FOOD
STANDARDS
AGENCY

TSE RESEARCH SEMINAR

REPORT

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INTRODUCTION

The FSA held a seminar to discuss the ongoing programme of research on TSE's in relation to it's policy objectives and to take a forward look and identify any gaps in the research programme. Presentations were made by research contractors, FSA personnel and invited scientists from the diagnostic industry.

Abstracts of the presentations were available to participants who consisted of officials from FSA, DEFRA,DH,MRC DARDNI and BBSRC ,research contractors and the MLC.

This report summarises information from the presentations in addition to that outlined in the abstracts provided by the speakers together with points made in the discussion sessions An overview summary was provided by Mr Mike Attenborough of the MLC and an assessment of the research in place against the policy research requirements is made.

2. PRESENTATIONS

2.1 FSA POLICY

2.1.1 Review of BSE controls and Research Policy Objectives
David Carruthers FSA

At the request of ministers the Food Standards Agency undertook a review of BSE controls. This was carried out in public and encompassed extensive consultation. The three main controls were considered

- The over thirty month rule
- Specified Risk Material controls
- Feed Ban

The report produced identified research requirements in several areas.

Tests and Screening
- Need for a rapid screening method for BSE in sheep
- Mass screening of slaughtered sheep for TSE's
  - It is important to establish the prevalence of scrapie in the national flock
- Mass screening of slaughtered sheep with a test to distinguish BSE from scrapie
  - This information is required for sheep going into the food chain
- Real time tests for TSE's of proven efficiency at clinical and sub clinical levels in sheep and cattle
  - A test in live sheep is desirable to eliminate TSE's from the food chain
- Screening of deer for chronic wasting disease (CWD)

Confirming the basis for the current food safety controls
- Checking that lymphoid tissue is removed in production of casings from sheep intestines which are used for sausage casings
- Further work to indicate any risks in this use of sheep intestines
- Expansion of bovine tissue infectivity studies using the most sensitive tests for material going into the food chain
  - The mouse bioassay is not the most sensitive technique. The calf bioassay is more sensitive and this should be supplimented with other techniques as they become available for example in vitro multiplication and molecular methods
- Testing of cattle and sheep for carrier status
  - It is known resistant mice can harbour infectivity, could this be an issue re resistant genotypes in sheep
- Further work on infectivity in pigs and poultry
  - So far there is no evidence that pigs or poultry contract BSE via the oral work however more evidence is still required

Other Issues

- Developing a method of analysis to detect presence of MRM in meat products
    - This requirement is not correct, as it is not possible to determine a method for MRM. The safety issue is whether MRM contains CNS tissue and methods are being developed to test for this. There is also the issue of labelling, as consumers would like to know if food contains MRM.
- Work on prophylactics for TSE's in animals

Developments since the review have identified other areas where research is required

Safety of imports

- Risk assessments for imports from other EU countries.
    - BSE has been reported in other EU countries for the first time.
- Bovine head meat
    - In the EU the head is not all treated as SRM and head boning still exists.

BSE in sheep

- Does BSE occur naturally in sheep
- What tissues are infective and when
- Does sheep to sheep transmission occur
- Safety of sheep milk and dairy products – there is much uncertainty surrounding this important issue. FSA policy on safety needs to consider these products along with meat and there is little data to help.

Recommendations from SEAC

- Historic human exposure
    - MRM – studies are required to help assess human exposure and epidemiology
    - Historic butchery practices – what happened in the abattoir concerning the treatment of brain
- Sheep risk assessments – necessary to look at the risk with the available information and to identify key gaps in knowledge.

In the discussion a question asked about deer, was whether there should be a concern limited to farmed deer which were fed concentrates containing MBM or should wild deer also be considered a potential risk. The conclusion was work on deer in general, their susceptability to TSE's together with more knowledge of the routes of deer products into the food chain was needed

2.2 SESSION ONE

SLAUGHTER, MEAT PROCESSING AND DETECTION OF CNS TISSUE

2.2.1 Dispersion of CNS material during splitting of cattle and sheep carcasses

Andy Knight Silso Research

The presentation covered three closely related projects (including one extension) concerned with the removal of spinal cord.

- Measures to reduce contamination of meat and environment with CNS tissue during slaughter and processing of cattle and sheep (REMCOLM) – M03008*
- Contamination of meat, and exposure of abattoir workers, by CNS material during standard butchering processes prevalent in the member states of the European Union (EUCNSRISK) – M03009*
- Transfer of CNS material to subsequent bovine carcasses at splitting – extension of M03009
- Removal of spinal column from cattle and sheep carcasses (REMCOLM) – M03017*

The collaborators for the projects are from the UK, Ireland, France, Germany and Norway and included universities, Research Institutes and Commercial companies. Following commercial carcass splitting samples were taken both from the carcass and aerosols and tested for CNS specific proteins Syntaxin (S-100â) and GFAP. The results showed CNS presence on the following areas with the estimated concentration given as ng/mg CNS tissue on a wet weight basis

- Operators Apron — <100ng/mg
- Hand held screens — 250-500ng/mg
- Cut vertebral surfaces — 100-150ng/mg
- Whole carcass — 100-200mg spinal cord per carcass

Experiments investigating washing the cut faces showed the contamination could not be removed.

Samples were taken from 8 EU Member States abattoirs and although a wide variation was seen the maximum contamination using the syntaxin assay was <1000ng/mg on the cut vertebral surface. Work is currently underway to attempt to correlate contamination with observational data. A video was shown highlighting operators hands as a route of cross contamination when moving carcasses in cold stores

Experiments looking at carcass to carcass contamination were undertaken dressing a male carcass followed by female carcasses and looking for male specific tissue. Results showed 0.1% of the tissue was male on the first female carcass and sporadic contamination was seen on subsequent carcasses.

In a research abattoir the splitting saw has been shown to be a source of cross contamination with 10% of the tissue on the saw identified as male after splitting four females. The next step is to carry out a study, under commercial conditions, of the splitting saw as a route of cross contamination.

Alternative methods to carcass splitting are

- Hot deboning
- At splitting – involves two offset cuts or a twin blade saw
- Suction to remove the cord prior to spliting
- Intact column removal

Intact column removal is being investigated using an oval saw . Following encouraging experimental results a demonstration project with proving trials has just commenced. Saws will be constructed and installed in European abattoirs and carcass and meat safety and quality, engineering integrity, and economics investigated.

The main conclusions from the project are

- Dispersion of spinal cord during splitting
  - Little ariel dispersion
  - Cut vertebrial surfaces most contaminated
  - Washing does not remove contamination

- Carcass to carcass transfer
  - Contamination can be sporadic
  - Saw cleaning can be improved

- Alternative Approaches
  - There are problems with existing methods
  - Complete column removal is undergoing proving trials

In discussion the following points were raised

- ***Further work was needed to establish if all the dorsal route ganglia are removed with the oval saw.***
- ***contamination estimates were carried out on swab samples which could recover only 1/5th of the material present.***
- ***contamination during transfer of carcasses to and from the chiller could be a problem due to cross contamination via operators hands. This could be easily managed by good working practices***

2.2.2 <u>Prevalence of CNS embolism and the determination of its potential for visceral dissemination at stunning and slaughter in cattle and sheep</u>

Alan Fisher Bristol university Veterinary School

Previous work has raised concern about stunning and slaughter methods and contamination by neural tissue.
Experimentally blood was collected from the jugular vein and the aorta following stunning and tested for CNS tissue using immunohistochemistry methods looking for GFAP,syntaxin and neurofilament antibodies.

Results showed brain fragments staining strongly for syntaxin present in venous blood with levels of syntaxin detected rapidly after stunning by cartridge operated stunning with pithing in cattle, cartridge operated stunning in sheep and pnuematically operated air injection stunning cartridges in cattle and sheep.

To demonstrate the potential risk from neuronal embolism, marker bacteria were inoculated into the stun wound of cattle and the presence or absence of the organism determined in a range of tissue samples. Positive samples were recorded for blood, liver, spleen, lung, kidney, lymph nodes and deep muscle as well as the carcass surface.

Further work is planned to look at:

- incidence of CNS detection in venous blood
- transfer of CNS into arterial circulation and edible tissues
- occurrence with cartridge operated stunning with no pithing

The issue of whether marker bacteria are a good model for prion dispersion was raised. The presenter explained that the experiments demonstrated risk and the results obtained justified further investigation. The discussion concluded that the number of cattle and sheep looked at needed to be increased before the riska of captive bolt stunning could be quantified

2.2.3 <u>Historical Entry of SRM into the Food Chain</u>
Stephen Dixon FSA

The background and progress with this work as described in the abstract was presented.

Professor Ironside noted that information made available to SEAC from New Zealand would be available to assist the study. It was noted the study would focus on bovine and the question was asked if there were plans to incorporate other species such as sheep and deer. The agency replied that proposals to include ovine had been prepared but the priority was to carry out the work on historical routes of bovine SRM entering the food chain.

2.2.4 <u>Evaluation of Immunological test kits for the detection of CNS in meat and meat products</u>

Mary Howell FSA

The needs for a CNS test were presented as twofold

- to exclude SRM tissues of CNS origin from the food chain to reduce human exposure to BSE and other TSE's
- to label non SRM CNS on packed meat products as a response to consumer expectations and GMP.

To enforce food labeling law, and to demonstrate compliance with the SRM ban any method needs to be reliable, validated and fit for purpose. These methods would be used by official food control laboratories and so would need to comply with the requirements of ISO 17025.
The comparison of two commercially available kits undertaken by the agency concluded that levels of 1% of brain or spinal cord or CNS tissue could be reliably detected in raw and cooked meat and meat products.
A similar study undertaken by the EU Joint Research Centre with one of the kits but involving 26 European laboratories reached the same conclusion.

The results show that both methods appear to be fit for the purpose of the second need i.e. labeling of non SRM CNS tissue but are not currently sensitive enough to effectively enforce the SRM ban.

Validation trials still need to be undertaken on an international basis to complete the fitness for purpose study.

Professor Ironside commented that the two target proteins although occurring in CNS tissue at a much higher level than other tissues are not exclusively specific adding weight to the conclusion that these types of methods are suitable for detecting considerable contamination i.e. >1% but not for trace contamination.

2.2.5 <u>Molecular tools for detection of CNS in meat and meat products</u>
Neil Harris LGC

The objective of this work is to develop a robust DNA based method to detect neuronal tissue from cattle sheep and goat and to differentiate it from muscle in processed food products.
The basis of the approach uses methylation specific PCR to detect promotor regions of genes coding for tissue specific proteins eg GFAP.
So far a test has been developed for the detection of cattle and sheep neuronal tissue and transferred to a real time platform to enable quantification to be carried out. Current sensitivity in raw and cooked products is in the region of 1% .
Further work is planned to improve the sensitivity using nested PCR and Taqman MGB probes which fit into the minor groove of the DNA helix and potentially will give a greater level of specific differentiation. Further work will also explore the potential technique as a species specific test.

It was noted that GFAP is also transcribed in Schwann cells and that CNS is only SRM in sheep over a certain age. Both these points could limit the application of the technique

2.3 SESSION TWO

TRANSMISSION OF TSE's TO FOOD ANIMAL SPECIES

2.3.1 <u>Transmissibility of BSE to domestic fowl</u>
Steve Hawkins VLA

The two objectives of this work were to determine

- If BSE can be transmitted to domestic fowl by parenteral injection of brain homogenate from BSE cattle
  - 12 chicks were challenged and 14 control chicks were injected with only saline.
- If BSE can be transmitted to domestic fowl by oral challenge with brain from BSE cattle
  - 11 chicks were given 5g brain stem at 4,5 and 6 weeks by gavage.

Birds were sacrificed at 2 and 5 years or when clinical disease was apparent.

Motor disturbance syndrome was observed in male birds in the challenge groups however at the onset of clinical signs there was only one surviving male bird in the control group.
The Motor disturbance was further investigated with the objective of determining transmissibility into inbred mice or domestic fowl. No evidence has been found either clinically or histologically.
Current work is looking at CNS tissue from the primary challenge, and first passage immunohistochemically using a range of antibodies.

The discussion raised the issue of numbers of birds in the experimental groups being small. The species used were those normally reared as broilers in the UK with a life span of 8 weeks. The agency needs to consider if more work should be done with higher numbers or birds kept for less time and using the improved immunological methods of disease diagnosis available today.
The syndromes seen and the high intercurrent disease could be attributed to boredom and welfare aspects of being caged for up to five years. The question of a carrier state was raised and also if the birds ate infected

material and it remained in the gut when they were rendered this could be a source of recycling.

2.3.2 Transmissibility of BSA and scrapie to pigs by oral exposure to brain homogenate
Steve Hawkins VLA

Ten 2 week old pigs challenged by a combination of intercranial, interperitoneal and intravenous routes with pooled brain homogenate from field cases of BSE in cattle developed disease after 17months.

Ten 2 month old pigs challenged 3 times at one to two week intervals with 4kg of pooled brain homogenate from 29 clinical cases of BSE showed no signs of disease after 84 months either clinically or histopathologically.

Twelve 2 month old pigs challenged 3 times at one week intervals with 4.8 kg of pooled scrapie brains from 370 field cases also showed no signs of disease after 84 months.

Samples of tissues from both orally challenged groups and the parenterally challenged groups were tested for infectivity in the mouse bioassay. Tissues from the orally challenged groups did not show infectivity, whereas in the parentally challenged groups the CNS and alimentary system were infective.

Some scrapie challenged tissue samples are still being assayed.
The conclusions are

- Pigs are susceptable to BSE infection via the parental route
- There is no evidence to date of infectivity of either BSE or scrapie via the oral route.

2.3.4 Further studies on the transmission of BSE to pigs
Steve Ryder VLA

Vacuolation in normal control pigs was observed during the oral and parental challenge studies. In the Hypothalamus this was readily distinguishable from BSE but in the rostral coliculi the vacuolation was indistinguishable.
Further studies have observed this phenomenum in most UK pigs being most extensive in the older pig. It has also been observed in animals from Germany. Australia and New Zealand but to a lesser extent compared to UK pigs. Immunohistochemical analysis for disease specific Prp and astrocytosis has been negative.
The most sensitive test for infectivity is to bioassay material within species so the following experiment has been set up with 4 groups of pigs challenged by the IP/IC and IV route.

- Rostral coliculi from pigs born pre 1996
- Rostral coliculi from pigs born post 1996
- Rostral coliculi from New Zealand pigs
- BSE bovine brain (positive control)

The experiment has been going for 17 months and there is currently no sign of clinical disease. Brains from all animals will be examined for disease specific PrP.

Additionally with the BSE control a supply of porcine material should be generated which will be available for research and surveillance purposes.

In the discussion for both presentations covering pigs it was agreed that the occurrence of vacuolation in the rostal coliculi was strange and it was unclear as to whether the discovery should sound alarm bells as to if there is an endemic TSE in pigs. The results of the ongoing experiments are therefore important.
The parenteral challenge work had demonstrated that immunohistochemical staining was as sensitive as the mouse bioassay. It was suggested that experiments should be undertaken to find out what happens to the large amount of infectivity given by oral challenge using this tool and concentrating on peripheral tissues.
In the discussion it was questioned if there were any plans to sub passage material from challenged pigs enabling higher amounts to be tested for infectivity without the species barrier i.e. 1.2 kg per pig in 3 doses.
Professor Ironside also suggested that the parental routes should be teased out and separate IC, IP and IV challenges undertaken.
The question was asked as to whether other species should be investigated. Salmon were fed MBM and it was understood some work was underway in the EU with salmon and trout. The agency was recommended to find out what these studies and other European studies with pigs were covering.

2.4 SESSION THREE

RISK OF HUMAN EXPOSURE TO TSE's

2.4.1 The pathogenesis of experimental BSE

Steve Hawkins VLA

Thirty 4 month old calves were dosed with a single 100g dose of brain pool from 75 field cases of BSE (1991). Groups were killed from 2 to 40 months post challenge and a range of tissues tested for infectivity in mice. Neural, Alimentary and Lymphoreticular tissues together with kidney, lung, nasal mucosa, pericardium, heart, blood, bone marrow and bone were tested.

Infectivity was detected in distal ileum, CNS sensory, triminal and dorsal root ganglia and bone marrow.
Experiments have been undertaken to compare the efficiencies of the bioassay of BSE infectivity in cattle and mice and determine if a cattle bioassay can detect infectivity in tissues that are negative by mouse bioassay. The following objectives are being addressed

- Measure the underestimate of infectivity due to the species barrier
- Produce a dose incubation curve for infectivity in cattle and mice

The results have enabled the following conclusion to be drawn

- The underestimation of infectivity of BSE brain tissue titrated across a species barrier in mice is 500 to1000 fold

In the following discussion the occurrence of infectivity in distal ileum and bone marrow was noted, the latter with some sceptism. Work to repeat the finding is ongoing and not yet complete. The distal ileum is the first tissue where infectivity is detected followed by the CNS. Infectivity is thought to get to the brain via the peripheral nerves. Due to the information gained from sheep other tissues are still suspect however all results have never demonstrated infectivity in muscle. Professor Ironside remarked that detection of infectivity in blood would be important in the context of edible tissues and it is important the tests are carried out using the most sensitive bioassay system. The age of infectivity is important together with the titre of infectivity for policy considerations. This information will be obtained from the dose response curves. The method of sacrifice was questioned bearing in mind the earlier work on neural embolism and it was confirmed all animals were killed by intravenous injection.
It was concluded that this work is crucial and is being carried out well. All available diagnostic tests should be applied to tissues as they become available.

2.4.2 Studies on milk from cattle experimentally infected with BSE
Roy Jackman VLA

The determination of abnormal prion protein in milk of cattle infected with BSE is a project that was submitted to MAFF in response to the Animal Health and Welfare Research Requirement Document 2000. The project was transferred to the FSA and following several negotiating meetings the detail was agreed in February 2002.
The requirement was to collect and store milk from animals developing disease and develop and apply a method for determining PrPbse to the milk samples. The milk samples were available from another MAFF experiment studying maternal transfer. Animals were challenged in September 1998 and were calved in September 2000. Results are needed for risk analysis and it is required to show the presence of PrPbse in the minimum volume of milk or the non detection of PrPbse in the maximum volume of milk. One of the immediate problems is that

the form of PrPbse is not known. It could theoretically be soluble, monomeric or non-aggregated.
The study design allows milk to be collected from 0-7 days and then at 10, 20, 30 and 40weeks post calving. The milk collected so far has been pasturised and homogenised to simulate normal practice. As PrP is known to be membrane bound a cellular fraction has also been collected and all samples are stored at –80°C awaiting analysis. Initially, EU approved tests will be applied and the maximum volume of milk able to be presented to the assay with minimum maxtrix effect determined. The tests to be applied will be the Prionics check and the BioRad test.
The future plan is to investigate immunoaffinity extraction using antibodies that bind native prion. Both existing antibodies and new monoclonal and recombinant antibodies will be investigated.

In the discussion the question of sensitivity was raised. A target of a test with no matrix effects that gave a negative result for 20litres of milk was proposed. If a positive test is recorded what would be the significance for human health. The tests will measure prions that bind to antibodies and not infectivity. Currently there are no plans to store milk for infectivity studies and there are only 19 animals left in the study as they are starting to come down with clinical disease. It is a one off chance to take samples for infectivity however there will be an additional cost and possibly a storage problem. It is also probable that any infectivity bioassay will be less sensitive than the chemical test. The agency urgently needs to make a decision concerning storing samples for infectivity.

2.4.3 <u>Risk of Exposure to BSE Infectivity in UK Sheep</u>

Philip Cromer DNV Consulting

It is known that sheep can be infected with BSE after experimentally feeding bovine brain from BSE infected animals and that sheep were fed rations containing MBM during the BSE epidemic. This study assesses the potential exposure of the UK population to BSE from lamb and sheep meat should BSE be present in the UK flock. Previous work showed the total exposure of sheep to BSE infectivity via MBM was about 3% of that for cattle and that the average dose per sheep was at least 100 fold less than for cattle. The assumption has been made that between 0.01% and 10% of scrapie cases could be BSE and that the UK prevalence of scrapie is 0.1%. From ongoing studies of experimental oral transmission to sheep it has been established that the time course and pattern of infection are different to those for cattle and more in line with scrapie. An important factor is that infectivity is found early in the incubation period in different tissues to cattle in particular lymph nodes. As levels of infectivity of tissues from BSE in sheep is not available for this study the oral dose to humans is assumed to be the same as the infectivity of CNS tissue from cattle with BSE. All sheep tissues that may contain any level of infectivity as determined from scrapie data and are consumed as food were considered. The overall exposure of the Human population has been estimated to be 3.5 human oral ID50 units per

year with a 95 percentile range from 0.02 to 650. The exposure of any one individual is therefore low but not insignificant. There are high levels of uncertainty in the estimates due to the lack of data.
Most of the exposure is due to animals older than one year with the exposure coming mainly from lymph nodes.
Four possible risk reduction measures were evaluated using the exposure risk model.

1. No animals older than I year allowed in food for human consumption
2. 95% lymph nodes removed from the carcass.
3. Use of intestines banned from a)animals older than one year b) all animals
4. All offals from animals older than one year banned

I and 2 singly effect a reduction in risk of 75% and combined 90%. 4 combined with 2 also effects a 90% reduction. 2,3b and 4 combined give the greatest reduction of 97%

The discussion considered if it was important to look at the minor routes of potential infectivity or concentrate on the major routes. A steering group has been established to advise on the areas of uncertainty but due to the time pressures imposed by the agency to complete the risk analysis they group have been unable to contribute as envisaged. The group will meet on 13th December 2001 to consider how to assist the studies . It must be noted that the analysis has been calculated using the medium scenario that 0.1% scrapie cases are BSE and no consideration of genotype has been taken. Accurate measurement of this prevalence would substantially reduce the uncertainty of the model, and if surveillance shows cull ewes are predominantly a susceptible or resistant genotype the analysis would be significantly effected. Major lymph nodes can be removed on a commercial basis but it is a time consuming process. It could be made simpler with a change in traditional cuts of lamb.

2.4.4 Estimating the risk to human health posed by possible entry of BSE infection into the GB sheep flock

Neil Ferguson Imperial College

The risks to human health have been analysed assuming that BSE has entered the sheep population in the UK. The implications of different scenarios of BSE spread in sheep from rapid decay to endemicity have been considered. Currently there is no data to indicate that BSE entered the sheep flock so it remains uncertain if transmission occurred from contaminated MBM. A small number of sheep brains collected from 1996-2000 from animals diagnosed with clinical scrapie have been tested by bioassay and shown to be negative for BSE but this would still allow a considerable prevalence to be present. Large scale testing of sheep brains for TSE and when a method is available for BSE is required for proper scientific assessment. Estimates of infectivity in animal tissues during disease incubation are crucial for risk assessment ,and although data is limited a profile can be constructed that shows a more rapid rise in overall infectivity early in the incubation period in a wider range of tissues compared to cattle. As for

BSE in cattle host survivorship is important given the long incubation period of the disease. Best estimates for sheep of 1.5 years have been used but more precise data are urgently required.
The risk analysis presented incorporated the available information into a single integrated framework however the data is limited and further studies to measure the following are required

- Scrapie and BSE prevalence in sheep stratefied by age and employing a large enough sample to detect low prevalence
- Sheep survivorship
- BSE infectivity in sheep quantitatively by stage of incubation, tissue and sheep genotype
- Age dependant susceptability to TSE infection in cattle and sheep
- Historical trends in ovine and bovine tissue consumption

The question was asked, whether stratefication of sheep had been taken into account in the analyses, it was confirmed it had not and the model assumes all sheep flocks are the same. The MLC offered to supply their data to assist the analyses. It was queried why the analysis only included UK meat and did not consider imported meat. It was assumed the risk from imported meat was negligable compared to the risk from UK lamb and so the analyses had solely considered UK sheep. How much MBM had been fed to sheep and at what age? Was there a geographic variation in the feeding pattern? There was information to show the feeding pattern of highland compared to lowland sheep was very different. The MLC have data to show the majority of lamb consumption is by people over 45 years of age, however it must be noted that this did not mean they were necessarily at risk.
The SSC's opinion on risk was that one infected bovine would have a certain number of infective doses and so can estimate how much could be in each cut of meat. If the same situation is applied to sheep the risk is higher due to the tissue distribution . However the data on BSE in sheep is so limited and it is important to carry out a transmission experiment in sheep using tissue from BSE infected sheep.
The question was raised concerning numbers to test the scenarios presented. The presenter indicated 100,000 sheep needed to be tested and the test should be carried out on the most appropriate tissue which was not brain in young animals. There would be some benefit looking at reported scrapie cases but due to the massive under reporting screening sheep entering the food chain for TSE's and then trying to see if BSE can be identified in the positive samples was a sounder scientific approach.
It was queried whether during the recent mass slaughter of sheep in the FMD epidemic any information was able to be obtained concerning TSE's. It was confirmed although recognised as a scientific window of oportunity due to resource implications it was impossible to organise.

2.5 SESSION FOUR

DIAGNOSTICS

2.5.1 Institute of Reference Materials and Measurement and their role in the evaluation of testing methods

Mary Howell FSA

The Institute if Reference Materials and Measurement(IRMM) are part of the EU Joint Research Centre and are responsible for the production of reference materials for use in testing methods. In 1999 they organised the evaluation of test methods for BSE in cattle CNS. Each test developer analysed 1400 blind brain or spinal cord samples under the supervision of an IRMM scientist. These consisted of 1064 negative and 336 positive samples. This resulted in the three approved EU tests.

An investigation to look at the concentration distribution along the spinal cord was undertaken with the Enfer and the BioRad tests which are both semi-quantitative. It was shown there was a clear drop in signal with increasing distance from the obex with the maximum abnormal PrP 0.5-1.5 cm anterior to the obex.

An original plan was for IRMM to have mouse titrated brain pools available to test developers to enable investigation of equivalence of test signal. This has not proved possible however limited experiments have been undertaken using the BioRad test and shown that brain pools with similar titres gave comparative readings and the sensitivity of the test is at least one infectious unit for R111 mice.

Samples from the VLA pathogenesis have also been tested with the BioRad test to investigate pre clinical diagnosis. Samples were negative at 26 months and positive at 32 months. Clinical symptoms appeared at 35 months. It could be concluded that the test was at least as sensitive as immunohistochemistry, SAF and mouse bioassay.

Proficiency testing is distinct from other interlaboratory trials as the laboratory analyses the test material using their method of choice. Proficiency testing has been undertaken with the EU reference laboratories the aim being to identify problems with the implementation of the EU approved tests. It was also hoped the remaining test material could be used as a control sample. It is important that a reproducible and sustainable supply of material can be identified to enable the production of a reference material.

Currently 5 further tests are being evaluated for BSE in brain. There are plans to evaluate all eight tests for scrapie detection. Negative samples of brain, spleen, ileum, blood and lymph nodes have been obtained from New Zealand. Corresponding positive samples are being sourced from the UK. All tests will be evaluated on brain and then suitable tests evaluated on lymphoid tissue. Finally 2 tests will be evaluated for their potential to distinguish BSE and scrapie.

The question of the potential of the tests to diagnose human disease was asked. The tests have only been evaluated on cattle brain but the antibody used in the Prionics test is broad spectum with regard to prion specificity and is also used in human brain analysis albeit in a different format.

2.5.2 Bio-Rad Rapid BSE test.

Emmanuel Comoy CEA

Transmissible spongiform encephalopathies (TSEs) in ruminants are neurodegenerative diseases presenting a real risk in term of public health. Bovine spongiform encephalopathy (BSE), which is believed to be responsible for new variant form of Creutzfeldt-Jakob in Human, seems to persist even well after the ban on meat and bone meals, suggesting other potent ways of contamination. These diseases having a long and silent incubation period and many studies have underlined the existence of asymptomatic but potentially infectious animals, which could enter in the human food chain. Therefore, use of TSE diagnostic tests constitutes an important tool in food safety.

The Platelia BSE test, which has been developed by Bio-Rad in collaboration with research teams of CEA (Commissariat à l'Energie Atomique), is based on the post mortem detection in bovine brain of the abnormal form of the prion protein (PrPres), which is the only specific biochemical marker of BSE. According to the European evaluation study (DGXXIV, 1999), this test shows 100 % of specificity and 100 % of sensitivity on clinically affected animals. The test is an ELISA employing antibodies to the denatured prion. No antibodies suitable for use in detecting native PrPres are available .

Studies on samples from experimentally orally-contaminated cattle using the Platelia BSE and coordinated by IRMM detected pre clinical animals 32 months post-exposure as positive animals. Comparisons with other diagnostic tools on the same samples showed that some samples which were positive with Platelia BSE were found negative by histology, fibril detection and/or immunohistology (Grassi et al., Vet. Record 2001).

The test was shown to a have very low limit of detection, close to the reference test of bio-assay in mouse (Deslys et al., Nature 2001). Rapid and adapted to high-throughput screening, this test is used in the frame of the systematic analysis set up in the slaughterhouses by different European countries since January 2001.

2.5.3 The Enfer TSE test

Riona Sayers Enfer Scientific

The Enfer TSE Test has been developed as a rapid, high throughput and accurate post-mortem testing system for the detection of the abnormal prion protein in cattle and sheep, 45 samples can be tested in 3.5 hours. Samples for

testing are cut from the obex or upper cervical spinal cord. It is important to cut a cross section of the sample as prion is present mainly in the grey matter found in the centre of the spinal cord.The procedure itself is a three-step process.

- sample disection and preparation using a triad buffer system,
- enzyme immunoassy with a chemiluminescent based detection system,
- calculation and interpretation of results, light intensity is proportional to abnormal PrP in the sample.

The assay principle is based on highly specific isolation of PrP^{sc} using three buffers, Enfer Buffers 1, 2 and 3, combined with a specialized capture technique to bind aggregated PrP^{sc} to the ELISA plate. Enfer Buffer 1 acts to solubilise prion protein in the tissue sample and to reduce the effect of proteinases from bacteria. Enfer Buffer 2 contains a digestive protease(proteinase K), which destroys all normal prion protein (PrP^{c}) in the sample homogenate. Enfer Buffer 3 is a denaturing buffer, which results in epitope exposure thereby facilitating anti-PrP binding, it also deactivates proteinase K . The test has been independently evaluated as 100% specific and 100% sensitive by the European Commission and has continued to yield these performance characteristics in the field, no false positives having being reported to date. Random selections of Enfer negatives from the field have also been examined using standard confirmatory methods, and no false negatives have been detected. However, sensitivity has been found to be adversely affected by homogenization of samples before entry to the Enfer assay, due to protein conformational effects, and use of such samples is not recommended. Such samples are not routinely experienced and no adverse affects have been recorded in the field using fresh, frozen or autolysed tissue samples. The effect of homogenisation which can reduce sensitivity by 10 fold was discovered during work with the EU when trying to produce a homogenous sample for proficiency testing. The conditions do not apply under normal testing conditions but the problem needs to be overcome as proficiency testing and reference samples are an important part of the future development of validation and quality assurance of the application of the test in other laboratories. The Enfer facility in Ireland is currently the highest throughput TSE testing laboratory in the world having tested in excess of 100,000 samples in duplicate in October 2001 alone. In all, 115 positives have been detected in 2001 to date, 105 bovine, 10 ovine, all confirmed by histopathology or immunohistochemistry. The Enfer assay has proven itself to be Rapid, Reliable and Reproducible in the detection of TSE.

2.5.4 Prionics Check
Bruno Oesch Prionics

Diagnostic tests work by detecting the disease specific form of the prion protein. The EU approved Prionics check is based on WesternBlot detection. A development test The Prionics check LIA is a lumminescence immuno assay that has better potential for automation and is currently undergoing EU evaluation.

The best sample for diagnosis is in the obex region of the brain and is the same area as used for alternative diagnosis by histology or immunohistochemistry. Normal PrP is degraded by proteinase K wheras disease specific PrP is proteinase K resistant and only a small fragment is digested. The assay conditions are designed to have optimal conditions to destroy the normal form of the prion whilst retaining the pathological form. This is achieved with a combination of homogenisation and digestion buffers. The test does not include a centrifugation test and concern was expresses that centrifugation could cause other tests to miss the detection of small aggregates of prion that may be important in pre clinical disease. Following homogenisation and then digestion the sample is seperated by Western blot and visualised using a monoclonal antibody 6H4 which recognises a conserved part of prions and apart from chicken,marsupials and rabbit detects prions from all other species tested. Samples are tested without digestion and a prion band is seen, after digestion the prion band in normal samples disappears and in samples containing abnormal prion a band with a lower molecular weight is visualised. This means there are three criteria than are used for identification of abnormal prion.

- Immunological signal
- Molecular weight
- Glycosylation pattern

The assay has been adapted to a new immunoassay platform to enable automation and speed of screening to be improved. An evaluation of the LIA test has been carried out with the Swiss Veterinary service looking at 1400 samples. 336 positives all gave a signal greater than the 1164 negative samples. A dilution curve of BSE homogenate in non BSE homogenate gave a dose response curve with a 1in 300 dilution easily detected making the LIA more sensitive than the western blot. The possibility for automation can be demonstrated with a homogeniser processing 240 samples per hour and a robot carrying out 1700 tests in 14h. The first test result takes 5 hours with results for 450 samples in duplicate available in 8 hours.
Samples of scrapie positive and negative samples have been tested by both Western blot and LIA and 100% agreement achieved.

Prionics have recently developed a dipstick format for end detection of prion following digestion. The simple immunochromatographic system is similar to the pregnancy test. The strips are designed to be dipped into the wells of 96 well plates following sample digestion. Their use reduces the analysis time by 20mins and removes the need for a luminometer. Sensitivity has been estimated as 1in100 dilution of brain homogenate and 5 ng/ml of recombinant Prp. Some work with the EU has been undertaken but the strips were not included in the recent evaluation.

In the discussion the issue of concentration steps in the assay procedure was raised. The VLA have looked at centrifugation prior to Western Blot but the concern that must be considered is what form the prion would be in the matrix to be examined. Experimental work using the most sensitive tests

with concentration steps could be used on samples from the pathogenesis experiment to see if enrichment improves diagnostic sensitivity. This is important to consider when developing tests for other tissues together with the limited value and potentially misleading results that could be obtained by solely spiking with brain material.

2.5.6 <u>BSE Testing in Europe – The Eurofins Approach</u>

Bert Popping Eurofins Scientific

Eurofins are a global organisation offering bioanalytical services in over 50 laboratories in USA, France, UK, Germany, Switzerland, Netherlands, Denmark and Norway.
In 1999 they acquired a BSE testing laboratory in Germany which had a low throughput until November 2000 when the first German BSE case was reported. Since then testing facilities have been widely established at short notice to meet consumer demand. A facility was established in France in one month with government approval including an audit and successful testing of check samples in three months. This laboratory has 13 staff and processes 6-700 samples per day.
The company has a scientific information officer who deals with all relevant scientific and regulatory information aspects and informs and coordinates all the testing laboratories. This role is seen as key to their success as a provider of test facilities.
Currently the Prionics check and BioRad test are undertaken. It has been noted that the quality of sample taking i.e. distance from the obex is more important than sample age. In both tests digestion with proteinase K is a key step that needs to be controlled and monitored. With the western blot samples have been seen that are negative but have bands that stain but are too low to be BSE, demonstrating detection judgement is required for this test. It is also important to have a sample labeling system of the highest integrity between the abattoir and the laboratory.

2.5.7 <u>The role of the EU reference laboratory: Research developments in antibody technology for concentration of prions</u>

Roy Jackman VLA

The VLA as the first institute to work with BSE and identify the disease in cattle are the EU reference laboratory. The main roles and work programme of the reference laboratory are

- Confirmatory techniques

    - Harmonisation of methods including SAF(Scrapie Associated Fibrils), Immunohistochemistry and Western Blot and application to autolysed tissue samples
    - Proficiency testing of techniques
    - Training
    - Provision of materials
- Clinical Disease – what is pre-clinical ?
- Feed Controls – the recent change in legislation for the temperature of the rendering process from 128 to 133 has made policing the MBM ban difficult as the existing immunological tests use antibodies that do not work detecting material processed at the higher temperature. There is a need for new antibodies or a PCR test for bone.

The VLA was involved in the development of the DELPHIA test which although it did not pass the EU evaluation as a screening test has major advantages over the other tests. Proteinase K is not used and so there is potential for the test to be developed for body fluids and milk. With the issue of pre-clinical disease should this include a test for carriers if this does exist, diagnosis and surveillance are closely tied up in this respect. For pre-clinical detection it is important to improve diagnostic sensitivity not test sensitivity. The pathogenesis experiments have found infectivity in the ileum early in the disease but no other tissues have been identified before the brain. There have been suggestions that a disease marker can be identified in urine and studies are ongoing at the VLA using Immunocapillary Electrophoresis (ICE) to test blood. Using a comparison of bound and free ratios non TSE exposed animals show a ratio of 1 and in Scrapie infected animals the ratio reduces to 0.7 and does appear to follow a time course.
For analysis of milk it has been decided to try to develop an affinity chromatography concentration step. Currently three antibodies are available that recognise the native form of prions and their use in an affinity application is ongoing. Work is also ongoing to produce further monoclonal and recombinant antibodies .

The discussion noted that it was important to have reliable tests and all three EU tests gave results showing no false positives however the issue of false negatives was the important issue in terms of consumer safety. As all the EU approved tests focus on CNS tissue it is likely there will be false negatives as the pathogenesis tests show other tissues are infective before CNS tissue is positive. This is why the tests are used in combination with the SRM rules and not alone for the consideration of food safety. For the future it is important to understand the infective dose particularly from pre clinical tissues. Risk analysis needs to indicate the level of sensitivity the diagnostic tests need to achieve equated with risk.

3 SUMMARY OF THE SEMINAR

Mike Attenborough Meat and Livestock Commission

3.1 Session one

The paper presented on carcass splitting gave some very interesting findings concerning the potential risk of contamination of meat with spinal cord and therefore, potentially prions.

- Further work on alternative splitting techniques and risk reduction measures for existing techniques needs undertaking.

The paper on neural embolism indicated that a captive bolt stunning technique that has widespread use and acceptance from the welfare aspect, may have some safety implications concerning distribution of brain tissue within the carcass.

- Further work should be carried out to assess this concern.

The new work to be undertaken on historical routes of entry of SRM will be challenging but needs to be done. The MLC will give as much assistance as possible.

The paper on immunological tests for CNS detection in meat and meat products concluded that a detection limit of 1% was achievable which is not low enough for policing the SRM ban. The paper on DNA methods offers some hope to improve on this.

- Further work on development and application to a species specific test needs to be undertaken.

3.2 Session two

The paper on transmission to poultry showed that birds caged for 5 years had serious welfare problems.

- Experiments on a larger number of birds for less time should be undertaken

The papers on pigs showing disease could be induced via the parental route but not the oral route raised questions

- Experiments to investigate what happens to the large doses given orally and what is the route of parental infection should be considered.

- **Other species should also be investigated including fish and deer as priorities**

3.3 Session Three

The paper on cattle pathogenesis presented some fascinating work, this project should continue to provide information on risk reduction.

- **It is important find out what happens in between the distal ileum and the brain becoming positive in infectivity tests.**
- **All available diagnostic tests should be used to test tissues**

The paper on prion analysis in milk raised the interesting issue of samples for bioassay. Current thinking indicates abnormal prions are unlikely to be present in significant or infective forms in blood. However if abnormal prion is identified the question of infectivity will be asked. Current estimates suggest 3000litres of milk need to be stored in case bioassay is required.

- **Some guidance on the necessity of this is urgently required.**

The sheep risk assessment undertaken by DNV consulting, estimated 3.5 Human ID 50 units of infectivity could have been consumed last year assuming the medium risk scenario for BSE in sheep, the range of uncertainty for this estimate is 0.2 – 650. A range of practical risk reduction measures were considered.

The risk assessment undertaken by Imperial college highlighted several research needs

- **understand the prevalence of TSE infection**
- **the relative infectivity of different edible tissues**
- **more detailed consumption and demographic data.**

3.4 Session Four

The introductory paper detailed the role of IRMM in the evaluation of diagnostic tests and highlighted the need for the provision of reference materials for test evaluation and comparison. The work of IRMM coordinating the application of the test kits with infectivity studies and effect of sample location was important.

The three EU approved kit manufactures gave detailed and interesting presentations both on their current tests and future developments.

Eurofins Scientific highlighted the world of high throughput screening using approved kits and servicing the needs of abattoirs. The screening system set up in Ireland described by Enfer was also impressive in terms of sample turnaround. Eurofins highlighted the need for detailed sample

identity, a situation that will be applicable to the UK if the OTM rule is changed to allow tested animals into the food chain.

The final paper explained the role of the VLA as the EU community reference laboratory and then gave an excellent review of where things are analytically including an interesting update on the application of ICE methodology as a screening method for detection of scrapie in blood.

Overall the seminar had provided a good forum for discussion and had taken an overview look at the research in place to meet the policy needs of the Food Standards Agency.

4 ASSESSMENT OF FSA RESEARCH IN PLACE AGAINST THE RESEARCH REQUIREMENTS IDENTIFIED IN THE RESEARCH POLICY

4.1 Tests and Screening

Tools are available to undertake the mass screening of sheep for TSE's.

Currently there are no tests to distinguish ovine BSE from scrapie and more importantly ovine BSE if it has occurred in natural sheep flocks is not characterised and it is not known what it would look like. Results from ovine BSE sub passaged into sheep are needed together with more information on other breeds . This work is being undertaken by DEFRA

Research on a live animal test is being pursued via the joint funders group recent call for research proposals. Further work on test development and specifically tests to distinguish TSE's should be undertaken.

4.2 Confirming the basis for the current food safety controls

Work on removal of lymphoid tissue from sheep intestines and risk assessment for the use of sheep guts and other parts of sheep need to be initiated.
Stunning slaughter and dressing techniques will continue to be investigated for risk and alternatives researched and proposed.

The cattle pathogenesis study will explore the application of new sensitive detection methods.
Milk from cattle with experimental BSE will be stored and tested for infectivity if required.

Carrier status is being investigated by DEFRA.

Infectivity studies with pigs, poultry and other species (deer, fish) will be continued.

4.3 Other Issues

Method development for CNS detection at low levels in animal tissues and with age differentiation need to be supported.

4.4 Safety of imports

Risk assessments need to be undertaken

4.5 BSE in sheep

DEFRA are undertaking extensive animal studies to address the occurrence, infectivity and transmission requirements. Experiments to determine infectivity titre of different tissues with time are not in place and need to be considered to assist the risk assessment studies.

Risk assessments on sheep milk and dairy products need to be considered Experimental studies to look for prion protein and possibly infectivity in milk from sheep and goats with experimental BSE need to be considered.

4.6 SEAC Recommendations

Historic human exposure and sheep risk assessment are being addressed

Mary Howell
Meat Science and Strategy Division
December 2001

SEMINAR DELEGATES LIST

TITLE	SURNAME	FORENAME	TYPE OF ORGANISATION
Dr	Howell	Mary	FSA
Mrs	Trowe	Carol	FSA
Mr	Walker	David	FSA
Professor	Ferguson	Neil	Imperial College
Dr	Davies	Jeremy	Eurofins Scientific
Dr	Praekelt	Uta	University of Leicester
Ms	Sayers	Riona	Enfer Scientific
Dr	Gough	Kevin	University of Leicester
Dr	Pugh	Ruth	FSA
Mr	Hewson	Peter	FSA
Mr	Davies	Owen	FSA
Dr	Pryde	Susan	FSA
Dr	Fergson	Carolyn	FSA
Mr	Attenborough	Mike	MLC
Mr	Fisher	Alan	University of Bristol
Dr	Helps	Chris	University of Bristol
Mr	Hindell	Philip	HSL
Mr	McCurdy	Gerry	FSA
Dr	Ghani	Azra	Imperial College
Dr	Hill	Irene	FSA
Mr	Comer	Philip	DNV
Dr	Huntly	Paul J	DNV
Dr	Allman	Avril	BBSRC
Mr	O'Neill	David	Silsoe Research
Dr	Harris	Neil	LGC
Mr	Tinker	David	Silsoe Research
Mr	Lumley	Ian	LGC
Dr	Dixon	Stephen	FSA
Dr	Knight	Andy	Silsoe Research
Dr	Pitman	Mark	MRC
Dr	Oesch	Bruno	PRIONICS
Dr	Mennim	Georgina	Roche Diagnostics
Mr	Harvey	Alan	DOH
Professor	Ironside	James	SEAC
Ms	Conroy	Adrienne	FSA
Ms	Gates	Hilary	DEFRA
Mr	Holley	Paul	FSA
Dr	Leigh	Antonia	DOH
Professor	Smith	Peter	SEAC
Mr	Matthews	Danny	VLA
Mr	Carruthers	David	FSA
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Dr	Cheeseman	Michael	VLA

Dr	Popping	Bert	Eurofins Scientific S.A.
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TITLE	SURNAME	FORENAME	TYPE OF ORGANISATION
Mr	Miles	Tim	MLC
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Mr	Stephenson	John	DOH
Sir	Arbuthnott	John	FSA Board
Dr	Ryder	Steve	VLA
Professor	McMurray	Cecil	DARD