Proceedings

International Conference: TSE in Animal Populations – Fact and Fiction

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Fort Collins, Colorado USA

Conclusion/Abstract

This conference was the second in a series of scientific communication programs on Transmissible Spongiform Encephalopathy (TSE) that were initiated by TAFS, whose purpose is to establish a line of communication between scientists, policy makers, regulators, and the public on topics related to TSEs in animal populations. The conference focused on the similarities and differences of scrapie with Bovine Spongiform Encephalopathy (BSE) and Chronic Wasting Disease (CWD). Three topics were addressed during this conference. Below is a short summary of the presentations and the discussions.

Pathogenesis

There is a larger knowledge base for scrapie and for BSE than for CWD. While BSE shares some characteristics with scrapie, both CWD and scrapie have more in common with each other than either disease has with BSE. The differences are well-recognized and can assist with differentiation of diseases.

BSE has a limited route of transmission mainly via feed. Vertical transmission of BSE has been investigated in experimental studies and appears not to be significant in the maintenance of an epidemic under natural conditions. There is no strong evidence for genetic involvement. Pathogenicity and virulence of BSE is consistent and BSE has limited tissue involvement outside the central nervous system. BSE tests for live animals are unlikely to become available in the near future.

Several cervid species, both farmed and wild, have been identified as natural hosts for CWD. CWD appears to spread via several horizontal routes but the significance of vertical transmission is unclear. Genetic involvement for CWD is also uncertain. Pathogenicity and virulence of CWD is undetermined. Like

scrapie, CWD involves multiple tissues. For both diseases, live animal testing is possible via tissue biopsy and there is potential for development of screening tests in live animals.

There is a commonality in the pathogenicity between scrapie, BSE and CWD. Advances in understanding and control will be most promising if the established knowledge of commonalities and differences of all TSEs are applied.

Investigations are completed or are underway regarding the potential for infection to cross species, tissue location and infectivity. There is, however, still a need to conduct studies to observe the natural exposure of animals to these agents and there are a number of questions that have not been answered.

Diagnosis and Epidemiology

Easy and reliable screening tests for use in live animals are needed for all TSE diseases. While techniques based on tissue biopsy have been developed for testing live sheep for scrapie and some cervid species for CWD, the BSE status of a bovine animal can only be ascertained after death. For CWD, there is limited validation of rapid tests and uncertainty in interpreting the test results depending upon the stage of disease. These tests must be used with the appropriate tissue to be valid. Due to the nature of prion diseases there is an extreme complexity for diagnosis and confirmation of TSE disease with current tests.

When a surveillance program is developed, it should be targeted to the appropriate "high risk" population. A surveillance plan that is properly structured to address the distribution of high risk animals will provide more information on the status of the entire population than a plan based on sampling at random from the population. In order to conduct a targeted surveillance program, there must be some knowledge of risk of contracting these diseases. Current surveillance programs are limited to the varied sampling protocols used by individual countries, states or provinces. At this time, it is difficult to determine disease freedom from BSE or CWD. Status is only known when a positive case is found. Increasing the sample size does not ensure a representative sample for BSE/CWD.

There is a need to expand the knowledge about TSEs in a natural setting. Information from the United Kingdom has provided the basis for most current information on BSE epidemiology and ecology and this has generally been well supported by the success of intervention strategies. Information gathered from Colorado and Wyoming, on captive cervids and wildlife observations, is the basis for current knowledge on CWD. Captive cervid herds have provided limited information due to intervention strategies.

Management and Regulations

For BSE there has been interaction between research findings and implementation of policy. There is a difference between the EU and OIE approaches to the classification of country status and the risk analysis process. BSE regulations have had a positive impact on other livestock health programs.

For CWD, as for many animal diseases, there are many differences in the management of disease in captive and free-ranging populations. Multiple agencies are involved and all have different priorities and agendas. Diagnostic assays and surveillance systems for captive herds have been standardized and the United States Department of Agriculture has developed a nation-wide plan to deal with CWD in wildlife.

Risk management is an essential component in dealing with these diseases. Particular emphasis should be on explicit communication in order to build public confidence. Policy makers could learn from early BSE experiences in handling other TSE diseases.

The conference concluded that some fictitious ideas become factual "statements" due to the lack of scientific assessment. These statements, then, need to be evaluated with scientific evidence before they are conveyed as facts. Public perception and public health issues dominate the priority of dealing with such diseases. Therefore, there is a need to assess the validity of approaches in terms of practicality and their economical values. The risk analysis process is an essential component in assessing preventive and eradication measures. Trust and transparency are essential components for a successful effort to handle these diseases. Standardization of protocols for sampling and surveillance systems requires a team effort and thorough planning. It is necessary to conduct further research with an open-minded attitude.

Purpose and format

The purpose of this conference was to establish a line of communication between scientists, policy makers, regulators, and the public on topics related to BSE and CWD in animal populations in order to improve future potential preventive measures. Scrapie in sheep was used as a base line disease for contrast and comparison. The potential zoonotic aspects will be included in these topics. This would require an understanding of current scientific findings and their implication in preventive measures as well as an understanding of the future impact of these diseases on the food chain supplies. Thus, scientists, policy makers, veterinary and public health regulators, wild life biologists, public, livestock producers, the food industry, and food suppliers were part of this dialogue and communication.

The conference was a combination of scientific and professional presentations in sessions in which a previously prepared set of questions was addressed. Participants were from both the resource personnel and the user contingent.

The two day conference was divided into three panels to answer the specific questions that were submitted from the participants during the sessions. Major emphases were on BSE and CWD. However, scrapie was used as a baseline for comparison and contrasts. Each panel was asked to address the facts and myths of a specific topic for each of these two diseases.

The three panels applied the following topics:

- Pathogenesis
- Diagnosis and epidemiology
- Prevention and regulations

The conference was sponsored by the Canadian Food Inspection Agency, US Department of Agriculture (USDA:APHIS), the International Office of Animal Health (OIE), and International Forum for TSE and Food Safety (TAFS). The Animal Population Health Institute (APHI) of Colorado State University - College of Veterinary Medicine and Biomedical Sciences hosted this event. Experts from around the world presented perspectives on the diagnosis, transmission and epidemiology of the TSE diseases as well as regulations and issues of public perception. Approximately 180 individuals from 23 countries participated in this conference.

Presentations

Dr. Mo Salman, APHI, Colorado State University, introduced the speakers and set the stage for the conference's objectives.

Dr. Ron DeHaven, Deputy Administrator, USDA:APHIS, Veterinary Services, then welcomed the participants in this conference. He spoke about the reality that the TSEs have generated widespread attention at all national and international levels. He emphasized however, that there is still too little exchange of information and, at times, some of the historical records of countries have been overlooked, e.g. the case of BSE in Canada did not consider the other aspects of risk relevance. This conference is meant to dispel myths and highlight the science that is so needed. The TSEs are often lumped indiscriminately in spite of their distinct and unique differences. Dr. DeHaven spoke of the need to better understand the challenges of TSEs and open dialogue and perspective as we examine the policies in our collective efforts at prevention and control.

Dr. Alejandro Thiermann, USDA:APHIS and the Chairman of the Code Commission of OIE inferred that there is a lot of confusion in trade related issues as the TSEs are considered in one context. The challenge is how to re-phrase the chapters in OIE to minimize the political and public reaction. The exercise should be in risk communication and how to put it in the proper context of risk.

Pathogenesis

Dr. Linda Detwiler, a food safety veterinary consultant for USDA:APHIS, was the moderator for this session. The transmission and pathogenesis of BSE was presented by Dr. Danny Matthews, TSE Program Manager, Veterinary Laboratories Agency (VLA), Weybridge, United Kingdom.

When requested to compare the transmission and pathogenesis of BSE with that of scrapie, Dr. Matthews considered it necessary to also present some data on clinical signs. This is particularly true in countries where the diseases are rare, or have not been confirmed. The usual written formats for transmitting such information, possibly coupled with still photographs, rarely ensure true understanding of the signs to be detected, however complex or simple. Video footage highlights the importance of such a medium as an alternative to first hand experience. As the OIE reference laboratory, the VLA will be placing video clips of BSE on its website in early 2004. The address is www.defra.gov.uk/corporate/vla

When BSE was first recognized, and its epidemiology investigated, early evidence suggested that most cases were infected via consumption of contaminated feed. The inclusion of ruminant protein (mammalian meat-and-

bone meal) in calf and cattle feed ensured the recycling of infectivity that was present in the intestine and CNS of healthy infected cattle slaughtered for human consumption. In the absence of a species barrier it is clear that cattle to cattle transmission via feed was the primary route of transmission, irrespective of the origin of the epidemic.

It was not possible to detect infected cattle and thereby put in place measures to prevent horizontal transmission. The only measure taken was to ensure that down-calving suspects were required to calve in isolation. After isolation for 72 hours the isolation box was decontaminated and the bedding incinerated or burned. This action was taken on the assumption that, as with scrapie, calving presented a risk of environmental contamination and consequential horizontal transmission.

In the absence of evidence that BSE transmitted from dam to calf, no specific action was taken with regard to calves of BSE-affected dams. A research project was started to investigate whether or not calves born to BSE-affected dams were at greater risk of contracting BSE than were the calves of healthy mothers. In 1997 the outcome of that study indicated that calves of BSE-affected dams were at greater risk than the controls, but this risk, estimated to be approximately 10% greater, could not be confirmed due to direct transmission from dam to calf. A genetic component, determining greater susceptibility to infection, could not be ruled out as a component of this risk.

By 2003, controls have been introduced to close routes of feed-borne transmission in the United Kingdom. There is still no evidence of horizontal transmission, even in herds that experienced high incidence of disease at the peak of the BSE epidemic. Additionally, the evidence for a greater risk as a result of maternal transmission is now estimated to be no greater than 0.5%. Clearly, if maternal transmission actually occurs, it cannot sustain the epidemic.

Meanwhile, for scrapie, horizontal and maternal transmission were accepted facts at the beginning of the BSE epidemic, although further research is in progress to determine the mechanisms by which transmission occurs. Feed-borne transmission was rarely considered to be involved historically, but it is clear that sheep were potentially exposed to scrapie via feed in a similar manner to cattle with BSE. There has been no evidence of a feed related epidemic in parallel to that of BSE in cattle. Mammalian meat-and-bone meal was incorporated only rarely into sheep rations, and the provision of supplementary feed to sheep was, and is, an occasional event rather than the routine that exists with cattle.

With respect to feed-borne transmission of BSE to cattle it is still essential that all countries remain vigilant. Oral transmission experiments have already demonstrated that 1g of BSE-infected brain tissue can transmit BSE to a calf. A

follow-up study involves exposure to doses as low as 0.001g, and transmission has already been shown to 3/15 calves with 0.1g, and 1/15 with 0.01g. Incubation periods are still well within the range seen in natural cases (<65 months). These results highlight the difficulty of preventing low dose cross-contamination of feed or feed ingredients in transit within or between countries, or within feed compounding plants.

Turning to the pathogenesis of BSE, experimental challenge of calves and subsequent assay for infectivity in their tissues when sequentially slaughtered has added to the list of tissues found to be infected in naturally infected cattle. That short list, of brain, spinal cord and retina, has now been extended by the recognition of infectivity in the distal ileum of cattle, from the early months post-exposure. No infectivity was then detectable in other tissues until shortly before the first detection of clinical signs. At that point, 32 months pi in the study, CNS pathology and infectivity were detectable. Dorsal root gangia and trigeminal ganglia were also infectious, and at one time point during the clinical phase bone marrow was also found to be positive. How infectivity migrates from distal ileum to CNS has yet to be determined.

A subsequent continuation of this study involves the intracerebral challenge of calves with tissues derived from the pathogenesis study. Such challenges were initiated as early as October 1996, and yet the only clear-cut transmissions have been the positive tissues identified by mouse assay. One of five calves inoculated with tonsil collected 10 months post inoculation has succumbed to BSE, with an incubation of 45 months, but one year later no further animals have died.

In a parallel study involving the inoculation of homogenized pooled nictitating membranes derived from naturally infected cows, one of five challenged calves has died of BSE at 31 months post-challenge. Remaining animals are healthy at 42 months pi.

Despite the results on tonsil and nictitating membrane it is clear that there is little involvement of peripheral lymphoid tissue in BSE-infected cattle. In sheep meanwhile there is a clear widespread involvement of peripheral lymphoreticular tissues in TSE-susceptible sheep. This is not a uniform phenomenon, being dependent on genotype. Therefore, in ARQ/ARQ sheep, challenged with BSE by mouth, the gastrointestinal tract becomes infected early, progressing to involvement of the entire gastro-intestinal tract by clinical onset. Peripheral lymph nodes become involved early, as does the spleen, and entry to the CNS occurs at around 12 months post exposure (mid-incubation). This applies equally to scrapie in susceptible genotypes (e.g. VRQ/VRQ). In partially resistant genotypes, however, the distribution of infectivity is far more restricted, often barely detectable in the gut or peripheral lymph nodes. Infectivity may only be detectable in CNS at the time of clinical disease. Some sheep appear to be fully

resistant to challenge, with neither infectivity nor immunostaining detectable in any tissue, but it remains to be shown whether such sheep are fully resistant to infection.

Migration of infectivity from gut to CNS is clearly demonstrable in sheep. Transmission of BSE or scrapie in experimentally-infected sheep by means of blood transfusion identifies the likely involvement of blood and lymphoid cells in the distribution of infectivity around the body. The enteric nervous system and sympathetic and parasympathetic innervation are also recognized candidates.

How the pathogenesis of scrapie relates to likely routes of transmission remains to be confirmed. Placenta can clearly be infectious and contaminate the environment. Infectivity may subsequently gain entry by mouth, via nasal or occular mucosa, or by scarification. Other potential routes of discharge are faeces and nasal discharges, but these remain to be proved. Whether or not environmental vectors such as hay mites or gastrointestinal mites play a part in transmission also needs to be confirmed.

In summary, there are key similarities and differences between BSE in cattle and scrapie in a fully susceptible sheep. The differences are significant, and highlight both the obstacles and potential that the science presents with respect to control and eradication of both diseases.

Dr. Elizabeth Williams, University of Wyoming, Laramie, Wyoming, USA then presented the topic of "Transmission and Pathogenesis of CWD". (Her complete slide presentation is in Appendix C.) The host range of this disease in its natural occurrence are Mule deer (Odocoileus hemionus), white-tailed deer (Odocoileus virginianus) and Rocky Mountain elk (Cervus elaphus nelsoni). There is no evidence of natural transmission of CWD to cattle as indicated from findings of a six year study after exposure of oral inoculation to cattle. A number of species that are known to be susceptible to TSEs e.g. cattle, sheep and goats, have been in direct and indirect contact with CWD cases, but none has succumbed. So the origin and indeed maintenance of CWD remains an enigma, very much like that of scrapie. The PrP^{CWD} was detected three months in lymphoid tissues and six months in the obex of mule dear after oral inoculation of 5 grams of PrP^{CWD}. The PrP^{CWD} however, was detected in both lymphoid tissue and obex five months after oral inoculation in white-tailed deer. In the alimentary tract, lymphoid involvement is early in the process and widespread. Also, there was evidence of spread in the tonsil, Peyer's patches, and spiral colon, and later the central nervous system (dorsal motor nucleus of the vagus).

Transmission (specifically exit of infectious agent from affected animal) appears to take place through feces, saliva, urine and maybe other excreta. There is evidence against maternal transmission as being an important aspect in spread of

the disease. Horizontal transmission appears to be very efficient in the spread of CWD.

In mule deer, males appear to be more commonly affected than females, and the same pattern appears true in elk. These differences may be due to susceptibility, behavior, and the varying degrees of exposure that include the migration of animals and the influence of density in the transmission of the disease. This has been proven by the differences in the density of deer in Wisconsin that is far greater than Colorado and Wyoming, thus creating more opportunities for transmission of the disease in Wisconsin than in states like Colorado and Wyoming, or in the case of Canada, Saskatchewan. Dead carcasses can play an important role in the disseminating of CWD. The role of predators (coyotes, crows, magpies, wolves etc) could potentially play a role in the epidemiology of the disease.

CWD has been reported as a result of other facets of environmental contamination and research to determine more specifics are needed. There are some similarities of CWD to scrapie in sheep.

The challenge for the research and animal disease control officials at both the state and federal level is putting CWD in context. The media has hyped CWD to emotional levels approximating that of BSE. Headlines like "mad deer" disease, and the analogies that hunters have died of CJD-like or other neurodegenerative diseases that could be associated with the consumption of venison have been blown up for impact. This continues in spite of concerted efforts of health officials at all levels of government who continue to try to set the record straight. So the constant challenge remains – how to communicate risk based on the current known epidemiology and explain to skeptics that there is no proven link of CWD to human health.

The panel then assembled and composed of Linda Detwiler, moderator, Danny Matthews, Beth Williams, and Gerald Wells. Dr. Linda Detwiler highlighted some of the important points related to the pathogenesis and transmission of TSE diseases in animal populations. The main points from her presentation are:

- The TSEs must be examined from their similarities and differences and the distribution of the infectious agent in tissues.
- The pathogenesis of the disease influences the public health relevance, diagnosis, and possible treatment at a later date or how to block the spread of the disease.
- There are vast differences in transmission of the different TSEs – each disease is an entity, and should be treated as such.

- We need to beware of the existing mantras – what research has been done and how – is the research relevant, was there a species barrier, etc.
- In scrapie, there has been no proven infectivity in feces, saliva, colostrum, urine – even though the intestine is an early site of the location of the infectious agent.

A set of questions were submitted from the participants to the panel members. The list of the questions and the responses are below:

Q. Are there strains of BSE?

A. Dr. Matthews: We probably have not done enough research in strain typing. Research by Dr. Bruce (Animal Health Institute, Edinburgh, Scotland) indicates that there may be closely similar strains, but molecular tools could assist the further identification of strains. Early indications were that BSE has only one strain. But, the entire world of strain typing is complex and we should keep an open mind pending further research.

Dr. Wells commented that biological strain typing methods require "passaging" through laboratory animals (mice), and that this process produces a series of events to result in a strain. Some of the current methods e.g. phenotyping are cumbersome.

Q. What is the lymphoid tissue distribution of PrP^{CWD} in cattle intracerebrally inoculated with brain from infected deer?

A. Dr. Williams, supported by Dr. Janice Miller (USDA:ARS, Ames, IA.): It is not usual to find lymphoid distribution in cattle, or from a very conservative perspective, it is not consistent.

Q. Has v-CJD been found in vegetarians?

A. Dr. Matthews indicated that there has been one reported case. He further prefaced his comments to indicate that food consumption is difficult to determine, thus this can complicate the issue.

Q. Has CWD always been in free-ranging animals?

A. Dr. Williams theorized that the disease likely has always been around but with varying incidences in different locations. We really know nothing about the origin of CWD, and whether or not there are spontaneous cases are difficult to determine.

Diagnosis and Epidemiology

Dr. John Kellar of the Canadian Food Inspection Agency was the moderator for this session. He indicated that the purpose of this session is to develop and relay the significance of pathogenesis to the epidemiology and likely control of the TSEs, specifically BSE, because of its public health significance.

Dr. Dagmar Heim from the Swiss Federal Veterinary Office, Switzerland gave a presentation entitled "BSE Distribution: Surveillance and Diagnosis". A summary of her presentation is below. (Dr. Heim's slide presentation is not available at this time.)

Initially, BSE was seen as a purely British problem. Greater attention was paid to the problem after the occurrence of BSE in exported cases in 1989. Not until the end of 1989 were the first indigenous cases reported outside the United Kingdom (UK): in Ireland, France, Portugal and Switzerland. In the mid-90s, other countries reported cases of BSE: The Netherlands, Luxembourg, Belgium, and Liechtenstein. With the introduction of targeted surveillance in risk populations in 2000, a large number of countries in Europe and also the first countries outside Europe detected their first BSE cases.

The reported number of BSE-cases in a country has to be seen in the light of the quality of its surveillance system and the measures taken. Until 1999, surveillance systems for bovine spongiform encephalopathy (BSE) world-wide were primarily passive, i.e. relying on the examination of diseased adult cattle showing clinical signs that were reported to the veterinary authorities. Brains were examined by histopathology and immunhistochemistry. Histology and immunhistochemistry are reliable to confirm BSE in cattle, but the procedure is cumbersome, time consuming and therefore not suited for mass testing of animals.

The number of reported cases in a passive surveillance system is influenced by the degree of disease awareness of farmers and veterinary practitioners, and by the willingness to report a suspect case. The motivation to report suspect cases depends on the measures taken and the compensation paid. In such a system, the reported number of clinical cases underestimates the true number of clinical disease in the population. In summary, surveillance based on clinical symptoms alone is not sufficient to provide a true picture of the BSE situation in a given country and must be interpreted with caution.

The development of rapid BSE tests allowed the fast and uncomplicated testing of brain tissue for BSE on a large scale and the possibility to identify infected animals in the last stage of the incubation period. This made it possible to implement active surveillance programs in populations at risk. Unfortunately

these tests are not sensitive enough to identify animals that are infected but that do not yet have a high concentration of the BSE agent in the brain. A negative result is thus no guarantee that the tested animal is not infected. Nevertheless, through the use of these tests, it is possible to get closer to the true incidence of BSE and the tests have proven to be a useful screening technique.

With the availability of a rapid test for BSE, in 1999 Switzerland initiated an active surveillance scheme to enhance the detection of BSE cases in the adult cattle population. In addition to the mandatory reporting of all suspect clinical cases, all adult fallen or killed bovines and all emergency slaughtered cows must be examined. These two risk-populations were chosen because the probability of finding otherwise undetected BSE-cases seemed highest in them. Additionally, a random sample of cows is examined during routine slaughtering.

Based on the results of the Swiss targeted surveillance system, many countries have also implemented an active surveillance system; tests for BSE have been carried out in populations at risk since 1999/2000 in some countries and 2001 in others. Since January 2001, systematic testing is obligatory in the EU-member-states. According to the legislation in the EU, the surveillance should comprise all bovine animals > 24 months subject to emergency slaughter (accident or serious physiological and functional problems); all bovine animals > 24 months found at ante-mortem inspection to be suspected of or suffering from a disease or a disorder; all bovine animals > 30 months subject to normal slaughter for human consumption (only Sweden is allowed to take a random sample); all bovine fallen stock > 24 months, died or killed on farm or transport, but not slaughtered for human consumption; and all bovine animals suspected of being infected of BSE.

Based on the legislation, all EU member states should have a similar surveillance system. Between January 2001 and December 2002, more than 20 million cattle have been tested in the EU. However, the percentage of adult cattle tested has varied substantially among the member countries. Although among these countries differences in the production systems do exist, it would be expected that the variation in percentage of animals tested should remain fairly consistent.

The number of positive animals found within the different sub-populations of cattle indicates that targeting the risk populations (adult fallen and emergency slaughtered cattle) is the most efficient sampling approach.

		2001	2002
healthy slaughter population	no of tests	7670176	7511862
	no of positives	279	237
	rate of positives: 1 of	**27492**	**31696**
risk population (fallen stock + emergency slaughter)	no of tests	771272	1030484
	no of positives	744	938
	rate of positives: 1 of	**1037**	**1099**
clinical suspects	no of tests	3634	2230
	no of positives	1086	589
	rate of positives: 1 of	**3.3**	**3.8**

An evaluation of the surveillance systems for BSE for most other countries is difficult because detailed data are often not available. In the chapter on surveillance of BSE in the OIE Terrestrial Animal Health Code, it is recommended that surveillance should be determined by, and commensurate with, the outcome of the risk assessment. However, in many countries an up-to-date risk assessment for BSE in the country is not available. Additionally, there are no clear guidelines for an "adequate" surveillance program.

In conclusion, to estimate the extent of the BSE problem in a country, a comprehensive, scientifically based risk assessment must be carried out. Based on the result of the risk assessment, a surveillance program should be planned. Guidelines for an "adequate" surveillance are needed. Active surveillance in populations at risk plus passive surveillance improves the estimate of the true BSE situation in a country. Numbers of tested and positive cattle in the different sub-populations should be centrally collected and published.

Dr. Mike Miller of Colorado Division of Wildlife, Wildlife Research Center, Fort Collins, Colorado, USA, gave a presentation entitled "CWD Distribution: Surveillance and Diagnosis". A summary of Dr. Miller's presentation is below:

The clinical diagnosis of CWD includes the following signs: abnormal behavior, weight loss, emaciation, central nervous system signs, and a mixture of other signs including frequent urination. Histopathology the affected site is a typical spongiform lesion in the brain as seen in other TSEs. The Immunohistochemistry (IHC) is a confirmatory test and it indicates the presence of PrP (CWD) in both lymphoid tissue and obex. Last year rapid tests using ELISA principles were evaluated and they found that can be used in both obex and lymphoid tissue. Tonsil biopsy is applicable as a live animal test in deer but requires skill in obtaining the appropriate sample.

The aims of the CWD Surveillance in free-ranging cervids are to detect cases and estimate the geographical distribution of cases. The current surveillance is mainly mortality-based design with population-based surveys – geographically-targeted

and random sampling of the deer/elk population. The difficulty in the design and maintain a reliable surveillance is related to the uncertain population sizes and boundaries, e.g. the density of the herds could vary from region to region. In addition, there is constant movement of deer and elk and heterogeneous disease distribution.

The risk factors for this disease are variable and not fully defined. Collect and examine suspects throughout the state (or province). There should be a program of annual random surveys of endemic herds and systematic surveys of other herds to make comparisons.

Knowledge acquired in the process:

- The distribution is underestimated.
- Prevalence of the disease varies between the sexes – males show a greater incidence.
- The need to develop adaptive management strategies.
- Implications for Wildlife Management:
- Addressing CWD as a primary target may usurp resources.
- The existing technology may limit management options.
- Unrecognized factors may impede success of programs.

Then Panel B, "Epidemiology and diagnosis", was assembled to discuss and answer written questions from the participants. The panel consisted of John Kellar, moderator, Terry Spraker, Mike Miller, Lynn Herrmann, Dagmar Heim, Ueli Kihm.

Dr. Kellar initiated the discussion by summarizing some of the main points presented by the above two speakers. A look at new technology and rapid tests for the TSEs are developed, and some still need to be validated. A breakthrough would occur if we could find the infectious agent in tissues other than brain, especially with relevance to BSE. The gold standard of 100% sensitivity and specificity is difficult to accomplish with most tests. While the third eyelid test is great for sheep and shows encouraging results, the test does not work as well in older sheep, thus complicating diagnosis, to a degree.

There was an extensive discussion period on testing strategies of different countries, the impact on consumer confidence in general, and target testing of risk populations to detect the different TSEs.

One advantage of coming together as a community with a fervent interest in the TSEs at a conference of this nature is to continue raising the questions of the

many unknowns. It becomes readily obvious that many unknowns persist, but the progression of the last decade provides reason for optimism, even when many answers are still needed and dependent on research findings that take time. That makes these complex diseases all the more fascinating!

The following questions were presented and answered by the panel:

Q. Can the cost of surveillance be diminished by the pooling of samples?

A. Dr. Bruno Oesch (Prionics): Pooling of samples would have major disadvantages for an effective surveillance program. When samples are pooled there will be concurrent lowering of sensitivity, thus making the concept of pooling unacceptable.

Q. In Canada, diagnostic testing picked up one infected animal, how many others may be in the population?

A. Dr. Dagmar Heim: The answer is obviously difficult and is dependent on what Canada, as a country, does in surveillance of BSE. The country's other instituted controls must be considered, e.g. the feed "ban", etc.

Dr. Danny Matthews: The potential for more serious problems or cases in Canada could be very low because of the existing incidence and a whole lot of other factors. The scale of exposure in Canada was likely low.

COMMENT: Various comments and remarks from participants heightened concepts of transmission and risk of BSE. The Canadian case was used as a current reference for managing and communicating risk.

Dr. Gerald Wells, Consultant Veterinary Pathologist, Veterinary Laboratories Agency, Addlestone, Surrey KT15 3NB, UK, then reviewed the recent research findings in terms of the BSE pathogenesis. Below is the summary of his presentation. (See Appendix H for presented slides.)

The pathogenesis of scrapie has been studied principally by experiments in laboratory rodent species and by examination of tissues from cases of natural scrapie in sheep. In most experimental models of scrapie, after peripheral non-neural routes of infection, replication of the agent can first be detected in lymphoreticular system (LRS) tissue. Thereafter, there is a striking amplification of the agent in the LRS and spread by lymphatic/haematogenous routes, giving widespread dissemination throughout the LRS and the presence of agent in some other tissues. This precedes replication in the CNS, but is not, it seems, in most models, the means by which infection reaches the CNS. There is now substantial evidence from experimental models of scrapie, employing non-neural exposure routes that spread of infectivity to the CNS is by the peripheral nervous system

(PNS) pathways. In some models, employing oral exposure the earliest localised LRS replication is in the gut associated lymphoid tissue (GALT) and autonomic PNS routing to the CNS has been implicated. This seems true of at least some examples of natural sheep scrapie. However, it must not be concluded that this pattern of agent dissemination is necessarily applicable to all forms of natural exposure to TSE agents.

Studies of the pathogenesis of BSE have examined the development of infectivity in cattle after oral exposure to a single 100g dose of BSE affected brain homogenate at 4 months of age (Wells et al. 1994,1996, 1998, 1999 and EC 2002) and have been described in a previously presented paper (Danny Matthews, The transmission and pathogenesis of BSE; this meeting). These studies have indicated the presence of agent in the distal ileum and localisation of disease specific PrP in Peyer's patches from an early stage of incubation. In contrast to natural scrapie of sheep, cattle with BSE appear to develop only a very limited involvement of the lymphoid tissues. Significant infectivity is confined to the central nervous system where it accumulates from an undetermined, but probable late stage in the preclinical period.

This last statement, requiring an estimation of occurrence of infectivity in the CNS relative to incubation in cases of BSE is important for public health risk assessment purposes. In naturally occurring BSE, the age at which the CNS may first contain infectivity is not known. It is also not possible to predict when a case of BSE will show infectivity in the CNS from experimental study of BSE in cattle after oral exposure. This study showed only that the lower limit of the incubation period range was 35 months in animals that were kept to this point in the sequential kill study and that the first evidence of infectivity [by conventional mouse bioassay] in the CNS was at 32 months after dosing (Wells et al., 1998). These two observations, clinical onset and tissue infectivity, cannot therefore be related directly, since the two parameters cannot be compared in the same animal. An estimate from dose response data of cattle infected orally with a dose of BSE infectivity closely similar to that administered to induce disease in the oral exposure study (G. A. H. Wells, unpublished data) suggests a mean incubation of almost 45 months (range 33-55 months), but this study, by its very nature cannot provide information on infectivity of tissues relative to incubation. It appears however, that in BSE, unlike many experimental models of TSEs, after peripheral routes of exposure, in which infectivity may be first detectable in the CNS from about 50% of the incubation period, CNS infectivity occurs late in the preclinical period. This is also supported by epidemiological observations (J. W. Wilesmith, personal communication). The mean age of cattle diagnosed with BSE through active surveillance in Britain has not changed significantly, which is contrary to what would be expected if a substantial proportion of preclinical cases of BSE were being detected several months before clinical cases. However, many

questions with regard to the pathogenesis of BSE in cattle remain. Some are important to risk assessment issues. Are apparent differences in tissue distribution of infectivity between BSE and scrapie related to titre, or/and true pathogenetic differences?

What is the role of the autonomic nervous system or other PNS components in the pathogenesis of BSE (EC 2003; Fries et al., 2003)? What are the public health implications of additional potential positive tissue (e.g. muscle) results using more sensitive assays in future? An experimental oral exposure of calves to BSE agent has replicated the original study described here and together with other archived tissues is providing opportunities for further research:

Furthermore, an unprecedented proliferation of large animal containment facilities in Britain provides potential for additional studies in farmed animal species. With the probable under utilisation of such facilities in future, their availability to address experimentally issues of concern to other countries, where for reasons of apparent freedom from disease and/or biosecurity they do not wish to conduct such studies within the country, could form the basis of effective collaborations in this area of research.

Studies of tissue infectivity or disease specific PrP distribution in other species affected by BSE suggest that the restricted locations of agent in cattle is essentially a host influence on pathogenesis. For example, in the greater kudu (Tragelaphus strepsiceros) (Kirkwood et al, 1993) the infectious agent is present in a wide variety of tissues and the distribution suggests possible routes for transmission of the disease (Cunningham et al, in press).

In domestic cats in which feline spongiform encephalopathy has been attributed to infection with BSE agent, an epidemic, now declining to extinction, has resulted in 89 cases in the British Isles since 1990.

An immunohistochemical study of the distribution of PrP in the peripheral tissues of a small sample of cats has indicated limited and inconsistent LRS involvement (Peyer's patches and myenteric plexus in the intestine and spleen, but not lymph nodes). Interestingly, immunostaining of uncertain significance was detected also in the kidney (Ryder et al., 2001).

While early in the UK BSE epidemic, animal health measures were taken to prevent exposure of other domestic animal species to TSE agents, such measures were only partially effective. Legislation in 1996 substantially reinforced these measures by prohibiting the use of meat and bone meal in all farmed species feedstuffs. An equivalent ban on the use of meat and bone meal in the EU was implemented in 2001. (See Appendix H for "Possibilities of infection of UK livestock by SE agents, April 1996")

Pigs and poultry in the United Kingdom have undeniably been exposed to the bovine spongiform encephalopathy (BSE) agent. They consumed the same ruminant protein that gave rise to the BSE epidemic in cattle, but there has been no evidence of an epidemic in these species. Experimental investigations have shown pigs to be susceptible to infection by multiple parenteral exposure, but resistant to oral exposure with BSE-infected cattle brain. Current but incomplete evidence suggests that they are also resistant to oral exposure to sheep scrapie agent. (See Appendix H for "Studies of pigs exposed to BSE and scrapie agents")

It might be argued that the life-span of commercial pigs is insufficient for the manifestation of clinical disease in a detectable proportion of the population, that the clinical signs (as observed in experimentally infected pigs) are too subtle to attract veterinary attention and that sporadically occurring, slow degenerative neurological disease in older pigs, at least that which is readily distinguishable from the more acute features of some statutorily reportable infectious diseases, would not warrant veterinary treatment. This raises the potential for inapparent within species recycling of infection where no controls exist for the feeding of meat and bone meal derived from the same species (i.e. pig to pig). (See Appendix H, "Risk of intraspecies recycling of BSE agent in pigs")

Studies in domestic chickens indicate that they are apparently resistant to both parenteral and oral exposure to the BSE agent.

The conclusions from Dr. Wells' presentation are that the current methods of detection of the agent of BSE suggest that the pathogenesis of BSE, although essentially similar to that of scrapie of sheep, lacks widespread LRS or other non-CNS tissue involvement, but studies to date have been limited. Examination of a greater range of tissues from larger numbers of animals, with more sensitive methods of agent detection, at appropriate time points in incubation is required to further underpin public health controls and improve an understanding of the pathogenesis of the disease in cattle. Studies of the distribution of infectivity/PrP in other species, naturally or experimentally infected with the BSE agent show important host differences which may have significance for the potential for transmission among species where appropriate controls are not in place. While in pigs and poultry, experimental studies to date indicate that the possibility of inapparent intra-species recycling of the BSE agent is remote, the issue also requires further research.

The third and final session was entitled " Preventive Measures and Existing Regulations for BSE". Dr.. Alex Thiermann, USDA:APHIS and president of OIE Code Commission, was the moderator for this session.

Dr. Ray Bradley, Guildford, UK, gave a presentation entitled "Preventive Measures and Existing Regulations for BSE". A summary of this presentation is below. The slides of this presentation are available in Appendix J.

BSE is a zoonosis. The reason is that the agent that causes BSE in cattle has the same biological properties (biological strain type) as the agent that causes variant (v) Creutzfeldt-Jakob disease (CJD) in man that is invariably fatal. vCJD is presumed to be contracted from the consumption of BSE infected cattle products derived from infected slaughter cattle. Only certain tissues in the body are infectious. Infectivity is likely to be highest in the tissues of the central nervous system (CNS) from late in the period of incubation. Infectivity may also be present in the distal ileum and tonsil soon after initial exposure. Collectively, these and CNS tissues form the most important part of the specified risk materials (SRM).

The most important immediate public health measure is to compulsorily notify, slaughter and completely destroy cattle suspected clinically to have BSE. The second and vital measure is an SRM ban that will ensure that tissues that carry a risk of being infected with the BSE agent, including tissues from those animals that are clinically healthy, are removed and destroyed. The third protection is enforcement of existing and new meat hygiene regulations using HACCP principles, thus reducing any risks there may be from cross-contamination in the abattoir. The production of mechanically recovered meat (MRM: also known as Advanced Meat Recovery) from ruminant animals should be banned to reduce the risk of introducing a risk from vertebral bones containing residual spinal cord and dorsal root ganglia.

Subsidiary regulations prohibit certain kinds of stun guns and pithing, regulation of bovine bone gelatin manufacture and production of tallow derivatives. The long term protection of public health is by elimination of BSE in cattle. The main measure to protect animal health is the prohibition of feeding mammalian protein (with a few exceptions such as for milk protein) to all food animal species including horses and fish. If this measure is perfectly enforced BSE is highly unlikely to occur, new epidemics would be prevented, existing epidemics would decline to extinction, recycling of infection via feed would cease and all species would be protected from exposure via feed. There is also a need to regulate rendering and disposal processes used to destroy waste materials of animal origin. The value of the species barrier as a natural asset should not be ignored, thus within-species recycling of most animal proteins should be prohibited.

The compulsory slaughter and complete destruction of suspect clinical cases and SRM removes highly infected material from all food and feed chains, prevents it being used for any purpose and ensures that risks to humans, other species and the environment are eliminated, or minimized to a negligible risk level. The feed ban

is directed at all food animal species because experience has shown that it is not possible to completely eliminate the risk of cross contamination of ruminant rations when such a rule is applied to ruminants only. Rendering controls are necessary to maximize the titer reduction in the end products, particularly MBM, since it is known that some methods of rendering have no effect and none is completely effective at inactivating the BSE and scrapie agents.

Meat hygiene regulations ensure complete separation of SRM from parts of the carcass intended for human consumption so that risks of cross-contamination of edible parts of the carcasses are eliminated or minimized. Where cross-contamination does accidentally occur or might have occurred, all relevant parts are condemned as SRM. Certain stunning methods and pithing are prohibited to reduce the risk that pieces of the central nervous system could enter the circulation and be found remotely from the brain such as in the blood, heart and lung. Controls on the production of tallow derivatives and gelatine maximize the achievable reduction in titre should any infectivity be unexpectedly present in starting materials. For disease control purposes and to establish or improve consumer confidence in the source and safety of the meat they are purchasing or eating in a restaurant, animal identification permitting trace-back to the farm and herd of origin is essential. Slaughter of cohorts of affected cases is theoretically sound but generally has a low cost/benefit ratio since most members of a feed cohort will be dead when the first BSE case in a cohort is detected. Slaughter of offspring of affected cases appears to have an extremely limited effect and possibly none at all as there is no plausible way in which maternal transmission can occur and no case has yet been proved. There is no scientific justification for herd slaughter following identification of a case. Suspicion of BSE must be officially notified and investigated thus demanding that those in contact with livestock are aware of the clinical signs of the disease. If BSE is believed to be the cause, a movement restriction order is placed on the animal until it is compulsorily slaughtered and removed to permit safe removal of the brain. This is done to confirm disease in an approved laboratory by an approved method. The remainder of the carcase is completely destroyed by incineration or in rare cases burial. TSE has not been reported in CERVIDAE species or free-ranging animals in Europe so there is no experience of this problem as it occurs in North America. SRM are tissues that in an effectively exposed animal are highly likely to be infected with the BSE agent. SRM includes tissues cross-contaminated with SRM or joined to them. Employing the precautionary principle SRM include central nervous system tissues, the eyes and associated ganglia, the skull or head, the vertebral column and the intestine from duodenum to rectum inclusive along with the mesentery and mesenteric fat. However, the list is varied to some extent by different authorities and in different countries depending upon the geographical BSE risk assessment (GBR) for that country. The higher the perceived risk the longer is the SRM list. If the geographical risk is regarded as highly unlikely no

bovine tissues are regarded as SRM. For some tissues the age of the cattle from which they are derived may be a criterion. SRM, bovine fallen stock and certain other materials are, for practical purposes, regarded as SRM. They must all be stained with an approved heat-resistant dye. Incineration or co-incineration is permitted directly. Alternatively, after reducing the starting material to an approved particle size, SRM may be rendered by a one of several approved methods. The end-products can be destroyed finally by incineration or co-incineration. SRM tallow can be licensed to be used as a fuel in rendering plants. MBM, if rendered using approved parameters may be buried in licensed landfill or otherwise burnt in purpose-built burners, power stations or in licensed incinerators. Alternative methods for carcass and SRM destruction that show an equivalent reduction in titre can be authorized. Such methods could include alkaline hydrolysis under pressure. Risk communication is very important and should be targeted appropriately to veterinarians, farmers, feed compound, the livestock and food industries in general, the waste disposal industry, hunters (re: CWD) and consumers, pointing out the uncertainties and that a zero risk is not provable. The OIE is a key organization and its role is crucial. By means of the Terrestrial Animal Health Code chapter on BSE and the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals the OIE enables the continued international trading of bovine animals and products in a safe manner. This necessarily restricts trade in some commodities that present a risk (like SRM) but does not restrict trade in other commodities like milk, semen and embryos that present a negligible BSE risk even from high BSE-risk countries. The Code chapter on BSE specifies the definition of the BSE status of a country or zone by virtue of the BSE risk (high, moderate, minimal, provisionally free and free) and states the conditions necessary to achieve this categorization. The EC has a similar way of classifying countries and has published opinions on the GBR for all Member States of the EU and several other countries that wish to trade with the EU in cattle and/or cattle products.

In conclusion, if BSE is discovered, due regard must urgently be paid to the protection of human health as well as animal health. The potential economic and social effects of a BSE epidemic in a cattle rearing country should also be considered. Any lost confidence of the consumer can only be regained by securing the immediate protection of public health and a declared intention to eliminate BSE. Lessons can be learned from the experience in the EC and particularly in the UK. Instrumental in the process will be guidelines of the WHO in regard to human health and those of the OIE in regard to animal health and trade.

Dr. Lynn Creekmore, USDA:APHIS:VS, Fort Collins, CO, USA, gave a presentation entitled " CWD – Preventive Measures and Existing Regulations".

Dr. Creekmore made the following points: The slides of this presentation are available in Appendix K.

The unique challenge of CWD is mostly the misconceptions and thinking of the public and media about the importance of the disease. CWD was detected in wild cervids in 8 states. Wild animals and farmed animals necessitate different regulatory controls. The farmed cervid industry is relatively new and represents about 1 million animals, which divided by species is about 800,000 deer and 200,000 elk.

The maximum incubation of the disease is unknown. The time from infection to shedding of the infectious agent is also unknown, including the route of shedding. Interestingly, the most efficient route of transmission is also unknown.

There are no existing techniques to measure levels of the agent in the environment. The minimum infectious dose is also unknown, and an adequate system for the cleaning and disinfection of premises is also unknown.

A challenge with CWD can be described as the 3 Ps – Politics, Public Perception, and Popular Culture. Efforts to communicate risk have been difficult in spite of extensive attempts to explain the public health relevance. Of interest is the difficulty to dispose of carcasses which creates a serious problem for regulatory agencies.

Current Program Activities (Farmed Animals) are surveillance and development/ identification of positive or exposed animals or herds. There is a proposed USDA:APHIS CWD Herd Certification Program to eliminate CWD from captive and farm deer/elk herds. The program is voluntary and will have established surveillance requirements of deaths in animals over 16 months of age. There will also be fencing requirements for herds. Response to positive herds for CWD will be depopulation or a mixture of quarantine and selective depopulation. Interstate movement of animals will apply only to farms participating in the certification program.

Dr. Conrad Brunk, University of Victoria, Victoria, British Columbia, presented a talk entitled "Understanding the Perception of TSE Risk". Dr. Brunk's slide presentation is available in Appendix L. Dr. Brunk presented the concepts of risk by posing the following questions and presenting a provocative and philosophical overview of the subject.

- What is the debate over safety all about?
- Public Risk Perception and Acceptable Risk!
- The Profile of BSE (what you see is what you would predict!)

- What can we learn!
- What tasks are involved in managing risks like the TSEs?
  - Assessing the risks
  - Limiting the risks
  - Managing public concerns re: the risks.
- The Science "Short Cut" to Safety
- The temptation of "science-based" risk management is to find a quasi-scientific solution to the issue of acceptable risk.
- Scientific temptations – if risk assessment is low enough, we can count it as "effectively zero."
- Find a quantitative algorithm (formula) for determining risk.
- The Risk of Science-Based Safety Analysis/Expert Solution for Public
- Risk Debates
- Improve science education in the schools and in the media.
- Engage in strategic communication. What is wrong with this solution? It fails to recognize that risk acceptability involves far more than simply the scientific assessment of risk and the possible outcomes.
- Unpacking the Concept of Safety
- The question of safety is fundamentally a question of the acceptability of risks.
- To Whom Is the Risk Acceptable? Options
- To those who benefit from the risk?
- To those who bear the risk?
- To the experts who assess and manage the risk (risk managers)?
- By What Standard Is Risk Acceptable?
- Zero risk (or de minimus)
- Actual acceptance
- Presumed or applied acceptance e.g. in the case of government's established generally recognized as safe (GRAS).
- Expert standards.

- How Risk Experts Tend to View Safety
- Prefer quantitative algorithms for risk acceptability.
- Expert Perceptions of Safety
- Experts prefer to withhold judgment until there is scientific affirmation.
- BSE/v-CJD –The trend factors
- High unfamiliarity about the diseases in general
- Invisibility – little known
- Dreadful diseases – always fatal
- Scientific uncertainties – what is the cause of the disease? the incubation period? treatment?

Panel C then assembled and was composed of Ray Bradley, Lynn Creekmore, Conrad Brunk, John Kellar, Sarah Kahn, Phillip Comer, Leonardo Mascitelli. This panel had questions and answers that covered a broad range of topics that were covered during the different presentations.

Some of the questions of special interest to the rendering industry were:

- What can the animal protein industry do to manage the perception of risk regarding animal products with the regulators?
- Why is landfilling not approved for the disposal of carcasses?
- How many deer/elk were killed in 2002 that were CWD-positive?
- What is the risk probability of v-CJD from eating beef from infected vs. BSE-free cattle?

Conclusion

Dr. Mo Salman, gave the conclusion for this conference. His summary is below.

This conference is a second in a series communications on the TSEs coordinated by the International Forum for TSE and Food Safety. It establishes a line of communication between scientific policy makers, regulators, and other stakeholders.

Scrapie is used as a baseline disease for comparison and contrasts within the TSEs. Both BSE and CWD share some similarities with scrapie pathogenesis. The similarities are not perfect. BSE has limited route of transmission that appears to be mainly via feed. There is consistency in the pathogenesis and

virulence of BSE and there is uncertainty in the genetic involvement for BSE, compared to scrapie with proven genetic implications.

Wild animals are natural hosts for CWD. There are potential broad routes of transmission for both CWD and scrapie. Also, there is uncertainty for genetic involvement for CWD.

The emphasis is on valid and reliable screening diagnostic tests for all three TSE diseases. The importance of representative samples of high-risk populations must be recognized. Value of the appropriate biological specimens is important for accurate results. The diagnosis and confirmation is a complex process.

The term surveillance tends to be used with several meanings and interpretations. Surveillance requires structured plans. A target population requires previous knowledge of risk.

Diagnosis – uncertainty of validation of current rapid tests for various stages of the TSEs. There is limited validation of existing rapid and other tests for CWD.

Surveillance – limited to sampling protocol used by a country for BSE/CWD.

Status – Know status if a case is found. This is limited to the sampling protocol for CWD.

Increasing the sample size does not ensure a representative sample for BSE/CWD. The population sampled must relate to risk.

Epidemiology – there must be knowledge of the natural setting of the disease.

The U.K. experience and observations are the basis for existing BSE epidemiology.

Intervention strategies provide further evidence of BSE epidemiology.

Captive cervids have not provided sufficient information for conclusive findings/determinations for CWD.