Compare test statistic with tabulated significance values.
   Note for a 2 x 2 table the no. of degrees of freedom (df) is 1.
   In general, df = (rows-1)*(columns-1)
f. Apply decision rule: If the test statistic is greater than the tabulated significance value (3.84 for χ^2 with 1df, P=0.05), then reject the null hypothesis
   Here χ^2 = 10.2 > 3.84, reject H_o and conclude that RR differs significantly from 1

Warning! If the data are dependent (matching used, or before and after measurements on the same individual), then a special form of the chi-square test (McNemars χ^2) must be used.

2. Confidence interval - an interval excluding 1 indicates statistical significance at the specified level of confidence. These can be calculated in programs such as Epi Info

Rule of thumb: A relative risk of say 4 and above, will not usually be completely explained by biases in the study - but the association might still be non-causal!

AR: Attributable risk (also called "risk difference") is the absolute difference between the 2 incidences from a 2 x 2 table:

The AR tells you the incidence of disease that is **attributable** to the exposure - in theory, it is the incidence of disease that could be removed/prevented if the exposure was removed completely from the study group. (If you get a negative AR, the AR is telling you the rate of disease that **was prevented** by the exposure.) The AR has the same units as the IR and can theoretically vary from -1 to +1; the null value is zero.

Example: For the example of confinement and PRV risk, we calculated:
Incidence in confinement group = 16.4 %
Incidence in non-confinement group = 1.4 %
Attributable risk =

Interpretation. The incidence% that can be attributed to the factor of confinement is _____ %. Note that this statement implies a causal relationship and an unbiased estimate of the effect of the factor on PRV risk.

Caution!! There are several variations on the "pure" attributable risk .. be careful when reading the literature. A common variation is the **etiologic fraction among the exposed** (AR_{exp}%) which expresses the AR as a fraction of the incidence among the exposed - in theory, this measure indicates the proportion of disease in the exposed that could have been prevented had exposure not occurred.

For the PRV example, AR_{exp}% = 15/16.4 = 91.5%.

In theory, 91.5% of the incidence of PRV in the confinement herds could have been prevented if they were non-confinement herds.

OR: Odds Ratio: One measure that is used in epidemiologic studies of all types (cohort, case-control, cross-sectional) is the odds ratio. As the name implies, this is a ratio of the **odds** of exposure:non-exposure in disease-specific groups or the ratio of the odds of disease:no disease in exposure-specific groups.

Using the same notation for the cells of the 2 x 2 table, as we used for the relative risk we get:

Odds of disease in exposed group = a/b
Odds of disease in non-exposed group = c/d
Odds Ratio = a/b ÷ c/d = ad/bc

Example: Confinement and PRV risk
Odds of PRV in confinement group =
Odds of PRV in non-confinement group =
Odds ratio =

Interpretation. The odds of PRV was ____ times greater for confinement herds than for non-confinement herds.

Evaluation of significance of the OR

1. Statistical test - usually a chi-square test
2. Confidence interval - an interval excluding 1 indicates statistical significance at the specified level of confidence.

Units and range of OR and RR values

1. RR and OR have no units, they are numbers.
2. Range of values 0 to infinity, null value (no association) = 1

When do RR and OR have similar values?

1. If there is no disease in the exposed group (a=0) and all other values are > 0, then OR = RR = 0.
2. When the disease is rare (say <10% incidence or prevalence)

c) Dose-response relationship

A demonstrable dose-response relationship (linear or curvilinear) between the risk factor is (like strength of association) strong evidence for causation if present, but only indeterminate evidence if absent. For the factor, confinement status of the herd, it might be difficult to do this although it may be possible to have an intermediate category of partial confinement between total confinement and no confinement.

Example:	Confinement	PRV incidence%
	Total	16.4
	Partial	3.4
	Nil	1.4

List some circumstances where it might be difficult (or impossible!) to show a dose-response relationship between a factor and a disease.

d) Consistency of the association upon replication

Have several studies found a relationship between confinement and PRV risk - this could of course be the first study of this relationship.

e) Biologic plausibility

Is there an underlying mechanism that makes biologic sense?

f) Specificity of the association

Specificity refers to the extent of "1- to -1" correspondence between the cause and the effect. **Perfect** specificity would imply that the risk factor has no effect other than the one being studied.

Also, perfect specificity would imply that the risk factor was both a **NECESSARY CAUSE** (the disease can't happen without the risk factor) and a **SUFFICIENT CAUSE** (the disease always occurs if the risk factor is present).

g) Analogy

It's easier to believe in the causal nature of an association if the situation is analogous to another one that we already know to be causal.

12. PROVING DISEASE FREEDOM

Although the term "freedom from disease" is commonly used, the term really means freedom from a specified pathogen rather than freedom from clinical disease. However, for simplicity we will use the term "freedom from disease" for today's discussion

Proof of freedom from disease theoretically requires that all animals in a population (single herd, state, region, or country) are tested with a perfectly sensitive test and no infection is detected. Testing all animals is impossible in most situations, and hence, surveys of a sample of herds and animals within each herd are done.

Surveys to demonstrate freedom from disease (or in the case of an infectious agent, freedom from the specific pathogen) are examples of hypothesis testing studies.

The factors, which need to be considered when calculating sample size for a survey to provide evidence of freedom are:

1. Confidence level (1-α)
2. Power (1-β)
3. Test performance (sensitivity and specificity)
4. Population size
5. Minimum detectable prevalence (given that infection is present).

No survey is able to guarantee that a population is free from disease. If a sample is used, it is always possible that a very small number (or even a single) diseased animal exists in the population and was not selected in the sample. Even if the entire population were tested, imperfect sensitivity means that any truly positive animal may have given a negative test result. It is easier to demonstrate freedom from highly contagious diseases with overt clinical signs e.g. foot and mouth diseases than it is for chronic infectious diseases such as Johne's disease which often occur at low within-herd prevalence.

This survey approach therefore does not attempt to prove absolute freedom. Instead,

the survey determines the likelihood (alpha or 1 – alpha) given random sampling that at least one diseased individual is included in a sample of size n if the study population prevalence exceeds a predetermined threshold prevalence.

If the probability is small, we can be confident that the disease, if present in the study population, has a prevalence less than that specified to calculate the sample size. Depending on the nature of the disease and the selected threshold prevalence, this may be widely accepted as proof of freedom. For instance, it is extremely unlikely that a highly contagious disease would have a very low prevalence in a naïve population. In other cases, disease may be present at low prevalence, but it is either impractical to detect it, or economically or biologically unimportant at those levels to warrant the effort to determine its "true" prevalence.

Additional evidence to support a conclusion of disease freedom can be often be obtained by using laboratory diagnostic data where the samples are obtained by passive surveillance.

The FreeCalc program in Survey Toolbox (http://www.ausvet.com.au/surveillance) can perform the calculation of sample size, and the formula used is described in Cameron and Baldock (1998).

Sample size for assessing BSE freedom based on surveillance data

Surveys for BSE in healthy adult cattle would be a waste of resources because of the disease's very low prevalence and lack of suitable ante mortem tests. Hence, inferences about BSE will be based on the type of cattle sampled (risk groups) and the number sampled by risk group.

Let's consider what has happened with recent active surveillance for BSE in the United States. In each of the last 2 years, 20,000 high-risk cattle (not further defined by risk category or geographic location) were tested with BSE with negative results. What inferences can be made about BSE?

Clearly the best guess of prevalence is zero but the numbers are limited given the low prevalence that is likely to present should BSE infectivity be cycling in the U.S. So one might ask, what is the upper 95% confidence limit for this estimate? It can be shown mathematically, that this is well approximated by the value 3/n (where n is the number of adult high risk cattle that are tested)

If we consider the 40,000 cattle as a single tested group for simplicity, the upper 95% CI is 3/40,000 or 75 per million adult cattle in the high risk population. If the proportion of high risk cattle in the total US cattle population were known, then this could be expressed as a rate per million adult cattle.

The current proposed recommendation is to test about 200,000 high-risk cattle over 12 to 18 months (about a 10-fold increase in testing frequency). We will consider the statistical basis for this decision and consider why USDA is proposing to test some healthy cattle as well.

Recommended sample sizes for BSE detection (probability of finding at least 1 positive) according to the likely prevalence (P) and confidence (C)level (from Scientific Steering Committee, European Commission, Nov 2001) – calculation based on n= log (1-C) / log (1-P)

Prevalence	90% confidence	95% confidence	99% confidence
1 /10 million	23 million	30 million	46 million
1 / 1 million	2.3 million	3 million	4.6 million
1 /100,000	230,000	300,000	460,000
1 / 50,000	115,000	150,000	230,000
1 / 10,000	23,000	30,000	46,000
1 / 5,000	15,000	15,000	23,000
1 / 1,000	2,300	3,000	4,600

13. REFERENCES

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