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Transmissible Spongiform Encephalopathies: Epidemiology,
Risk and Research Requirements**

**with the participation of
Office International des Epizooties**

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**World Health Organization
Communicable Disease Surveillance and Control**

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This report summarizes the presentations made to the World Health Organization on the occasion of a meeting held at its headquarters in Geneva from 1 to 3 December 1999, and provides the assessments and recommendations made by the consultation.

Opening

In 1986 the first case of a previously unrecognized neurologic disease of cattle was diagnosed in the United Kingdom (UK). Within a few years it was clear that there was an evolving epidemic outbreak of a new transmissible spongiform encephalopathy (TSE). To date over one hundred and seventy-five thousand cases of bovine spongiform encephalopathy in cattle (BSE) have been reported from the UK, and over eleven hundred in eight countries of continental Europe (ranging from one to over four hundred cases per country).

The TSEs are degenerative brain diseases seen in humans and some domestic and wild (captive and free-ranging) animals. The family of diseases is characterized by an incubation period usually measured in months to years. There is no treatment and no cure. The nature of the agent has not been fully determined, although the predominant theory is that a previously unrecognized pathogenic agent called a prion, an abnormally folded protein, is responsible. There is no early diagnostic test or screening test available for use on an easily accessed biological tissue in live animals, and to date, the gold standard tests to differentiate the strains of TSEs within an animal species require inoculation of mice and the observation of these mice over several years.

The scale of the BSE epidemic and the cost of the measures to control the outbreak are unprecedented. The UK anticipates spending £3.5 billion by the year 2000 in its attempts to control the BSE epidemic. The export of live cattle and cattle products (other than milk) from the UK was temporarily banned by the European Commission (EC) and trade in these products has been affected on a global basis. Epidemiologic studies were able to demonstrate that BSE was almost certainly caused by the practice of feeding cattle with meat-and-bone-meal (MBM) made from the carcasses of TSE infected animals, particularly sheep and cattle. The practice created a feedback loop that magnified the transmission of this newly recognized disease of cattle, creating an epidemic.

Strong measures were taken in the UK, later in Switzerland and the European region, to control the BSE epidemic. A decline in cases in the UK and Switzerland was observed. However, cases continue to occur, and some countries are currently seeing increases in incidence (the number of cases are, however, small). Virtually every country of the world has altered its cattle feeding practices although many did not do so until years after the epidemic was widespread within the UK. Nonetheless, to date, no endogenous cases of BSE have been recognized or reported from outside Europe; a small number of countries have imported infected cattle.

While BSE is an important animal pathogen, its relevance to WHO relates to its potential to cause human disease. WHO initiated a number of meetings to determine if there was a risk to humans, and early evaluations were reassuring. However, in March 1996, the UK CJD Surveillance Unit published a series of 10 case reports of a

new form of Creutzfeldt-Jakob disease (CJD), leading to speculation that these people's illnesses were caused by exposure to BSE. Strong experimental and epidemiologic evidence now supports a causative relationship between the new variant of CJD (vCJD) and exposure to BSE, making BSE the first known TSE zoonosis.

In addition, it is known that when brain tissues from cattle with BSE are experimentally fed to sheep or goats, they can cause a TSE that is difficult to distinguish from scrapie. Scrapie is endemic in some sheep and goat flocks of Europe, Asia and North America (possibly elsewhere) with the exception of a small number of countries (notably, Australia and New Zealand; some countries of South America and Southern Africa are believed to be free of disease as well). During centuries of human and animal cohabitation, there has never been a demonstrated risk to humans from sheep scrapie. However, now that it is known that sheep are susceptible to BSE, the EC, the UK and France have recently reviewed the risk and published discussion papers about the risk of transmission of BSE through sheep to humans. Countries with scrapie must determine if they have BSE in their sheep flocks. A test to distinguish BSE from scrapie is being sought and a policy of scrapie elimination is being considered in some countries. Even in the absence of evidence that BSE has been transmitted to goat or sheep flocks, some countries have adopted risk reduction policies.

BSE contaminated cattle products were fed to other animals. Infection has been identified in various captive wild animals in zoos, and in domestic cats, where it is called Feline Spongiform Encephalopathy (FSE). While human exposure to zoo animals is limited, many people live in intimate contact with their cats. A case of CJD in a man whose cat died from a spongiform encephalopathy (not FSE) and about 90 cases of FSE have been reported in the UK and other European countries.

Chronic wasting disease of deer and elk (CWD) is an emerging TSE. At this time, cases have been seen in wild deer and elk populations of Wyoming and Colorado, and in farmed elk in the United States (US) and Canada. Reports of CJD in three young deer hunters (two from the US and one from Canada) and an additional report of a case in a person who consumed venison led to speculation that CWD might be communicable to humans.

Humans may be exposed to human or animal TSEs through intimate contact (companion animals, animal husbandry), through food (cattle, sheep and deer), and through therapeutics. However, many therapeutics are made from human and animal biological materials – blood and blood products (including products containing albumin such as vaccines), biologics (i.e. human extracted growth hormone, no longer used, is known to have transmitted CJD), medications, human tissues (i.e. dura mater, known to have transmitted CJD), animal tissues (bovine pericardium, bovine insulin, bovine foetal serum), and health foods (antler velvet). Human and animal tissues may be used during the manufacture of some therapeutics, whether or not they are included as an active ingredient or excipient in the final product.

At this time there is considerable speculation as to the extent to which human TSEs may be caused wholly or in part by exposure to animal TSEs although, to date, the only established zoonotic TSE is BSE. WHO propose to review the evidence to

date and to consider whether there are appropriate public health actions that should be taken.

Dr. Lindsay Martinez, the Director of the Communicable Diseases Surveillance and Response, Department of Communicable Diseases, WHO, opened the meeting. Dr. Martinez began by remarking on the rapid evolution of the BSE epidemic and the implementation of control measures, saying that virtually every country of the world has had to review and revise its practices of animal husbandry with avoidance of this disease in mind.

WHO is, however, principally interested in human diseases. The first reports of new variant CJD in March 1996 destroyed the hope that BSE would not prove to be harmful to humans.

Dr. Martinez posed the following questions to the meeting:

- While BSE in cattle is generally accepted as a TSE zoonotic, are there other animal TSEs that pose a threat to human health?
- Are there any public health actions which should be taken now, based on available scientific information?
- Are there any clear information gaps which must be addressed in order to determine if human health is at risk?

WHO has a strong record in the TSEs, with nine consultations on the subject in as many years. Despite the waning of the reports of BSE in the UK and the tremendous steps taken in understanding the TSEs in human and animal populations, there are still numerous unanswered questions. Dr. Martinez emphasized the continued need for high quality coordinated surveillance of TSEs in humans and animals.

She welcomed the consultants to the meeting, and introduced the co-chairs Dr. Rosalind Ridley and Dr. Linda Detwiler. Drs. David Asher and Raymond Bradley kindly volunteered to be rapporteurs. The following document was prepared with their assistance and was subsequently reviewed by all participants. The summaries of the presentations are the opinions of the authors and do not necessarily represent the opinion of either WHO or the consultation. The recommendations of the meeting are presented at the end of the document. They were written at the time of the meeting, although some editing for clarity has occurred.

Section 1. Background

1.1 Trends in Bovine Spongiform Encephalopathy

Ray Bradley:

Bovine spongiform encephalopathy (BSE) was discovered in November 1986 and was originally regarded as a new disease with the first clinical cases recognized as having occurred from April 1985. Nothing has changed to alter this view.

Once the basic epidemiology was investigated and reported, a series of measures were introduced that aimed to reduce exposure of ruminants, man and other species to sources of infection. In BSE-affected countries, these measures included compulsory slaughter and complete destruction of suspect or confirmed BSE cases, various feed and offals bans (later specified risk materials (SRM) bans) and controls on rendering processes. In addition more specific measures were established to deal with any risks that were identified in products such as gelatin and tallow that were used in food, feed, medicines and cosmetics. Risk analyses, done in a variety of ways, for all these and other commodities have been conducted by product manufacturers, trade associations and national and international authorities, like the EC, the Office International des Epizooties (OIE) and WHO.

In order to standardize at least some aspects of risk analyses, the OIE and the EC have recommended the establishment of four categories of countries or zones in regard to BSE status: BSE-free, BSE provisionally free, low incidence and high incidence. The categorization of a country can be determined by the outcome of a risk analysis to a standard format agreed by the member countries. Different risk management strategies are proposed for trading in particular bovine commodities, depending upon the category to which the country belongs.

It is now 13 years since the discovery of BSE. The geographical distribution of BSE in the world and the current status of the epidemics in the UK and in other countries with cases in native-born cattle (currently confined to Western Europe) were described during the presentation. The clinical signs, the incubation period, the neuropathology and the biological strain type of the agents in isolates from cattle from different geographical areas have remained constant. These features are important because inconsistency in them could be associated with different agent strains and thus, potentially at least, with different risks.

The epidemic of BSE is declining in some countries and rising in others. Because of the long incubation period (mean 60 months) it is not possible to determine promptly the effect of new measures introduced to reduce risks. This creates uncertainty in their effectiveness of some year's duration. However, there is evidence from the UK that an epidemic of BSE can be contained, reduced and probably eliminated. It is now important to ensure that the lessons learned from the UK experience are applied everywhere the disease exists.

Also, as the OIE *International Animal Health Code* chapter on BSE states, it is essential for all countries to conduct a risk analysis for possible BSE occurrence, to

have an ongoing education programme, compulsory notification, a surveillance and monitoring scheme and an approved laboratory diagnosis capability.

What now needs to be done is to ensure complete reporting and investigation of all suspect or potential BSE cases. Detection of any concealed cases that are currently misdiagnosed or undiagnosed, could be assisted by using some of the more recently developed, rapid and validated testing procedures on targeted populations such as fallen stock or animals sent for casualty slaughter. These might include methods used to detect PrP^{Sc} in the brain of such animals. To aid this process of complete disease detection, the ongoing education programme for farmers and others in contact with animals recommended in the OIE *Code*, must ensure that the range of clinical signs that represents the full phenotype of the disease is fully appreciated. Once we are confident that all suspect cases are identified, reported and destroyed, all epidemics are controlled and declining, and that all measures are enforced, we might rest easier and have increased confidence that BSE is likely to be eradicated from the world. Assuming BSE eradication is successful, one remaining and important issue is to determine that BSE infection is also eliminated. Without this security we could never be sure that BSE could not arise again in the future if the guard was dropped, and in the meantime it could still be a danger.

1.2 Trends in variant Creutzfeldt-Jakob Disease

Bob Will:

To date, 48 cases have been reported from the UK, plus one case in each of France and the Republic of Ireland. The mean age at death is 29 years (17-53); mean age at onset is 28 years (14-52) and; the mean duration of illness is 13.5 months (7-38). The ratio of males to females is 21/25. A standardized questionnaire is conducted for every case. As yet, only three variables predict vCJD: young age, methionine-methionine homozygosity at codon 129 and residence in the UK. As the low age at death and onset is one of the most characteristic features of vCJD, it is notable that this is being verified through two studies. To determine if vCJD is occurring undetected among older populations, an investigation of the cause of death among all cases of dementia is underway. Secondly, a survey among children with neurological disease (Progressive Intellectual and Neurological Degeneration) is being conducted throughout the UK. Regarding codon 129, considering that persons with VV or MV typing may have a different phenotype, the research group has begun to examine cases of sporadic CJD among younger people, by strain typing in mice.

Trends over time are being examined through review of notifications by quarter, corrections for reporting delay, notifications by date of onset, and mathematical modelling. While none of these examinations indicate a statistical increase in the number or rate of reports, all are tending toward an increase in reports, without reaching biostatistical significance. Resultantly, it is still not possible to predict the overall trend in vCJD.

Several important investigations regarding possible routes of transmission of vCJD have been conducted. It is noted that vCJD does not occur among members of the same family. Neither occupation nor history of previous surgery or blood transfusion predicts disease. The role of blood transfusion in the transmission of vCJD continues to be actively investigated. At this time, among the 48 vCJD cases,

only six were donors. Twelve people received blood from these six donors. None have developed vCJD, although it must be noted that most of the transfusions are quite recent. The role of organophosphates (OP) has been recently reviewed by both the Spongiform Encephalopathy Advisory Committee (SEAC) and the European Union (EU) working group on TSEs, both concluding that OPs are unlikely to be the source of BSE or vCJD. Similar investigations of contact lenses have not been able to draw a link. Childhood vaccination histories have been examined. However, the majority of vCJD cases were born before 1980, hence any childhood vaccinations they received were extremely unlikely to have been made from materials contaminated with BSE.

Dr. Will left the group with a number of questions which he points out remain unanswered. What is the explanation for the age distribution? Why have there been no cases identified among valine-valine homozygotes, or among codon 129 heterozygotes? Given the exportation of cattle and cattle products from the UK and the EU during the endemic period, why are there so few cases in other countries?

Section 2. Bovine Spongiform Encephalopathy in Sheep

2.1 Transmission of Bovine Spongiform Encephalopathy into sheep: Experimental evidence and ongoing work in the United Kingdom

Nora Hunter:

BSE is transmissible experimentally by the intracerebral and the oral route to sheep, incubation period being controlled by PrP genotype of the challenged animal. Giving the genotype at codons 136, 154 and 171 (in order), animals encoding ARQ/ARQ had the shortest incubation period but disease also occurred in VRQ/ARQ, VRQ/ARR and ARQ/ARR with more prolonged incubation periods. When sub-passages are carried out from tissues of the terminally ill sheep, infectivity with the typical BSE fingerprint (mouse incubation period and lesion profile) is recoverable from both brain and spleen. Preliminary evidence therefore suggests that, like scrapie in sheep but unlike BSE in cattle, sheep BSE would produce infected peripheral tissues.

Glycoform analysis carried out on PrP^{Sc} extracted from tissues of sheep experimentally infected with BSE showed a similar pattern to that found in sheep challenged with source of scrapie known as CH1641, which is completely different to BSE in mouse transmission characteristics.

It is naturally of interest to know if BSE did infect sheep through contaminated protein concentrates in a similar manner to the infection of cattle and if it could be maintained as an endemic disease of the sheep population. It is recognized that sheep do not consume MBM in the quantity associated with cattle, and that flock management changes the exposure as well. For example, hill flocks are fed concentrates when they are sold on for finishing; however, early lambs are fed concentrates earlier. This said, lambs are slaughtered young, when the risk of clinical disease would remain quite low.

A series of experimental studies being carried out at the Institute for Animal Health in Edinburgh includes the following:

- (a) A pathogenesis study in sheep orally exposed to BSE to determine the spread of infectivity and identify tissues that retain infectivity is underway. Preliminary results identify heavy PrP staining in many tissues at terminal stages of disease.
- (b) A study of maternal transmission of BSE in sheep to their offspring is underway, and a preliminary and negative attempt to induce maternal transmission of BSE from affected goats to their offspring has been conducted.
- (c) Sheep with naturally occurring TSEs are being tested in mouse bioassay and glycoform studies are being conducted to determine if there are any signatures consistent with BSE. At this time, among ARQ/ARQ sheep, there are no signatures consistent with BSE.
- (d) Blood transfusion experiments from BSE-inoculated sheep to sheep imported from New Zealand are underway.

2.2 Estimating the epidemiology of Bovine Spongiform Encephalopathy in sheep

Linda Hoinville:

There is currently no experimental evidence to suggest BSE is actually present in the UK sheep population. These investigations currently rely on time-consuming, costly methods to distinguish the BSE and scrapie agents, although other methods of identifying BSE on clinical or pathological grounds or by using other strain typing techniques are under investigation. In addition, experimental investigations of agent strains rely on the assumption that BSE passaged through sheep would be identifiable as BSE.

Epidemiological analysis of data on the spatial and temporal distribution of scrapie in the UK enables effective use of existing data to assess the likelihood that BSE is present in the sheep population. Analysis of the number of cases and the number of flocks reporting cases each year has been undertaken. A large-scale anonymous postal survey was recently conducted in which questionnaires were sent to 11 554 farmers. The response rate was over 61% and the results revealed that only about 15% of farmers thought that scrapie had ever occurred on their farm. The data from this survey were also used to investigate whether there was evidence for a change in the incidence of scrapie over time. Such analyses have provided no evidence for a large transmissible spongiform encephalopathy epidemic in British sheep since the start of the BSE epidemic.

As BSE and scrapie cannot be distinguished easily using currently available tests, the only way to control the potential threat of BSE in sheep is to develop effective policies for the control of scrapie. The Veterinary Laboratory Agency (VLA) is currently investigating how scrapie may be controlled both within flocks and in the national population. A combined approach involving a detailed study of

flocks with a known scrapie problem and a case-control study to compare affected and unaffected flocks is being used to determine the risk-factors, such as PrP genotype, and mechanisms of transmission of the disease. Using the information collated to date, a mathematical model for the within-flock dynamics of scrapie has been developed which can be used to investigate various control policies such as use of scrapie resistant rams. VLA plans to extend this work by developing a model for the between-flock dynamics of scrapie, which will allow the effectiveness of different national control policies to be evaluated. Dr. Hoinville emphasized the importance of encouraging sheep farmers to report scrapie cases and to participate in research projects.

2.3 France: Status report on investigations of Bovine Spongiform Encephalopathy in sheep

Thierry Baron:

Dr Baron began by describing the incidence of scrapie in France (199 identified outbreaks since June 1996), noting that the rate of infected flocks is higher in some regions, particularly in the southwest of France. They undertook strain typing by transmission of the disease into mice. The recent acquisition of transgenic mice expressing the ovine prion protein allowed the initiation of similar work in these animals.

Glycoform typing of natural scrapie was also undertaken and results were obtained from 42 scrapie cases in 21 outbreaks, from different regions of France. These showed indistinguishable profiles in all these cases, similar in appearance to those observed in cattle with BSE, especially with regard to the glycoform ratios. In particular they showed, in both sets of data, high levels of di-glycosylated PrP and low levels of unglycosylated PrP. These data suggest that a larger number of field scrapie cases than previously expected could have glycoform features similar to those of BSE, and that the glycoform ratios will probably not be a useful tool for discrimination of BSE and natural scrapie in sheep. However, they then examined one case of experimentally infected BSE in a sheep, and noted a very similar disease to that found in some cases of natural scrapie, with intense pruritus and a long clinical course. Glycoform typing is being used to compare the glycoform profiles from 21 natural scrapie cases (from 21 different outbreaks) with this BSE infected sheep, with particular attention given to the fragment size of the unglycosylated PrP.

2.4 Netherlands: Status report on investigations of Bovine Spongiform Encephalopathy in sheep

Bram Schreuder:

Dr. Schreuder began with the premise that any scrapie control programme is core to control of BSE in sheep. The country's scrapie programme is focussing on genetic screening, especially of breeding rams. The control programme is on a limited number of farms supported by early pre-clinical detection, especially in the lympho-reticular system. He presented information relating to their investigations of scrapie in sheep, in particular the pathogenesis in the natural host.

To study the pathogenesis of natural scrapie, they used immunohistochemistry to monitor the deposition of PrP^{Sc} in various tissues, collected during a natural scrapie infection from sheep with VRQ/VRQ genotype. They have published in the past on the detection of PrP^{Sc} in tonsils, prefemoral nodes, mesenteric nodes and spleen. The tonsil biopsy becomes positive in VRQ/VRQ sheep at about four to five months, at approximately one-fifth of the incubation period in animals that are expected to develop scrapie at around 26 months. These sheep were purpose-bred for their known short incubation period for natural scrapie. He noted that other genotypes will have different incubation periods, and that scrapie strain variation may also influence incubation periods (for example, in VRQ/ARQ sheep, the tonsil became positive at 12 months, at approximately one-third of the incubation period, with disease developing at three years).

As noted earlier, they have found the PrP^{Sc} present in the lymphoid tissues of VRQ/VRQ genotype animals from the age of four to five months onwards. At this age, PrP^{Sc} was detected in the neural tissues in the enteric nervous system (ENS), only at the level of the duodenum and ileum. At the age of 10 months, PrP^{Sc} was not only found in the ENS but also in the ganglion mesentericum cranialis/coeliacum, the dorsal motor nucleus of the vagus and the intermediolateral column of the thoracic segments T8-T10. PrP^{Sc} was detected for the first time in the nucleus tractus solitarius and ganglion nodosus at 17 months of age and in the ganglion trigeminale and several spinal ganglia at 21 months of age.

Since the scrapie agent consists largely, if not entirely, of PrP^{Sc}, these results indicate that the ENS acts as a portal of entry to the neural tissues for the scrapie agent. Entry is followed by centripetal and retrograde spread through the sympathetic and parasympathetic efferent fibres of the autonomic nervous system to the spinal cord and medulla oblongata respectively. PrP^{Sc} accumulation in sensory ganglia occurs after infection of the central nervous system (CNS) and is, therefore, probably due to centrifugal and anterograde spread of the scrapie agent from the CNS through afferent nerve fibres.

Dr. Schreuder concluded that using brain-tissue based diagnostic tests in sheep would miss diagnosing the disease in the earlier half of the incubation period. In addition he commented on a danger which could arise if scrapie control programmes focus on breeding sheep for resistance – that, in fact, the programme could simply breed for a new strain of scrapie, and that they may be only breeding for sub-clinical disease. Dissemination of the infectious agent seems, in any case, reduced by the more resistant genotypes. In addition, he posed an intriguing question. If BSE is found in sheep, is this a finding of a newly introduced infection? Or is it simply the finding of the BSE progenitor scrapie strain?

2.5 Spongiform Encephalopathy Advisory Committee - Risk assessment of Bovine Spongiform Encephalopathy in sheep

Ann Nolan:

The SEAC risk assessment regarding BSE and sheep acknowledged that factors which must be considered were the experimental evidence of oral transmission, that sheep in the UK had been exposed to contaminated meat and bone meal, and that the

risk would be influenced by the genetics of susceptibility. In determining whether or not the national flock was infected and is maintaining the infection, issues such as the amount of exposure to contaminated feed (since sheep receive rather smaller amounts of MBM than bovines), the pathogenesis of BSE in sheep, and whether or not there could be sheep-to-sheep transmission had to be considered. In addition, SEAC asked the question, 'Is the BSE phenotype stable in sheep?' That is, if BSE had infected some sheep, would the phenotype change and how would it be detectable? Important questions about whether there is any risk to humans or to other species have not yet been answered.

SEAC recommended that ascertainment and diagnosis of TSEs in sheep needed to be addressed. Doing this would require that the case finding definition be reviewed, that surveillance systems be augmented, that past feeding practices be reviewed and that the sheep industry be consulted on how to undertake this work. Given the time since the initial exposures to contaminated MBM, surveillance must be able to detect serially passaged BSE in sheep populations.

Distinguishing between BSE and scrapie in sheep is currently dependent upon strain typing in mice, western blot and glycoform typing. However, improvements in the methods and speed of differential diagnostic methods would greatly improve the investigation of this problem. Pathogenesis studies to determine which tissues of sheep infected with BSE are infected were recommended.

Finally, SEAC supported the development of disease control programmes with the ultimate aim of eradication of scrapie in the long-term. Issues such as whether or not there is a carrier state and the question of environment persistence remain barriers to eradication at this time. The Ministry of Agriculture, Fisheries and Food (MAFF) is spending £13 million on research in this area this year, increasing to £15million next year. SEAC is reviewing its activities in this area; a subgroup on surveillance is being established.

2.6 European Union - Risk assessment of Bovine Spongiform Encephalopathy in sheep

Emmanuel Vanopdenbosh:

After over 250 years of observation, it has been generally accepted that the occurrence of scrapie in sheep and the incidence of CJD in humans are unrelated. However, the BSE epidemic in the UK in the 1980s, the recognition that BSE could infect humans, the realization that BSE can be experimentally transmitted to sheep and goats after oral challenge with brain material, and that the agent strain isolated from these sheep and goats was biologically indistinguishable from the BSE agent meant that a reassessment of the risk to human health from sheep and sheep TSEs was necessary.

In the first EU report¹ on this matter, in 1996, the Scientific Veterinary Committee (SVC) of the EC responded to WHO recommendations² that "Countries

¹ Scientific Veterinary Committee. 'Control Of Risks From BSE- And Scrapie-Infected Material In Regard To Protection Of Public And Animal Health'. VI/6665/96 October 1996.

should not permit tissues that are likely to contain the BSE agent to enter any food chain (human or animal)". The SVC determined that "...as for BSE in sheep, there is at present no evidence for a hazard, but it cannot be excluded as a possibility. The use of ovine and caprine SRM should therefore be prohibited in human food."

In October 1997 the EC approached the (now reorganized) Scientific Steering Committee (SSC): requesting that they

"... establish possible ways of transfer of the BSE agent to sheep and goats, identify the critical factors, assess the risk that the BSE agent exists in sheep and goats and evaluate the exposure of humans to the BSE agent through sheep and goats."

On the basis of the information reviewed, the following assessments were made:

- i. An assessment of the risk that sheep and goats have been exposed to the BSE agent and identification of the possible routes of exposure:

The SSC concluded that there is no evidence to suggest that BSE already exists in sheep and goats under field conditions. However, the evidence from transmission experiments and the likelihood that potentially BSE contaminated MBM had been fed to some sheep and goats suggests that BSE could have been introduced into the sheep and goat population. Although no experimental transmission via MBM has yet been successful, the oral route has to be considered as the most probable route of exposure for sheep and goats. Freedom from exposure via feed can not be assured until after the implementation of an effective feed ban in August 1996 in the UK.

In other countries, BSE risks in sheep must also be considered because of feeding practices, rendering practices, MBM exported from the UK, and recycling of raw infected sheep material. Other means of transmission such as the use of contaminated pharmaceutical or biological products are theoretically possible, as was recently illustrated by the accident with a scrapie contaminated *Mycoplasma agalactiae* vaccine in sheep in Italy. However, this event occurring elsewhere is regarded as unlikely provided currently recommended measures are in place and effectively enforced.

Finally, the theoretical spread of scrapie through nematodes or hay mites is difficult to assess at present.

- ii. Identification of critical factors contributing to the incident and propagation risks for BSE in small ruminants:

The same factors used by the EC to conduct the BSE geographical risk analysis in cattle, can be used for BSE in sheep:

² WHO Consultation on Public Health Issues related to Human and Animal Transmissible Spongiform Encephalopathies. Recommendation 2.2.3, p. 3. 2-3 April 1996.

- Structure and dynamics of the sheep population
- Animal trade
- Animal feed
- MBM bans
- Specified Bovine Offals (SBO) bans and SRM bans
- Surveillance of TSEs
- Rendering and feed processing
- BSE and scrapie related culling

In addition to this, special attention needs to be paid to the genotypes in the sheep population.

- iii. The assessment that BSE exists in small ruminants, is being maintained and spread:

If BSE in sheep behaves like scrapie in sheep, it is possible that the BSE agent has been maintained, propagated and recycled by horizontal and vertical transmission (n.b. maternal transmission of scrapie is unproven in goats). Hence, the risk could persist even after an effective implementation of a ruminant feed ban.

At this time, BSE and scrapie in sheep cannot be either pathologically or clinically differentiated. The appearance of scrapie-like disease in sheep of PrP genotypes in which natural scrapie does not frequently occur may be used as a potential indicator of the occurrence of BSE, although this needs further evaluation.

In a recent report of the 'Working Group on TSE Surveillance' of the EC, it is noted that no appropriate and reliable epidemiological data on scrapie incidence are available to assess whether there might have been an increase in the incidence of a scrapie-like disease caused by BSE infection in the different Member States. The working group therefore recommended that epidemiological work in each Member State is needed urgently to establish the true prevalence of clinical TSE in small ruminants and to identify the different genotypes in the different breeds.

- iv. The public health risk from BSE in sheep and goats:

The risk to humans would most likely come from eating infected food, using pharmaceutical products prepared from infected sheep and goat tissues, and the handling of these tissues. The risk could come from animals in the pre-clinical and clinical stage of the disease and possibly from silent carriers. By excluding SRM, the risk will be considerably reduced. Reducing the age at slaughter and application of validated processing methods to reduce any residual BSE infectivity could be useful. The development of rapid tests able to differentiate between scrapie and BSE in live small ruminants would assist in achieving effective control.

Conclusions:

The SSC has stressed that pro-active actions are needed throughout the EU, before the hypothetical is converted to reality.³ Three main barriers were identified:

1. Validation of large-scale, low-cost, specific and sensitive tests for *post mortem* and *ante mortem* TSE detection in small ruminants;
2. Development of large-scale, applicable, low-cost, specific and sensitive tests differentiating scrapie from BSE;
3. Definition of the parameters for a high quality epidemio-surveillance network for TSE in small ruminants.

A number of actions were proposed in the fields of assessment of the geographical risk, epidemio-surveillance and research:

1. Assessment of the geographical risk of BSE occurring in sheep.

The risk that sheep and goats have been fed BSE-contaminated MBM should be assessed urgently for all EU countries and a method for the assessment of the geographical risk of BSE occurring in sheep by an appropriate adaptation of the SSC method for the geographical BSE risk assessment in cattle, should be developed.

2. Surveillance.

An epidemio-surveillance system for TSE in sheep should be started in all EU member states, beginning with an awareness programme. A statistically representative number of sheep should be tested for TSE, as soon as tests become available. Furthermore, a scientifically justified regulation for the certification of TSE-free sheep flocks should be developed.

3. Research.

Research should be focused on the validation of large scale testing methodologies for TSEs in sheep (*post mortem* and *in vivo*) and on modelling BSE in sheep. Information networks should be created between research groups to keep all the concerned groups continuously informed on each other's progress. Even though the SSC felt that the theory that orally TSE-inoculated asymptomatic non-ruminants could transmit TSE infection through these tissues was unproven, some attention should be paid to this area.

As a general conclusion, the existence of BSE in small ruminants cannot be excluded, although there is currently no evidence to suggest that it occurs naturally under field conditions. A large number of UK's MAFF and EC-funded studies on different aspects of BSE in small ruminants have started and should give valuable information in the near future. This matter should be taken very seriously, and it is reassuring that both the EU Scientific Steering Committee and the SEAC Subgroup on Research and Surveillance for TSEs in sheep came, independently, to the same conclusions.

³ Research and Surveillance of TSEs in Sheep and Goat in Relation to The Risk of Infection with the BSE Agent and Actions to be taken at EU Level, adopted at the SSC meeting of 27-28 May 1999.

2.7 Office International des Epizooties - Measures relating to BSE

Thierry Chillaud:

The chapter on bovine spongiform encephalopathy (BSE) in the *International Animal Health Code* (the *Code*) of the OIE results from the application of procedures for the development and adoption of animal health standards for international trade in force at the OIE. Requests for updating may come from many different sources: the International Committee, i.e. the general assembly of the Delegates (representatives designated by their Governments) of OIE Member Countries, individual Delegates, the OIE Regional Commissions, other OIE Specialist Commissions (Standards Commission and Foot and Mouth Disease and Other Epizootics Commission), or other sources.

Suggestions received at the OIE Central Bureau are transmitted to the International Animal Health Code Commission, which examines them during its meetings and seeks, if necessary, the advice of the other Specialist Commissions or of internationally renowned specialists. When the Code Commission has sufficient information to reach a conclusion, it prepares draft articles or chapters for the *Code*. The draft documents are sent to the Delegates of all OIE Member Countries for examination and comment, frequently accompanied by a supporting document setting out the latest scientific knowledge on the subject in question.

The Commission examines the written comments by the Delegates on the report of the September meeting of the Commission when it meets in January. The report of the January meeting serves as the basis for discussions within the International Committee during its General Session in May. During this Session the Committee decides whether to adopt the texts prepared by the Code Commission as they stand or subject to amendment. It may also consider that a proposal deserves examination in greater depth, in which case it requests the Commission to prepare a fresh draft during its subsequent meetings to take into account the Committee's observations. The decision to adopt, expressed in the form of a Resolution, is generally by consensus.

The OIE International Committee adopted a *Code* chapter on BSE for the first time in May 1992. Since then it has been amended each year, to take into account the latest scientific knowledge⁴. It is associated with a supporting document, which was last updated in January 1998.

The chapter begins with two articles listing the criteria (risk factors, components of surveillance) and conditions (incidence of the disease, measures relating to management of identified risks) allowing the animal health status of a country or zone to be determined in respect of BSE. Through a combination of these criteria and conditions, a country or zone can be attributed one of the following 4 animal health statuses: free from BSE, provisionally free, of low incidence, or of high incidence. This is followed by an article listing the commodities that do not present a risk, whatever their origin, and an article stating what certification is required to export other commodities from a BSE-free country or zone.

⁴ Chapter 3.2.13 *Bovine Spongiform Encephalopathy*. OIE International Animal Health Code. Eighth edition, 1999.

The following articles stipulate the animal health conditions under which potentially dangerous commodities may be the subject of international trade, where such commodities are from a country or zone that is not BSE-free. The poorer the status of the country or zone of origin, the more stringent the conditions that must be met. The commodities of bovine origin covered by these articles are: live cattle, fresh meat and meat products, embryos/ova, meat-and-bone meal, specified risk material, gelatin and collagen (prepared from bones), tallow (other than protein-free tallow) and products derived therefrom, ingredients or reagents used in the manufacture of medicinal products.

In May 1998, the OIE International Committee adopted a Resolution⁵ requesting the Foot and Mouth Disease and Other Epizootics Commission to develop a procedure to enable the OIE to recognize the BSE-free status of certain of its Member Countries. The Commission is due to present its proposals on the subject to the International Committee in May 2000. If these proposals are accepted, the procedure for examining the documentation presented by Member Countries in support of their application for recognition can begin, and an initial list of countries recognized by the OIE as being BSE-free could be adopted in May 2001.

Section 3. Scrapie Elimination

3.1 Principles, practice and experience of scrapie elimination

Linda Detwiler:

Scrapie is an insidious degenerative disease affecting the central nervous system of sheep and goats. The disease was identified over 250 years ago in Great Britain. Scrapie is the prototype of the diseases known as the sub-acute transmissible spongiform encephalopathies that affect humans and some animal species. Philosophies about scrapie control/eradication have varied greatly from country to country. Certain countries have acted aggressively and utilized broad depopulation measures, taking not only the infected flocks but also all flocks containing exposed animals. Other countries have implemented partial flock depopulation (e.g. bloodlines, high-risk) programmes, certification programmes, and genetic control programmes or have chosen not to include scrapie as a regulated disease. To date, attempts to control and eradicate scrapie in most countries have not been successful unless there has been early detection and wide-scale depopulation measures employed immediately after discovery.

Lack of a live animal pre-clinical test for scrapie has been the largest impediment for eradication. Without this tool, disease detection has relied on owner or veterinary practitioner reporting. There are many factors which influence reporting. These include, but are not limited to, knowledge of the disease and where to report, consequences, amount of indemnity, and stigma. The lack of a pre-clinical test has also allowed for the questioning of disease freedom in a country, region, etc.

⁵ Resolution No. XII Recognition of the bovine spongiform encephalopathy status of Member Countries. Adopted by the International Committee of the OIE on 29 May 1998.

Scrapie was first detected in the US in 1947. Regulatory actions were not introduced until 1952 and the disease has become endemic. Over the past 50 years a number of different approaches to scrapie control have been attempted. The measures and reasons for less-than-total success were discussed, as were new technology and how it may be utilized in future control/eradication programmes.

3.2 Transmissible Spongiform Encephalopathies in sheep – Surveillance programmes

Danny Matthews:

Dr. Matthews emphasized that whether searching for scrapie or BSE in sheep, careful planning is essential if surveillance programmes are to deliver interpretable results. The design of a surveillance system will be different if the objectives are for research, or protection of animal health or protection of human health. In addition, as in the case of BSE in sheep, if the disease being sought is expected to be rare, or must be identified in the pre-clinical state, then the design of the surveillance system must reflect this. In addition, researchers must be aware that all attempts to interpret results and redefine objectives must await completion of pre-planned milestones.

Currently used methods of surveillance in the UK include compulsory notification (with slaughter and compensation), strain typing of scrapie isolates, postal surveys and abattoir surveys. Results were provided, with a critique of the weaknesses of currently used methods. These methods are probably not adequate to meet the goals identified above. Other important factors include the size of the native population of sheep (almost 40 million) and the large number of outlets for disposal of fallen stock (there are about 370 renderers, knackeries and hunt kennels).

Furthermore, there are important limitations in the traceability of individual sheep. Dr. Matthews noted that case definition is crucial whether targeting clinically affected or healthy sheep. The current state of knowledge regarding pathogenesis, pathology and the interplay between genotype and strain, and their consequential effect on the selection of appropriate test samples and diagnostic tests mean that at present there is no single definitive tool that meets global needs. Dr. Matthews stated that it was important to take into account the economic and social consequences of diagnosis if surveillance and consequential control are not to be undermined.

3.3 Breeding scrapie resistant sheep: The genetics of Transmissible Spongiform Encephalopathies in sheep

Nora Hunter:

The incidence of TSEs in sheep is linked to PrP genotype, with codons 136, 154 and 171 being of major importance. Most scrapie-affected sheep are homozygous for glutamine (Q) at codon 171 and succumb to disease, in order, with ARQ/ARQ, VRQ/VRQ, VRQ/ARQ genotypes. The details of the genetic links with disease are complex, and are breed and probably country specific. However the common forms of scrapie do not represent a genetic disease as the susceptible PrP genotypes can be found easily in sheep from scrapie-free countries such as Australia and New Zealand. For an animal to develop scrapie, therefore, a susceptible PrP genotype and exposure

to infection are both required. It is not understood how natural transmission of scrapie occurs and maternal transmission of disease and environmental reservoirs of infection are both possibilities that are being investigated at the moment.

Breeding for resistant PrP genotypes is now being carried out in the UK and elsewhere, bringing in with it worries about the potential long-term effects. Scrapie resistant animals could, for example, be less healthy in other respects or could be more susceptible to other diseases. In addition, it has been suggested that the development of an entire national flock of ARR/ARR genotype (the most resistant known) could result in inadvertent selection of rare scrapie strains able to cause disease in this genotype. Although there is no evidence for any of these suggestions yet, other options for disease control are being investigated in a major epidemiological study of UK sheep flocks.

3.4 Industry and scrapie elimination: Perspective of the Scrapie Information Group

John Thorley:

The Scrapie Information Group (SIG) is a UK Ministry of Agriculture sponsored group, established in 1997 to foster understanding and collaboration between different parties involved in the day-to-day surveillance and control of scrapie. The Group keeps a watching brief on scrapie research and development and offers advice to industry. It includes representatives of government departments, research institutes, veterinary organizations, and of industry bodies.

The long-term objective of scrapie control for SIG is elimination. Time, and tools such as diagnostic tests in live animals, further research and particularly improved surveillance and control of scrapie are all necessary before this goal can be achieved. Mr. Thorley noted that in sheep farming communities, scrapie is perceived as a declining animal health problem, not a potential human public health problem. He drew attention to the fact that even with 40 million sheep on over 75,000 farms in the UK, most sheep farmers have never seen scrapie. In addition, he pointed out that the impact of an ill-informed 'news' story erroneously declaring scrapie to be a human health problem could precipitate a real economic disaster for the livestock industry, sheep farmers and their families. The effect would be to undermine the willingness of sheep farmers to cooperate in the research and surveillance effort. It is vital therefore that the research goals and the motivations for the research are properly explained to farmers. The reasons for needing the help of sheep farmers to conduct this work and the potential benefits to sheep farmers must also be explained. In particular, it must be explained why it is in everyone's interest to participate in scrapie/BSE research and surveillance.

SIG provides a dedicated forum for informing and educating sheep farmers in this way. For example, early in year 2000 it will host the first workshop of sheep farmers, veterinarians and others, in which experts will brief participants on research, surveillance and control work. The workshop participants will provide a report to SIG on the barriers to farmers' reporting of scrapie and participation in research projects. SIG has supported UK Ministry initiatives to improve surveillance, which include publication of a video, distribution of an advisory note on the clinical signs of

scrapie to sheep farmers, and articles to raise the general awareness in the sheep farming community of scrapie.

Mr. Thorley ended his talk with three watchwords: *Communicate*, *Consider* and *Collaborate*. He emphasized the importance of cultivating goodwill and dialogue with the industry, saying that these efforts would pay dividends in scrapie control.

3.5 Animal vaccine-related Transmissible Spongiform Encephalopathy risks: Scrapie outbreak in Italy

Maurizio Pocchiari:

Historically, Italy has had a low incidence of scrapie; however, in 1997 there was a dramatic increase in the number of reported flocks. This increase in reports included a relatively high proportion of goat flocks, generally considered more resistant to natural scrapie than sheep. Details of the timing of the flock outbreaks, composition of the flocks and vaccination status were provided. It was noted that vaccination for *Mycoplasma agalactiae* was provided to a large proportion of the flocks developing scrapie, and that this vaccine is made from sheep brain and mammary gland. Small batches of brain and mammary glands are mixed, and given subcutaneously to adult and young animals, providing considerable possible exposure to contaminated material. Some flocks receiving this vaccine did not develop scrapie, and western blot and transmission experiments are underway.

However, the consultation agreed that the epidemiologic evidence points toward a vaccine origin for the scrapie disease seen in some of the flocks.

Section 4. Chronic Wasting Disease

4.1 Epidemiology of Chronic Wasting Disease

Michael Miller:

CWD is a naturally occurring TSE of native North American deer (*Odocoileus* sp.) and wapiti (*Cervus elaphus nelsoni*), and is apparently endemic in a few deer populations residing in western North America. While a chronic wasting disease of deer was first recognized by biologists in the 1960s as a disease syndrome of captive deer held in wildlife research facilities in Fort Collins, Colorado, it was not recognized as a TSE until the late 1970s. To date, approximately 500 CWD cases (including free ranging, privately owned, and research cervids) have been documented.

Targeted surveillance (examination of clinical case suspects) has revealed an endemic focus of CWD in free-ranging deer and wapiti in north-eastern Colorado and south-eastern Wyoming, US. CWD has probably been occurring in this area for well over 25 years. Based on random surveys conducted to date, CWD does not appear to be widespread in free-ranging cervids: brainstems from >4500 deer and wapiti harvested or road-killed outside CWD-endemic portions of Colorado and Wyoming examined via histopathology or immunohistochemistry (IHC) have shown no evidence of CWD infection. In contrast, about 5% of mule deer, 2% of white-tailed

deer, and 0.5% of wapiti from endemic portions of Colorado and Wyoming show evidence of pre-clinical CWD infection.

There is no clear epidemiological connection between CWD cases in free ranging and privately owned cervids, although some historical relationship is possible. Cases have been detected since 1996 in farmed wapiti herds from Colorado, Montana, Nebraska, Oklahoma, and South Dakota, US, as well as from Saskatchewan, Canada. Additional cases are likely to be detected in the cervid industry in the near future as surveillance efforts increase.

Of the animal TSEs, epidemic dynamics of CWD most closely resemble those of scrapie in sheep. Lateral transmission (direct and/or indirect) appears to drive the epidemic dynamics of CWD. Experimental and circumstantial evidence suggest infected deer and elk probably transmit the disease through animal-to-animal contact and/or contamination of feed or water sources with saliva, urine, and/or faeces. CWD seems more likely to occur in areas where deer or elk are crowded or where they congregate at man-made feed and water stations. Although CWD does not appear to be transmitted via contaminated feed, artificial feeding of deer and elk may compound the problem. This may in part explain the intensity of infection in some cervid populations housed in farm or research settings. Despite similarities with scrapie, there is no evidence suggesting either scrapie or BSE are caused by contact with wild deer or elk, or that wild deer or elk can contract either scrapie or BSE in countries where these diseases occur.

As a general precaution, public health officials recommend that people avoid contact with deer, elk, or any other wild animal that appears sick. Deer and elk affected with CWD show progressive loss of body condition accompanied by behavioural changes. In the later stages of disease, emaciation, excessive salivation, increased drinking and urination, stumbling, trembling, and depression may precede death. The clinical course of CWD appears to be progressive and irreversible, ultimately leading to the death of affected animals. Because the clinical signs of CWD are relatively non-specific, laboratory examination of clinical suspects is essential for confirming this diagnosis.

At present, the diagnosis of CWD is based on microscopic examination of brain tissues (specifically, the medulla oblongata at the obex) from suspected cases. Both histopathologic examination and immunohistochemistry (IHC) are used in routine diagnosis of clinical cases, and may also be used to detect pre-clinical cases in surveillance and monitoring programmes. Western blots and negative-stain electron microscopy have also been used to further confirm diagnoses. There are currently no validated live-animal tests for diagnosing either clinical or pre-clinical CWD in either deer or elk; however, research is underway to evaluate several promising avenues for antemortem diagnosis.

Both sexes and all ages appear uniformly susceptible to infection in all three host species; there is some evidence of genetic resistance to CWD among wapiti, but not among either deer species. Although its unique features bear consideration, tools and approaches generally used in studying other TSEs, particularly scrapie, appear to apply to CWD epidemiology. Dr. Miller recommended that additional surveillance to discern the true distribution of CWD be encouraged. Potential strain variation,

interrelationships with other TSEs, and the role of environmental variables in epidemic dynamics, natural spread, and persistence of CWD were also noted as meriting further study.

4.2 Diagnosis and pathogenesis of Chronic Wasting Disease

Beth Williams:

CWD is a prion disease naturally affecting mule deer, white-tailed deer, and elk (wapiti). Research by a number of agencies and institutions is in progress on many aspects of the disease. Several pathogenesis studies are nearing completion: mule deer and elk have been orally inoculated and the progression of disease is being followed sequentially.

Dr. Williams described three major studies underway in her laboratory, involving oral exposure of both mule deer and Rocky Mountain Elk to brain material from elk with clinical disease. These studies are being used to study the incubation period of CWD and to examine tissues of deer at sequential periods after infection, to determine which tissues are infected and when. Preliminary results to date indicate that these species are readily infected orally, PrP^{res} may be detected by immunohistochemistry (IHC) in the parasympathetic vagal nucleus of the medulla oblongata of the brain by approximately 6 months post-inoculation, and that accumulation of PrP^{res} occurs in lymphoid tissue months prior to the ability to detect it in the central nervous system. Appearance of clinical signs is associated with development of spongiform change. The pathogenesis of CWD in mule deer and elk appears to be similar.

Dr. Williams provided detailed information on the methods used for histopathology and immunocytochemistry, and a comparison of the efficacy of a number of antibodies for Western blotting. She states that immunohistochemistry is an extremely valuable diagnostic and research tool for CWD and is more sensitive than standard histopathology and western immunoblotting. Approximately 40% of deer diagnosed with CWD in surveillance studies show only accumulation of PrP^{res} in the brain by IHC and do not have spongiform lesions. Optimization of the techniques for conducting IHC for CWD is necessary for each laboratory. A mouse monoclonal primary antibody (89/160.1.5 prepared by Dr. K. O'Rourke) gives excellent results.

Strain typing by mouse passage suggests that CWD is unique and does not resemble BSE, CJD, or scrapie (Dr. M. Bruce, personal communication).

A series of studies regarding cattle susceptibility are also underway. Cattle from non-CWD endemic areas have been challenged intracerebrally and orally with inoculum from a mule deer brain pool. In addition, cattle have been left to co-habitate with clinically ill deer. Finally, the brains of cull cattle are being examined. At this point, CWD has not been diagnosed in any of the cattle experimentally exposed to CWD, nor in the contact and cull populations.

Other studies of CWD in progress include additional strain typing, development of techniques for antemortem diagnosis, and investigations of PrP genetics in deer.

4.3 Assessing interspecies Transmissible Spongiform Encephalopathy transmission using the cell-free conversion assay

Gregory Raymond:

Wild or captive deer and elk in certain areas of the US (Colorado, Wyoming, South Dakota, and Montana) are infected with CWD. Little is known about the transmissibility of CWD from deer and elk to other species. At least one animal transmissible spongiform encephalopathy (TSE) strain, BSE, has infected humans and many other mammalian species through the food supply. Hence, it is important to consider the possibility that CWD could also be transmitted to humans or other mammals exposed to CWD-infected cervid species. The transmissibility of CWD to animals can be tested directly; however such experiments will take years to complete because of the long incubation periods encountered in interspecies TSE transmissions. In addition, since CWD transmissibility cannot be tested directly in humans, we have sought alternative methods to help provide clues to the potential interspecies transmissibility of CWD.

The conversion of the normal protease-sensitive host prion protein (PrP-sen) to a disease specific protease-resistant isoform (PrP-res) is a key biochemical event that occurs in the pathogenesis of all TSE diseases. We have developed a cell-free system in which PrP-sen is converted to PrP-res⁶. The conversion efficiencies of various combinations of PrP-res and PrP-sen that have been analyzed correlate remarkably well with known *in vivo* transmissibility, suggesting that this assay could be useful as an indicator of the transmission potential between species⁷.

Previously we used this conversion system to gauge at the molecular level the potential transmissibility of scrapie and bovine spongiform encephalopathy (BSE) to humans. We observed weak conversion of the normal human PrP-sen to the abnormal PrP-res isoform when incubated with PrP-res isolated from the brains of either scrapie-infected sheep (ov-PrP^{Sc}) or BSE-infected cattle (PrP^{BSE}). However, the efficiencies of these heterologous conversion reactions were much lower than homologous human, bovine, and sheep conversions. The similar but poor efficiency of PrP^{BSE} or ov-PrP^{Sc} to convert normal human PrP-sen suggests that, at least at the molecular level, the inherent ability of these diseases to affect humans following exposure is likely to be finite, but similarly low.

Using the cell-free assay, we have found that the CWD-associated PrP-res (PrP^{CWD}) of cervid species induced the conversion of cervid PrP-sen molecules robustly, correlating with known transmissibility of CWD amongst cervid species. In contrast, PrP^{CWD} induced moderate conversion of ovine PrP-sen and similarly weak conversions of human and bovine PrP-sen molecules. The efficiency of PrP^{CWD}-induced conversion of human PrP-sen was similar to that induced by PrP^{BSE} and less than that induced by ov-PrP^{Sc}.

The implication of these results regarding the potential transmissibility of CWD to humans is unclear because, while BSE appears to be at least weakly transmissible

⁶ *Nature*, V.370:471-474; *Chem. & Biol.* V-2:807-817.

⁷ *PNAS*, V.92:3923-3927; *PNAS*, V.94:4931-4936; *Nature*, V.388:285-288.

to humans, there has been no epidemiologically measurable transmission of sheep scrapie to humans. Nonetheless, these results suggest the existence of a molecular incompatibility that is likely to limit the transmissibility of cervid CWD strains to these non-cervid species.

4.4 Centers for Disease and Control and Prevention investigations relating to Chronic Wasting Disease exposures

Ermias Belay:

During 1997-1998, three CJD patients 30 years of age and under were reported to Centers for Disease Control (CDC), Atlanta, Georgia. Two of the cases were hunters and one case was reported to have regularly consumed venison. The occurrence of these unusually young CJD cases created a concern about possible zoonotic transmission of chronic wasting disease CWD of deer and elk.

Review of the clinical records and pathologic studies of brain tissues of the patients indicated clinicopathologic features consistent with other sporadic CJD patients. None of the cases were reported to have consumed deer meat obtained from the known CWD-endemic areas of Colorado or Wyoming. Unlike patients with vCJD, these patients did not have a unique characteristic pathology, clinicopathologic homogeneity among the cases, uniformity in the codon 129 of the prion protein gene, or protease-resistant prion protein immunoblot characteristics different from classic forms. Although the occurrence of these unusually young CJD cases suggested a possible relationship with CWD, the ongoing investigation, to date, does not support a causal link. Deer brain samples from the areas where the patients were reported to have hunted or obtained their venison will be tested to determine the presence or absence of CWD.

Section 5. Feline Spongiform Encephalopathy

5.1 Pathology and epidemiology of Feline Spongiform Encephalopathy

Gerald Wells:

The occurrence of the scrapie-like disease of domestic cats, FSE, in Great Britain has been monitored neuropathologically and epidemiologically by voluntary notification of cases since the report of the first case, with a clinical onset in 1989. To November 1999, a total of 87 cases of FSE have been recorded in Britain:

Total number of cases: 87 (+3)¹

Year	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999
Cases ²	1	16	11	14	10	14	4	7	8	1	1

¹ N Ireland (1), Norway (1) and Liechtenstein (1)

² By date of onset of clinical signs

Descriptive epidemiological features were determined by questionnaire to identify potential sources of exposure. The pathology of FSE has been defined by studies of the lesion profile of vacuolar changes and by PrP-immunostaining in the brains of confirmed cases of FSE.

The vacuolar lesion profile of FSE in domestic cats, based on examination of 60 neuroanatomical areas of the brain from 25 cases reported in the period 1990-92, is consistent among cases, but differs markedly from that of BSE in cattle. In cats the changes of FSE are invariably characterized by the most severe changes in the head of the caudate nucleus, medial geniculate nucleus and frontal cortex. Diagnostic criteria based on the profile have, as in the diagnosis of BSE, proved valid in all subsequent cases throughout the epidemic.

Detailed neuroanatomical profiling of PrP-immunohistochemical staining was carried out in five randomly selected confirmed cases of FSE in domestic cats using automated image analysis to determine the percentage area of PrP immunostaining according to 35 neuroanatomical sites throughout the brain. The PrP profile was consistent between cases but, in common with studies in other species, PrP distribution did not invariably coincide with the precise distribution of vacuolar changes.

Because cats with a wide range of neurological signs were referred by veterinarians for surveillance of FSE, neurohistological differential diagnostic examinations were made on unconfirmed cases to improve case definition.

From over 270 cats submitted between 1990 and 1996, the brains from a sample of 190, which on initial histological examination were considered negative for FSE (and were suitable for further examinations), were examined to determine differential diagnoses. By examination of routinely stained histological sections, brain changes of probable diagnostic significance were found in 67%. The remaining 33% of cases had no significant histological changes in the brains. No additional cases of FSE or other vacuolar encephalopathies were identified by this study.

Immunohistochemical examination for PrP in the brains of a sample of 214 "suspect" cases of FSE indicated no significant underascertainment of cases of FSE as a result of using histopathology as the sole laboratory diagnostic method.

Epidemiological findings were based on a sample of 80 cases. The most frequent clinical signs were ataxia, hyper-reflexia and behavioural abnormalities. Cases occurred in a wide geographical distribution with some apparent geographical variation in risk. Although there was incomplete ascertainment, peak animal incidences of approximately 4 cases per million of the cat population were estimated. Age-specific incidences were consistent with an incubation period similar to that for BSE of approximately 60 months. Data supported a foodborne source of exposure to a single, stable strain of scrapie-like agent.

Section 6. Transmissible Spongiform Encephalopathies in Other Species

6.1 Transmissible Spongiform Encephalopathies in captive wild bovids and felids

Gerald Wells:

In the period 1986-1998 light microscopic neuropathological examinations were conducted for the investigation of spongiform encephalopathies (SE) on approximately 124 submissions of non-domestic animals, representing some 50 different species. The majority of species from which material has been examined were zoo or wildlife species. Most were mammals but material from three avian species was also examined. Submissions were mainly from the UK but included also several from other countries. Material was submitted voluntarily from animals showing neurological or other clinical signs suggestive of SE or, from animals culled from collections, usually exhibiting signs of other disorders.

The total number of cases in which lesions consistent with a TSE was diagnosed is 37. The total number of species affected is 12. These species are all members of the families bovidae or felidae. Affected members of the family bovidae fall into two sub families, boviniae and hippotraginae.

Comparisons of the life span of the affected bovids and affected felids show interesting differences, with survival times of only 18-48 months for bovids but 5-18 years for felids. This may reflect possible differences in age at exposure or in susceptibility with age.

Lesion profiling of the changes in the brains of affected exotic bovids has been carried out. The profiles, albeit conducted of necessity on a very small number of cases, differ from that of BSE in domestic cattle. Confirmation of the accumulation of PrP by immunohistochemical examinations has added to the case definition of TSE in exotic species.

The high incidence of disease in the Cheetah (*Acinonyx jubatus*), nine cases to date, suggests relatively greater susceptibility of this species. The recent occurrences of TSE in the tiger (*Pantera tigris*) and lion (*Panthera leo*) suggests that there may be differences in incubation period among species of the family Felidae.

The occurrence of cases contemporaneously and geographically in association with the BSE epidemic, the potential for exposure of all the ungulate species affected to feeds containing meat and bone meal, and the history of feeding the carnivore cases with raw parts of cattle carcasses containing central nervous system tissues provides strong circumstantial evidence of a causal link with BSE. Evidence from mouse biotyping of isolates from two of the bovid species and of the domestic cat has further confirmed infection with an agent indistinguishable from that of BSE.

6.2 The implications to humans of zoo primates infected with Bovine Spongiform Encephalopathy

Noëlle Bons:

In 1996 a spongiform encephalopathy was described in macaque rhesus and large prosimians (lemurs) living in the zoological park of Montpellier. The abnormal isoform of the prion protein (PrP^{Sc}) was immunocytochemically demonstrated in the brain. PrP^{Sc} accumulation was also found in the digestive tract and lymphoid organs in these diseased animals and in healthy primates living in other French zoos. These observations led to suspicions that the diet (comprised in part by industrial food) contained BSE-infected meat. However, similar infections in primates in the UK and other EU countries where exposure could be anticipated have not been reported. The difference in findings between France and the other exposed countries is not fully explained. Transmission experiments from the PrP (+) primates have not been conducted.

The announcement of a new variant of the vCJD in 10 humans in the UK, implicating an oral exposure to BSE by meat, has shown the urgency to undertake experiments on the BSE transmission to the primates. Because of interest in the development of a primate model for TSEs, a group of non-human primates, *Microcebus murinus*, were intracerebrally or orally infected by BSE-contaminated brain of bovine or macaque. Behavioural changes and clinical signs were observed at 3, 7, 13 and 17 months after the inoculation, in the experimental lemurs. Microscopic examination of the central nervous system, the digestive tract and the lymphoid organs during incubation period and in diseased animals was performed, detecting the progression of the BSE agent. The existence of PrP^{Sc}, resistant to the proteinase K, signature of the infectious agent, was confirmed by Western blot.

This study has shown the susceptibility of the microcebe to the BSE infectious agent. The primary BSE transmission has been possible via intracerebral and also oral routes and the incubation periods were very short compared to the simians. Moreover these experiments confirm the ability of the BSE agent to cross the species barrier and its infectivity regarding the primates after oral exposure. Dr. Bons concluded that it is imperative to carry out more large investigations using the microcebe in order to elucidate not only the physiopathology of the prion diseases, particularly of vCJD, but also to evaluate the risks related to BSE transmissibility and the conditions of a vertical and horizontal contamination for the humans, and non-human primates bred in facilities.

6.3 Are there prion diseases of fish?

Liana Bolis:

Dr. Bolis was unable to attend the meeting. Dr. Pocchiari kindly agreed to summarize the material provided to the consultation in advance of the meeting.

The recent description of a normal form of the PrP isoform in the brain of a salmon triggered a proposal to study the susceptibility of fish to the agents of BSE and scrapie. If infection can be established, the expression level of these agents in various

fish tissues will be evaluated. A series of experiments is underway in a number of European laboratories (funded by the EU) to study the normal isoform of the prion proteins in normal fish, and to study the abnormal isoform in fish experimentally exposed to the TSE agents. The methods of exposure and assessment of TSE infection are being developed. No results were presented. The project is expected to run for two more years; some results will be available shortly.

6.4 The case for the zoonotic origins of Creutzfeldt-Jakob Disease

Robert Rohwer:

Dr. Rohwer began by suggesting that there may be many as yet unrecognized TSEs, pointing out that it is suspicious that we are only aware of TSEs in domestic and captive animals, and humans. He noted that a number of emergent diseases were actually only recognized when new reagents or diagnostic tests became available, or when the motivation to look for new diseases became very high. Moreover, wild animal reservoirs of TSE would be hard to recognize because the onset of disease is insidious, and affected animals would be subject to culling by predators or environmental factors before the disease could progress to an easily recognized state. Furthermore, we might not recognize TSEs that present with symptoms that are outside the spectrum of CNS signs that we currently attribute to TSEs.

He pointed out that CWD in deer was studied for a decade as a metabolic disorder before it was recognized as a CNS disease, let alone a TSE disease. He questioned the sheep origins of BSE and explained how the BSE epidemic could instead have originated in a single rare transmission from a wild animal source that was then amplified and widely disseminated through several generations of subclinical or unrecognized infection, rendering and feeding, so as to eventually present as an extended common source epidemic. The long incubation times and dose responses of TSE infections could, in this way, have conspired to hide a focal origin for the BSE epidemic in an extended common source presentation.

He pointed out that transmissible mink encephalopathy (TME) is clearly not an endemic disease of mink; rather mink acquire this disease from some unknown reservoir. If the proximal source of the mink infection is cattle, then cattle must also be a rare intermediate host between the true reservoir and mink as there has not been another outbreak of TME in the US since 1986 in spite of the continued feeding of cattle to mink.

Given the knowledge that there are a number of strains of CJD in humans, it could be that humans have more than one source of exposure to TSEs, and that the disease we currently call CJD could actually be a series of different diseases, acquired from different zoonotic sources. Transmission rates would be governed by size and prevalence of the reservoirs, the amount of human exposure to the reservoirs and the transmission efficiencies once exposed. A bottleneck at any one or more of these points could account for the low infection rates indicated by the sporadic form of the disease. Familial forms could be explained by increased genetic susceptibility to either endemic reservoirs or to person-to-person transmission within the intimate confines of the family.

Section 7. Evaluation of Animal Transmissible Spongiform Encephalopathy Zoonotic Potential

7.1 Diagnostic tests for Transmissible Spongiform Encephalopathies: Status and update of the European Union initiative

James Moynagh:

The EC carried out an evaluation of rapid diagnostic tests for TSE in bovines. Ten tests were submitted for evaluation. Of the ten tests, four were selected for detailed evaluation designed to assess their specificity, sensitivity and to determine their detection limits.

Positive samples were obtained from brains and spinal cord of cows which showed signs of BSE and where the disease had been confirmed histologically. Negative material was obtained from adult cows from New Zealand. Based on 1064 positive samples and 336 negative samples, three tests achieved 100% sensitivity and 100% specificity. The detection limits of the tests was assessed by testing diluted brain homogenate which had been previously titrated in mice to give a titre of $10^{3.1}$ i/c i/p LD50 doses. The detection limits of these tests varied from 10^{-1} to $10^{-2.5}$ of this homogenate.

On the basis of these results they concluded that the three tests had excellent potential for use for diagnostic purposes or for screening casualty slaughtering or dead animals being sent for rendering. The high specificity shown also indicated the possibility for using these tests for more general screening. The ability of the tests to detect the diluted material has indicated the possibility of the tests detecting diseased animals before the display of obvious clinical signs. However, it is not yet possible to quantify such effects.

Further work including the assessment of the behaviour of diagnostic tests in samples from asymptomatic infected animals is underway. The EC will also be launching a second evaluation of diagnostic tests in the very near future. This evaluation will be targeted at tests that show promise for pre-clinical use; tests that could distinguish BSE from scrapie or that offer advantages over those tests that have already been evaluated.

7.2 Potential of diagnostic tests to distinguish between Bovine Spongiform Encephalopathy and scrapie

Jean-Philippe Deslys:

Pr^{Pres} has been reported to exhibit specific electrophoretic patterns depending on the infecting strain of TSE agent, theoretically opening new avenues for strain typing by biochemical methods. However, this application of the western blot seems difficult to standardize and is still not amenable for uses as a large-scale screening test.

Promising results have been obtained with another approach based on the controlled degradation of Pr^{Pres}, and detection of the remaining Pr^{Pres} by a two-site

immunometric method, allowing distinction between BSE and scrapie associated Pr^{Pres}. The original method of Pr^{Pres} detection has already been validated as the « CEA test » within the framework of the BSE-test evaluation conducted by the EU. The next step towards the development of a test in sheep is the validation of the method using samples from BSE-infected sheep to determine if the characteristics of Pr^{Pres} are similar to those found in BSE samples from other species.

7.3 Diagnostic protocols for Transmissible Spongiform Encephalopathies: Rationales and their applications

Gerald Wells:

The selection and application of appropriate protocols for the case finding and diagnosis of TSEs or prion diseases is an increasingly complex issue. In this presentation only pathognomonic features of pathology, PrP detection and infectivity were considered. The selection of a protocol must be related to the spectrum of purposes for which diagnosis is required. These will extend from the confirmation of clinical diagnosis in the control of overt disease to the screening of healthy populations for covert or pre-clinical disease.

Crucial also to the selection of a diagnostic method is the case definition that is to be adopted in relation to the purpose of the diagnosis. The case definition used will vary according to that purpose, dependent on the species, phenotype/strain of the TSE and the epidemiological context (epidemic/endemic disease or surveillance for disease freedom). A detailed knowledge of pathogenesis relative to phenotype of disease will be required if appropriate neural and non-neural tissues are to be targeted as samples for detection of pre-clinical disease. Assumptions, for example, that the presence of PrP^{res} equals infection must be stated in the case definition to be adopted. It is not practical to employ detection of infectivity by bioassay as a diagnostic approach but it will be important that bioassay be used in case definition of newly identified disease phenotypes and for reference control sample purposes.

Excluding bioassay, methods currently available for the diagnosis of TSE/prion diseases are:

- Histopathological examination of brain
- SAF detection by electron microscopy
- Immunohistochemical demonstration of PrP^{res}
- Western blotting for PrP^{res}
- Other immunoassays for rapid screening for PrP^{res}

The validation of any diagnostic method must complete five stages:

- Feasibility
- Development and standardization
- Characterization of assay performance
- Monitoring assay performance
- Maintenance and extension of validation criteria

The variables affecting assay performance will include:

- Sample - selection and quality
- Assay system - can the system detect the specific analyte?
- Test results - can the test predict the status of the host (sensitivity and specificity) relevant to the target population (predictive value)?

All current methods used in TSE diagnosis have applications dependent on the context. New approaches require to be completely validated against appropriate standards of comparison. In the selection of tests for introduction into surveillance programmes there are further practical considerations which include the infrastructure of the country and its testing capacity, cost/benefit issues and the feasibility of being able to re-validate a particular test for a new target population. The availability of reagents and the ability to maintain expertise and quality assurance also feature in this decision process. Standardization of a test procedure, although desirable, may require to be sacrificed for the “robustness” of that test on transfer to other laboratories.

7.4 Diagnostic tests and standardized reagents: WHO initiative

Ana Padilla:

On 22-23 March 1999, WHO held a Consultation on Diagnostic Procedures for Transmissible Spongiform Encephalopathies: Need for Reference Reagents and Reference Panels.⁸ The goal of the meeting was to assess needs for standardization and make recommendations for WHO activities in this area. The consultation realized that there was an important need for reliable non-invasive tests to detect asymptomatic subjects during the long incubation period of CJD. They recommended that WHO form a Working Group on International Reference Materials for Diagnosis and Study of TSEs.

This group was formed and met for the first time on 21-22 September. A report documenting this meeting, listing the terms of reference and identifying the members has been published.⁹ Among their conclusions are recommendations to form reference panels and to titrate infectivity in each candidate biological reference material. Panels will be prepared in replicate coded randomized dilutions of one or more candidate biological reference materials. The next meeting will be in May 2000.

7.5 The Rendering Industry – Current sourcing and current product uses in animals

Dr R. Oberthür and Mr D.A. Franco:

The rendering industry plays a major role in the recycling of animal fats and proteins. Those not used for human consumption go mainly into products used in animal feed, and in industry, e.g. in fat and protein chemistry (detergents, functional proteins and amino acids) and ceramics (bone china).

⁸ *WHO Consultation on Diagnostic Procedures for Transmissible Spongiform Encephalopathies: Need for Reference Reagents and Reference Panels, March 22-23, 1999.* Blood Safety and Clinical Technology. WHO, Geneva, June 1999.

⁹ *Report of the Working Group on International Reference Materials for Diagnosis and Study of TSEs.* Blood Safety and Clinical Technology. WHO, Geneva, 21-22 September 1999.

Worldwide, 68 million metric tons (Mt) of pure protein are fed yearly to animals, mainly to pigs and poultry, most of which become meat for human consumption. Eighty-six percent of these feed proteins are of plant origin, mainly from soy beans, but of the remaining fourteen percent (9.5 Mt), 5.5 Mt are animal proteins processed from by-products of the meat production and 3.9 Mt are fish proteins from catches not used for human consumption.

Animal protein comes from the recycling of by-products from meat production in industrialized countries, mainly North America, western Europe and Australia. In developing countries, most of these by-products are used in human food. As a consequence, almost all of the world's 10 Mt of animal protein meals are produced in North America, western Europe and Australia, and used either at home or exported into the rapidly developing areas of eastern Europe, the near East and South-East Asia.

After the emergence of BSE in the UK, the European rendering industry was affected in several ways, as the use of ruminant-derived proteins in ruminant feed was prohibited (1988) and SRM (bovine CNS-tissue) were excluded from rendering (1990). These measures were initially restricted to countries or regions with the highest BSE incidence (England and Wales) and later extended to other regions and countries with significant BSE incidence (UK, Switzerland, and Portugal are the countries with the highest BSE incidence).

Pilot plant experiments were initiated to explore the effectiveness of rendering systems to inactivate the TSE agent (1990 and 1991). Based on the obtained results, initially some systems (1994) - later all systems except pressure cooking at 133 °C - were excluded from rendering of mammalian tissues (1996). A joint European project¹⁰ began in 1999 to systematically determine the inactivation kinetics of TSE-agents under various technologically relevant but well-defined conditions.

¹⁰ FAIR J-CT98-7019.

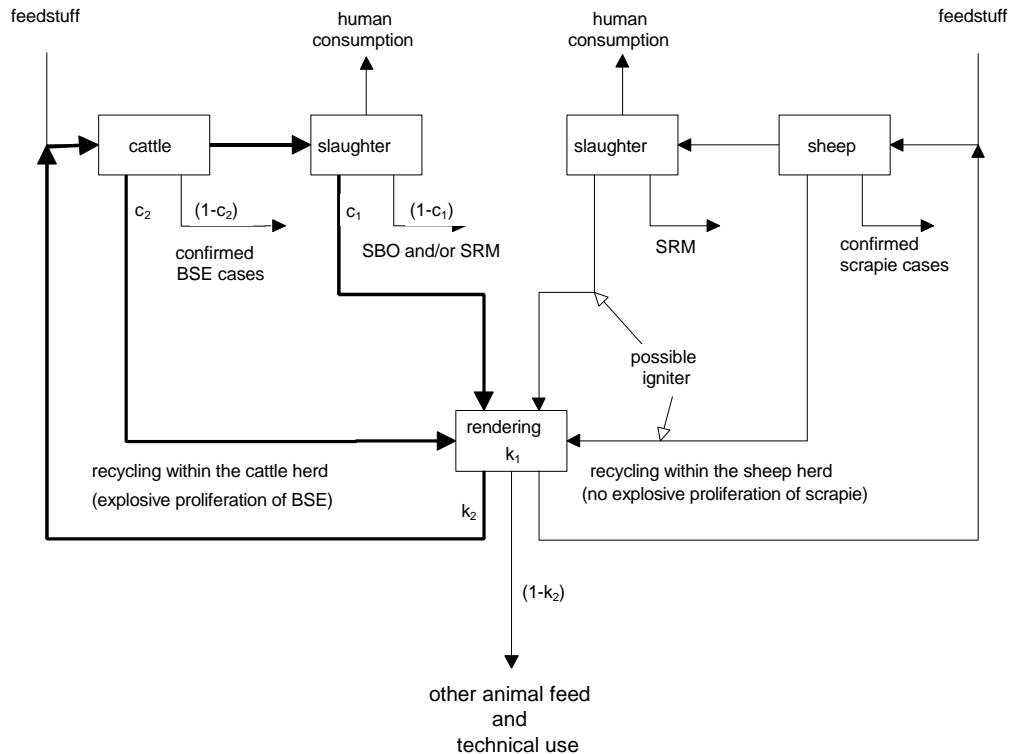


Fig. 1: Flow sheet for the recycling of animal by-products from sheep and cattle. Recycling within the cattle population is shown by the thick lines. The letters along the flow lines indicate the percentage removal of confirmed BSE cases (c_2) from the fallen stock, the percentage removal of SRM (c_1) from the slaughterhouse offal, the reduction of infectivity within the rendering process (k_1), and the percentage of processed cattle protein used in cattle feed (k_2).

The different measures taken to prevent transmission of BSE have been evaluated using epidemiological calculations, creating as a criterion the so-called reproduction number.¹¹ When this number exceeds one, magnification of the epidemic occurs. When it is less than one, magnification does not occur. From these data it was modelled that measures already taken decreased the probability of propagation by 2.2 orders of magnitude¹² leading to an exponential decrease of the BSE incidence in the UK from 1993 onwards. In fact, if these later conditions had been present when the first case occurred, an explosive outbreak would not have taken place. However, because a great number of infected, but undetected, animals are still present in countries with high incidence, even a low probability of infection will create an appreciable number of new infections. This makes regionalization of measures reasonable.

It is interesting to note that although the same processed proteins were fed to sheep as to cattle, an explosive outbreak of scrapie in sheep did not occur. This is

¹¹ Diekmann O., Heesterbeek H. (2000), *Mathematical Epidemiology of Infectious Diseases: Model Building, Analysis and Interpretation*. Ed. John Wiley & Sons. ISBN 047 1986828.

¹² de Koeijer A.A., Heesterbeek J.A.P., Oberthür R.C., Schreuder B.E.C., de Jong M.C.M. *BSE Risk Assessment. Calculation of the reproduction ratio for BSE infection among cattle*. Agricultural Research Department. Institute for Animal Science and Health, P.O.Box 65, 8200 AB Lelystad (The Netherlands), 1998

likely due to the much lower percentage of ruminant protein fed to sheep than to cattle.

In comparing former rendering practices in different countries, it appears that several were close to the critical situation where a propagated epidemic with exponential proliferation could have occurred. However, using the model, it can be predicted that a reproduction number of less than one can easily be obtained by: less feeding of bovine proteins to cattle (safer use); a more effective rendering system with respect to the inactivation of the BSE agent (safer processing); or either less scrapie occurrence in a given country or less inclusion of bovine brain and spinal cord for rendering (safer sourcing); all in comparison to the situation in the UK before 1988.

Also predicted from the model is that, of the various restrictions introduced after the discovery of BSE, the ruminant-to-ruminant feed ban alone stopped the explosive proliferation (reduction by a factor of more than 100). However, the same would have happened with a removal of the highly infectious CNS material from all processed bovine raw material alone (reduction by a factor of about 100, if done properly), or by more stringent processing of animal proteins.

The pilot plant inactivation trials¹³ have shown that the rendering systems in use in Europe in the beginning of the 1990s yielded deactivations between zero and four orders of magnitude, where four orders of magnitude was the limit of detection. The only technique able to yield inactivation levels of four orders or more is pressure cooking of the raw material at 133 °C and three-bar water vapour pressure. This is about the same as could be achieved by specified risk material removal at the source and the ban of ruminant proteins in ruminant rations for the final use.

Doubts about pressure cooking have come from experiments suggesting that TSE-agents might survive in stabilized niches in their environment.¹⁴ New experiments are therefore planned to check whether stirred-matrices, as in rendering processes, yield better inactivation than matrices-at-rest.

The industry recognizes a number of factors relevant to safety: safe sourcing, safe processing and safe use, all combined with tailoring measures to reflect the incidence of BSE. Restrictions in animal protein and fat processing and marketing should only be based on best scientific evidence with respect to the safety of human and animal health. At the same time an ecological reasonableness must be observed. Economic, political and ethical considerations vary from country to country and change with time. However, a recognized quality assurance is essential for producers who want to market their product worldwide for the long term. Along these lines the European rendering industry seeks cooperation with WHO to develop an optimal

¹³ (a) Taylor D.M., Woodgate S.L., Atkinson M.J. Inactivation of the bovine spongiform encephalopathy agent by rendering procedures. *Veterinary Record* 137, 605-610, 1995.
(b) Taylor D.M., Woodgate S.L., Fleetwood A.J., Cawthorne R.J.G. The effect of rendering procedures on scrapie agent. *Veterinary Record* 141, 643-649, 1997.
(c) Schreuder B.E.C., Geertsma R.E., van Keulen L.J.M., van Asten J.A.A.M., Enthoven P., Oberthür R.C., de Koeijer A.A., Osterhaus A.D.M.E. Studies on the efficacy of hyperbaric rendering procedures in inactivating bovine spongiform encephalopathy (BSE) and scrapie agents, *Veterinary Record* 142, 474-480, 1998.

framework for the recycling of animal fats and proteins not used for human consumption for a sustainable prosperity of human health.

7.6 Could vaccines prepared from animal brain tissue pose a risk of transmission of Transmissible Spongiform Encephalopathies to humans?

François Meslin:

Over 40,000 deaths due to rabies are reported annually worldwide and each year seven to eight million people receive antirabies vaccine treatment following dog bites. Dog rabies poses a significant public health problem in Asia, as 85% of the human deaths due to rabies reported worldwide and 80% of the vaccine doses applied in developing countries come from this part of the world.

In many Asian countries such as Bangladesh, India, Nepal and Pakistan, sheep-brain based Semple vaccine¹⁵ is the only vaccine available free of cost. It represents 50 to 95% of all vaccine doses used for rabies post-exposure treatment, depending upon the country. A complete treatment consists of 10 subcutaneous daily injections of 2 to 5 ml (depending mainly on patient size and nature of the exposure) plus booster doses; that is a total of 25 to 50 ml of the 5 % sheep brain suspension injected over a 10-day period.

According to the literature, the reported rate of neuroparalytic complications following the use of this vaccine varies from 1:600 to 1:1575 administrations, and 20-25% of these lead to death. The exact incidence of neuroparalytic complications throughout India or other countries in the area is not known. However, in the State of Karnataka, India, 112 cases of neuroparalytic accidents were admitted in the past 20 years following Semple vaccine administration. In contrast, the newly developed cell culture or embryonating egg vaccines are effective and safe, with lower and less severe complication rates.

In many Asian countries, Semple type vaccine has been used for the past 90 years. In India forty million ml of this vaccine are produced in this country to treat at least 500 000 persons each year. In Pakistan 450 000 and in Bangladesh 60 000 people receive Semple type vaccine after possible exposure to rabies. There is a theoretical risk of TSE transmission to humans through parenteral administration of these products. Although there is to date no evidence of such occurrences in human medicine, recent events in the TSE field have demonstrated that an animal TSE agent could affect human beings.

The situation is very similar regarding rabies vaccines for animal use. For example various Indian veterinary vaccine institutes prepare 100 million ml of Semple vaccine for use in both rabies pre-and post-exposure prophylaxis in dogs and food production animals each year. Scrapie could be theoretically transmitted to animal vaccine recipients, especially ruminants, through sheep-brain based vaccines such as Semple type vaccine. This could happen because scrapie infectivity, if present, would not be inactivated by the manufacturing process. In this connection, a recent

¹⁵ β -propiolactone inactivated or phenolized antirabies vaccine containing 5% suspension of sheep brain infected with a fixed strain of rabies virus.

publication strongly suggests that scrapie was transmitted to sheep and goats through the administration of a veterinary vaccine whose method of preparation is similar to the Semple type vaccine. In addition, various Asian countries have begun to use animal tissues as feed supplement for intensive sheep and dairy cattle production. This introduces an additional, though still theoretical, possibility that scrapie, or even BSE, could spread among the sheep population and enter the sheep flocks that are used as a source of rabies vaccine production for human or animal use. In areas where the status of animal TSE is not well documented, this risk cannot be totally ruled out, though it may be remote, as there is no test available at present to detect pre-clinical cases of prion disease in sheep.

7.7 Surveillance and eradication of animal diseases: Principles from human diseases

Pierre Cattand:

At a conference held in Berlin in March 1997, under the auspices of the Donor's Association for the Promotion of Sciences and Humanities, a report concerning the eradication of infectious diseases was developed. This report was eventually published¹⁶ and the following definitions are taken from it. Elimination was defined as the "Reduction to zero of the incidence of a specified disease in a defined geographic area as a result of deliberate efforts. Continued intervention measures are required". Eradication is defined as the "Permanent reduction to zero of the worldwide incidence of infection caused by a specific agent as a result of deliberate efforts. Intervention measures are no longer needed".

To determine whether the goal should be eradication or elimination, the following decision elements are considered:

- Importance of the disease in terms of impact and burden
- The technical feasibility of either eradication or elimination
- Political issues
- Logistic issues
- Legal issues

In addition, the following epidemiologic characteristics need to be considered. What is the source of the infection? What is the reservoir? Are the diagnostic tools available? Can the disease be treated or cured? Can the transmission of the disease be reduced or interrupted? In addition, one must consider the availability of the means to do so, in terms of material and equipment, human resources, affordable tools, and appropriate methods. Among animal diseases, rinderpest is scheduled for eradication.

Dr. Cattand concluded that, in his opinion, it is impossible to consider eradication of scrapie since there were a number of important problems, for example the absence of a simple pre-clinical diagnostic test, absence of information about

¹⁶ Chapter 5 Group Report: How is Eradication to be Defined and What are the Biological Criteria in the Eradication of Infectious Diseases? In: *The Eradication of Infectious Diseases*. Ed. W.R. Dowdle and D.R. Hopkins. John Wiley & Sons Ltd 1998

asymptomatic carrier states, absence of treatment, lack of knowledge about transmission routes, and others. He was less certain about BSE eradication, but encouraged a systematic review of the issue.

8. Recommendations

Section 1: Background on new variant Creutzfeldt-Jakob Disease and Bovine Spongiform Encephalopathy

Recommendation 1

Surveillance of CJD and related human TSEs must remain a priority (or become one where it is currently not a priority), particularly where risk assessments indicate possible exposure to the BSE agent from any source. Surveillance should enable the detection and subsequent investigation of changes in the epidemiology of known human TSEs and recognition of new phenotypes of human TSEs. Novel or previously undefined TSEs, at this time, must be confirmed through neuropathological examination and transmission experiments. It is noteworthy that surveillance may be useful for demonstrating the absence of a hazard.

Section 2: Bovine Spongiform Encephalopathy in small ruminants such as sheep and goats

Since in some countries small ruminants may have been exposed to the BSE agent through contaminated feed, there is a theoretical possibility that small ruminants may have developed BSE infection and that the infection may have been maintained, even in the absence of continued exposure to contaminated feed, through maternal or horizontal transmission. The theoretical, but potentially serious, direct risk to public health from BSE infection in small ruminants is important, as are the indirect socio-economic consequences of taking actions that may adversely affect the small-ruminant industry and rural communities. Working with the sheep industry will be essential. The following recommendations were made:

Recommendation 2

Eradication of BSE must remain the principal public health objective of national and international animal health control authorities. At this time, the only known food animal species with BSE is cattle, and hence the principal target for BSE eradication is cattle.

Recommendation 3

The consultation encourages all countries to conduct a risk assessment to determine if they are at risk for BSE in small-ruminant populations. Further, if analysis suggests a risk of BSE in small ruminants, then suitable risk management procedures should be instituted in order to protect public health. This recommendation is in line with a previous WHO recommendation:¹⁷

“In countries where sheep and goats may have been exposed to ruminant protein potentially contaminated with the BSE agent, the risk of BSE

¹⁷ *Report of a WHO Consultation on Medicinal and other Products in Relation to Human and Animal Transmissible Spongiform Encephalopathies.* WHO/EMC/ZOO/97.3. Geneva, 24-26 March 1997.

occurring in sheep and goats should be assessed and appropriate legislative measures should be taken when necessary.”

Recommendation 4

Consideration of the need for legislative actions to address the theoretical risk of BSE infection in small ruminants and the nature of such measures will vary according to the circumstances in individual countries. Subject to the risk analysis recommended earlier, interim arrangements may be adopted to reduce the risk of exposure. Earlier recommendations of WHO pertinent to this discussion were reiterated by the consultation.

“No part or product of any animal which has shown signs of a TSE should enter any food chain (human or animal). In particular:

- All countries must ensure the killing and safe disposal of all parts or products of such animals so that TSE infectivity cannot enter any food chain;
- Countries should not permit tissues that are likely to contain the BSE agent to enter any food chain (human or animal).”¹⁸

The interim arrangements in a country of export should be taken into account to judge safety of exports when importing small ruminants or small-ruminant products.

Recommendation 5:

Diagnostic tests for the differentiation of BSE and scrapie in small ruminants should be utilized as soon as these become available.

Recommendation 6:

The consultants recommend that WHO should request from OIE a priority review of BSE in small ruminants through the Code Commission.

Recommendation 7:

The consultation recommends that WHO facilitate or coordinate research on the following issues relating the theoretical infection of BSE in sheep:

- Search for and identification of any field cases of BSE in small-ruminant populations. Methodologies such as case definition for surveillance must be addressed.
- Development and validation of tests to diagnose TSEs (i.e. distinguish BSE from scrapie) are a priority, as is determination of whether there is a carrier state in BSE infected sheep. The investigation of genetic resistance to BSE among small ruminants must be included in the study design.

¹⁸ *Report of a WHO Consultation on Public Health Issues related to Human and Animal Transmissible Spongiform Encephalopathies.* WHO/EMC/DIS/96.147 Geneva, 2-3 April 1996.

- Continued study of pathogenesis, addressing tissue infectivity and incubation period.
- Determination of whether BSE-infected small ruminants transmit BSE horizontally and/or maternally.
- Studies of the stability of the BSE phenotype when passaged in small ruminants, with the goal of determining if the BSE phenotype can still be identified after serial passage.

Facilitating experiments not yet underway should become a high priority activity, because infectivity studies require years to complete.

Section 3: Scrapie control programmes

Recommendation 8

The worldwide epidemiology of scrapie is poorly understood, hence the consultation recommended that scrapie surveillance, with the goal of establishing the prevalence rate of scrapie-affected flocks, should be conducted in all countries with small ruminants. It is noted that this information is an essential component in any quantitative risk assessment.

Recommendation 9

The long-term aim of TSE control programmes should be to eliminate scrapie. It is noteworthy that the hazard of BSE in small ruminants might be eliminated by this measure. The consultants emphasized that, in addition to research and surveillance efforts, awareness and education are essential components of scrapie control programmes (for example, education of farmers and others in contact with live animals as to the clinical signs of scrapie/BSE).

Recommendation 10

The consultation recommended genotyping of all scrapie cases at scrapie susceptibility gene loci (e.g. the PrP gene, and at least at the three known scrapie susceptibility gene loci) in order to act as an indirect warning of changes occurring in scrapie host-agent interactions.

Recommendation 11

The consultation recommends that WHO facilitate or coordinate research on the following issues relating to scrapie in small ruminants. Research on scrapie is important in its own right, with the following areas of priority recommended:

- A review of the role of scrapie elimination in scrapie control programmes, including a systematic comparison of scrapie with diseases that have been successfully eliminated. The consultation advised that a useful review of scrapie-control efforts would systematically compare examples of successful elimination programmes with those that failed, as well as considering innovative control strategies such as the introduction into flocks of scrapie-resistant genotypes,

perhaps combined with the preliminary culling of high-risk animals. Working closely with industry to determine acceptable methods is essential.

- Identification of effective surveillance strategies is essential.
- Determination of the role of genetics of any gene (including the PrP gene) found to influence scrapie susceptibility is essential.
- Determination of the route(s) of transmission of scrapie is essential.

Recommendation 12

A review of the evidence of a hazard of acquiring a TSE as a result of occupational exposures to sheep is recommended.

Section 4: Chronic Wasting Disease

The consultation felt that there is currently no evidence that CWD in Cervidae is transmitted to humans, however the following recommendations were made:

Recommendation 13

National authorities should be vigilant and international authorities should encourage awareness and surveillance for CWD around the world.

Recommendation 14

With reference to the *Report of a WHO Consultation on Public Health Issues related to Human and Animal Transmissible Spongiform Encephalopathies* (see Recommendation 4), no part or product of any animal with evidence of CWD or other TSEs should be fed to any species (human, or any domestic or captive animal).

Recommendation 15

Work should be continued to improve the understanding of CWD, where it occurs.

Recommendation 16

Regarding the movement of cervids (domestic or wild for repopulation purposes), precautionary measures should be taken to prevent the introduction and spread of CWD.

Recommendation 17

The consultants recommend that WHO should request from OIE a priority review of CWD through the Code Commission.

Sections 5 and 6: Feline Spongiform Encephalopathies and Transmissible Spongiform Encephalopathies in other species

Recommendation 18

For the purpose of identification of TSEs, maximizing reporting completeness, systematizing the collection of appropriate epidemiological information about TSEs, and to allow appropriate public health actions, the consultation recommended that surveillance for TSEs be strongly encouraged and facilitated through education and awareness programmes. In particular;

- data collection and analysis should be conducted at all appropriate sub-national, national, regional and international levels;
- reporting should be to appropriate medical and/or veterinary public health authorities;
- suspect cases should be reported to allow confirmation by TSE diagnostic reference laboratories.

Regional reference laboratories should be used to support confirmation of the diagnosis for novel or previously undefined TSEs. It is noted that at this time, neuropathological examination and transmission experiments are necessary for the confirmation of a TSE.

Recommendation 19

Surveillance networks enhance surveillance activities. Hence, where these are absent, networking at the national, regional, and international level should be encouraged.

Recommendation 20

International harmonization of surveillance case definitions for 'suspect' and 'confirmed' cases in human and animal TSEs is encouraged. It is noted that surveillance case definitions are included in earlier recommendations of WHO - for the human TSEs CJD, FFI, GSS and vCJD¹⁹, and in a second report for probable sporadic CJD and suspect vCJD²⁰. In addition, definitions for BSE in cattle are found in OIE documents.

Recommendation 21

The consultants recommend that WHO should request from OIE a priority review of other TSEs in animals through the Code Commission.

¹⁹ *Report of a WHO Consultation on Clinical and Neuropathological Characteristics of the New Variant of CJD and other Human and Animal Transmissible Spongiform Encephalopathies.* WHO/EMC/ZOO/96.1 Geneva, 14-16 May 1996.

²⁰ *Global Surveillance, Diagnosis and Therapy of Human Transmissible Spongiform Encephalopathies: Report of a WHO Consultation.* WHO/EMC/ZDI/98.9 Geneva, 9-11 February 1998.

Section 7: Evaluation of animal Transmissible Spongiform Encephalopathy zoonotic potential***Recommendation 22***

It is recommended that a test (or series of tests, such as those used in diagnostic protocols) to confirm the diagnosis of known and new TSEs be developed for surveillance and control. Ongoing work on development and validation of these tests for use in non-research laboratories, and of sensitive and specific test protocols to detect and distinguish TSEs is essential. It is preferred that such a test be validated on non-experimental cases, where possible.

Recommendation 23

It is recommended that since pre-clinical tests are needed for surveillance and control activities (particularly for live animals, and on easily accessed biological tissues) and since such tests would extend the opportunities to further reduce exposure of humans to TSEs, continued research work in this area is essential. Continued development to enhance robustness, particularly field testing, is required.

Recommendation 24

Movement of tests from research and experimental use into standardized, approved or commercially available test kits is recommended to allow better access to these tests.²¹

Recommendation 25

Human vaccines prepared from whole ruminant brains may carry the risk of transmission of animal TSE agents, because the inactivation processes usually applied to these products do not inactivate TSE agents. In particular, considering the recent emergence of vCJD in humans related to BSE in cattle, the consultation recommends that the use of these vaccines should be avoided if suitable alternatives can be made available. The Consultation strongly supported the recommendation made by WHO Expert Committee on Rabies, which states:

"The (Expert) Committee reiterated, as stated in its 1983 report, its support for the trend to limit or abandon completely - where economically and technically possible - the production of encephalitogenic brain-tissue vaccines, and strongly advocated the production and use of inactivated cell-culture rabies vaccines in both developed and developing countries."²²

²¹ Comment: It can be anticipated that test performance may appear to decline as use increases, for two main reasons: the prevalence of infection in the test populations may be lower, thus affecting the predictive value, and because of any technical problems arising while implementing the test. Nonetheless, as tests are validated and become available, their use in TSE risk assessment must be considered. The consultation discussed the value of quality control programmes to improve performance.

²² WHO Expert Committee on Rabies, *Eighth report*. TRS 824 Geneva, 1992.

Recommendation 26

The use of **veterinary** vaccines prepared from whole ruminant brains, for use in ruminants, should be avoided unless the process ensures TSE inactivation and/or removal, or the source animals have been demonstrated to be free of any TSE.

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Annex 1 Agenda**WHO Consultation on Public Health and Animal TSEs
Epidemiology, Risk and Research Requirements**Salle A (1st Floor)WHO Headquarters, Geneva, Switzerland
December 1-3, 1999 **December 1, 1999**

Time	Subject	Presenter
9.00-9.15	Introduction by Director	Director, CSR
9.15-9.30	Selection of chair and co-chair	Rosalind Ridley and Linda Detweiler
	Introduction	
9.30 – 10.00	Trends in BSE	Ray Bradley
10.00-10.30	Trends in nvCJD	Bob Will
10.30-11.00	Coffee	
	BSE in sheep	
11.00-11.30	Transmission of BSE into Sheep: Experimental evidence and ongoing work in the UK	Nora Hunter
11.30-12.00	Estimating the epidemiology of BSE in sheep	Linda Hoinville
12.00-1.00	Lunch	
1.00-1.20	France: Status report on investigations of BSE in sheep	Thierry Baron
1.20-1.40	Netherlands: Status report on investigations of BSE in sheep	Bram Schreuder
1.40-2.00	Questions	<i>All</i>
2.00-2.20	SEAC - Risk assessment of BSE in sheep	Ann Nolan
2.20-2.40	EU - Risk assessment of BSE in sheep	Emmanuel Vanopdenbosh
2.40-3.00	OIE - Measures relating to BSE	Thierry Chillaud
3.00-3.30	Discussion and recommendations	All
3.30-4.00	Coffee	
	Scrapie Elimination	
4.00-4.30	Principles, practice and experience of scrapie elimination	Linda Detweiler
4.30-5.00	TSEs in sheep – Surveillance Programmes	Danny Matthews
5.00-5.30	Breeding scrapie resistant sheep: the genetics of TSEs in sheep	Nora Hunter

Cocktail Party hosted by WHO


December 2, 1999

Time	Subject	Presenter
9.00-9.15	Industry and scrapie elimination: Perspective of the Scrapie Information Group	John Thorley
9.15-9.30	Animal vaccine related TSE risks: Scrapie outbreak in Italy	Maurizio Pocchiarri
9.30-10.00	Discussion and recommendations Is Scrapie transmissible to humans? Scrapie elimination to prevent BSE in sheep?	All
CWD		
10.00-10.30	Epidemiology of CWD	Michael Miller
10.30-11.00	Coffee	
11.00-11.30	Diagnosis and Pathogenesis of CWD	Beth Williams
11.30-12.00	Assessing interspecies TSE transmission using the cell-free conversion assay	Gregory Raymond
12.00-1.00	Lunch	
1.00-1.30	CDC Investigations relating to CWD exposures	Ermias Belay
1.30-2.00	Discussion and recommendations What is the zoonotic potential of CWD?	All
FSE		
2.00-2.20	Pathology and Epidemiology of FSE	Gerald Wells
2.20-2.40	Discussion and recommendations What is the zoonotic potential of FSE?	All
2.40-3.00	TSEs in captive wild bovids and felids	Gerald Wells
TSEs in Other Species		
3.00-3.20	The implications to humans of monkeys infected with BSE	Noëlle Bons
3.20-3.30	Discussion	All
3.30-4.00	Coffee	
4.00-4.20	Are there prion diseases of fish?	Liana Bolis
4.20-4.40	Evaluation of animal TSE potential	Robert Rohwer
4.40-5.30	Discussion and recommendations	All



December 3, 1999

Time	Subject	Presenter
	Evaluation of Animal TSE Zoonotic Potential	
9.00-9.30	Diagnostic tests for TSEs: Status and update of the EU initiative	James Moynagh
9.30-9.50	Potential of diagnostic tests to distinguish between BSE and scrapie	Jean-Philippe Deslys
9.50-10.10	Diagnostic protocols for TSEs: Rationales and their applications	Gerald Wells
10.10-10.30	Discussion	
10.30-11.00	Coffee	
11.00-11.20	Diagnostic tests and standardized reagents: WHO initiative	Ana Padilla
11.20-11.40	The Rendering Industry – current sourcing and current product uses in animals	Mr R. Oberthür (EURA) and Mr D.A. Franco (WRA)
11.40-12.00	Could vaccines prepared from animal brain tissue pose a risk of transmission of TSEs to humans?	François Meslin
12.00-1.00	Lunch	
1.00-1.30	Surveillance and Eradication of animal diseases: principles from human diseases	Pierre Cattand
1.30-3.30	Conclusions and Recommendations: TSE control requirements and human zoonotic potential of BSE in sheep, CWD, FSE, BSE in zoo animals, fish	All
3.30-4.00	Coffee	
	Meeting Adjourns	

Annex 2 List of Participants

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