

**INVENTORY OF NATIONAL RESEARCH ACTIVITIES  
IN TRANSMISSIBLE SPONGIFORM  
ENCEPHALOPATHIES (TSEs) IN EUROPE**

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# INTRODUCTION

In its meeting of 16 November 2000, the Research Council requested the Commission to establish a group of experts on research on transmissible spongiform encephalopathies (TSEs) in collaboration with the Member States.

The mandate for this group was as follows:

- to examine the state of TSE research across Member States
- to encourage exchange of scientific information between research teams and
- to identify on-going research activities, which need strengthening, as well as new research activities which need to be launched.

The group of experts met on 15 December 2000 and 16 February 2001 bringing together different actors involved in this specific field. The participants included those nominated by Member States (MS) and several Associated Countries (AC), some members of the TSE/BSE ad hoc group of the Scientific Steering Committee and some relevant co-ordinators of EU research projects.

When the Action Plan for TSE Research was launched with three subsequent calls for proposals, a special effort was made at the same time to create an inventory of national research activities which was updated most recently in 1999.

The inventory presented here gathers information received from Member States (except Luxembourg which has no research activities on TSE) on the current national research activities as well those from Iceland, Norway and Israel. It also includes activities undertaken by the Joint Research Centre (JRC) and those funded by the European Union.

This provides a comprehensive and up to date description of the scope of TSE research on going across Europe and has been structured as follows:

**1- Description of the main TSE research activities in each country**

**2- How these activities fit into the principal areas of the European Action Plan on TSE Research:**

- (a) Clinical, epidemiological and social research on human and animals TSE
- (b) The infectious agent and its mechanism of transmission
- (c) Diagnosis of TSEs
- (d) Risk assessment of SEs
- (e) Treatment and prevention

**3- Principal research teams and their areas of expertise**

**4- Collaboration with other countries and openness of programmes to collaboration.**

The degree of detail provided by each MS/AC has been variable but this reflects the size and importance of the research programmes going on in different countries.

This inventory will be updated on a regular basis as new information is received.

DG RTD would like to thank the contact persons in each country for providing this information in such a short time in order to comply with the mandate of the Council.

## National Contact Points

<b>Country</b>	<b>National Contact Point and Organisation</b>
Austria	H. Budka -University of Wien
Belgium	E. Vanopdenbosch- Veterinary Agronomic Research
Denmark	R.Hoff-Jørgensen – Danish Veterinary Laboratory
Finland	L. Sihvonen – National veterinary and Food Research Institute EELA
France	D. Dormont- Commissariat à l’Energie Atomique
Germany	J. Zachgo – Bundesministerium für Bildung und Forschung – Bonn
Hellenic Republic	T. Sklaviadis - Aristotle University of Thessaloniki
Iceland	Astridur Palsdottir – Institute of Experimental Pathology - University of Iceland
Rep. Ireland	M. McElroy –Veterinary Research Laboratory
Israel	A. Taraboulos – Hebrew University of Jerusalem
Italy	C.G. Bolis – University of Milan
Luxembourg	F. Wildschutz – Veterinary Services
Netherlands	B. E. C. Schreuder – ID- Lelystad
Norway	B. Bratberg – National Veterinarian Institute
Portugal	C. Lima - Hospital Egas Moniz
Spain	R Rodriguez-Bernabé – Ministerio de Ciencia y Tecnologia – Madrid
Sweden	T. Linne –BMC
United Kingdom	H. Gates – Ministry of Agriculture Fisheries and Food

# AUSTRIA

Responder: Prof. Herbert Budka, Institute of Neurology, University of Vienna, and ÖRPE, AKH Wien.

## **1. Description of main TSE research activities underway in AUSTRIA including Public Health aspects**

No national action or concerted plan on TSE research in Austria is yet in existence. Recently however, there are plans towards this aim.

Main TSE research activities underway in Austria including Public Health aspects include:

- Pro- and retrospective epidemiological study and surveillance of human and animal TSEs in Austria.
- Clinical, neuropathological and molecular genetic study of disease characteristics in human TSEs in Austria.
- Correlation of clinical, neuropathological [including distribution of the abnormal prion protein (PrP<sup>Sc</sup>) in brain tissue], molecular genetic and PrP<sup>Sc</sup> Western blotting data for disease type categorization.
- Clarification of the pathogenesis of TSEs in brain tissue, especially determination of specific cellular vulnerability and mechanisms of cellular damage in human and experimental disease.
- Distribution of the normal cellular prion protein (PrP<sup>C</sup>) in the body, especially in the skin and intestines ; this work is important when considering the possibilities for entry and spread of prions.
- The role of the normal cellular prion protein (PrP<sup>C</sup>) in neurodegeneration.
- Neuropathological diagnosis of TSEs, especially by detection of PrP<sup>Sc</sup> in tissues.
- Clinical laboratory diagnosis of CJD, especially by 14-3-3 protein determination in the CSF.
- Development of testing methods especially on blood.
- Decontamination procedures for industrial processes of biological materials, especially blood products.

**2. How these activities fit into the principal areas of the European Action Plan on TSE research:**

**a) Clinical, epidemiological and social research on human and animal TSEs**

- Prospective epidemiological study and surveillance of human TSEs in Austria as contribution to the European Surveillance Study of CJD.
- Networking of European neuropathological and other TSE laboratories for monitoring of definite TSE cases in Europe, confirmation of the diagnosis, up-to-date typing of all forms of human TSEs, identification of yet unrecognised disease forms, possibly including variant Creutzfeldt-Jakob disease (vCJD), atypical dementias and neurodegenerative disorders especially in the young, as contribution for recognition of all types of TSEs.

**b) The infectious agent and its mechanisms of transmission**

- Clarification of the distribution and function of the normal cellular (PrP<sup>c</sup>) and disease-associated (PrP<sup>Sc</sup>) prion protein in the body, as contribution to elucidation of pathways of the agent.

**c) Diagnosis of TSEs**

- Providing TSE tissues and body fluids as a unique resource of reference materials for research and in the development and validation of new diagnostic tests.
- Development of diagnostic methods, especially on blood as contribution to the respective European effort.

**d) Risk Assessment of TSEs**

- Prof. Budka serves as a member of the TSE/BSE AdHoc Group of the EU Scientific Steering Committee.

### **e) Treatment or prevention of TSEs**

- Clarification of the pathogenesis of TSEs in brain tissue, especially determination of specific cellular vulnerability and mechanisms of cellular damage in human and experimental disease, as rational basis for therapeutic strategies.
- Decontamination procedures for industrial processes of biological materials, especially blood products, as contribution to preventing animal-to human and human-to-human transmission.

### **3. Principal research teams and their areas of expertise: names, addresses, full details**

Four groups in Austria are involved in TSE research with the following major topics:

Prof. H. Budka, Institute of Neurology, University of Vienna, AKH 4J, A-1097 Wien:

- CJD surveillance.
- Disease characteristics in Austria.
- Characterisation and pathogenesis of tissue lesions in human and experimental TSEs.
- The role of the normal cellular prion protein (PrP<sup>c</sup>) in disease.
- Diagnostic methods, especially on CSF and CNS tissues.

Dr. J. Pammer and Prof. E. Tschachler, Departments of Pathology and Dermatology, University of Vienna, AKH, A-1097 Wien:

- Distribution in body epithelia of the normal cellular prion protein (PrP<sup>c</sup>).

Prof. H.P. Schwarz, Baxter AG, Industriestr.67, A-1220 Wien:

- Diagnostic methods, especially on blood and genetic testing.

Mag. H. Reichl, Haemosan GmbH, Kahng. 20, A-8045 Graz:

- Prion detection and decontamination in blood.

#### **4. Collaboration with other countries and openness of your programme to collaboration**

The following EU-supported research projects (as far as known to H. Budka) have been, or are being, performed with Austrian leadership or participation. They document the Austrian researchers' large EU collaboration including all major European TSE research groups:

- The human prion diseases: from neuropathology to pathobiology and molecular genetics (H. Budka, 1994-1996)
- Human transmissible spongiform encephalopathies: neuropathology and phenotypic variation (H. Budka, 1997-2000)
- European Centralized FACility for Human Transmissible Spongiform Encephalopathies (TSECFAC) (H. Budka, 1998-2001)
- Human Transmissible Spongiform Encephalopathies: The Neuropathology Network (PRIONET) (H. Budka, 2000-2003)
- Surveillance of CJD in Europe (EURO-CJD) (R. G. Will, 3 projects 1993-1996, 1997-1999, 2000-2003)
- Molecular biology and pathology of prion diseases (J. Collinge, 3 projects 1996-1998, 1997-2000, 1998-2001)
- Assessment and improvement of selected technologies to remove or inactivate TSE-causing agents (H. Reichl, 1997-2000)
- Low levels of TSE infectivity in blood: Determination of titre and evaluation of removal (H. Reichl, 1998-2001)
- TSE spiking experiments for process validations: evaluation of different sources of infectivity and spiking approaches (H. Reichl, 1999-2002)

Non-EU collaborations include A. Aguzzi (Zurich, Switzerland), P. Brown and L. Goldfarb (Bethesda, USA), P. Gambetti (Cleveland, USA), T. Kitamoto (Sendai, Japan), G. G. Kovács, C. Majtényi and I. Szirmai (Budapest, Hungary), P.P. Liberski (Lodz, Poland), and E.S. Williams (Laramie, USA).

## ADDENDUM AUSTRIA

There is no national action or concerted plan on transmissible spongiform encephalopathies (TSEs, prion diseases) research in Austria. TSE research has traditionally been centered at the Institute of Neurology, University of Vienna. According to the background of this group, their research concerns mainly TSE surveillance, the characterisation and pathogenesis of tissue lesions in human and experimental TSEs, as well as disease characteristics in Austria and diagnostic methods. More recently three other research groups entered this field, focusing on body distribution of the prion protein and prion detection and decontamination in blood, respectively.

Prof. Budka, Director of the Institute of Neurology, University of Vienna, led by Dec. 31, 1996, an EU Biomed-1 Concerted Action (CA) “The human prion diseases: from neuropathology to pathobiology and molecular genetics“ (Budka, 1995a; Budka, 1995b). It has networked almost 100 European neuropathological and basic research laboratories dealing with neuropathologic diagnosis of, and tissue-based research in, human TSEs. The final report of this very successful CA was published (Budka, 1997a). In two consensus reports, the CA has succeeded to define criteria for neuropathological diagnosis (Budka et al., 1995a) of, and tissue handling (Budka et al., 1995b; Budka et al., 1996a) in, human TSEs. The former consensus report is important for diagnosing human TSEs under generally accepted terms. The latter consensus report has greatly contributed to alleviate fears to perform autopsies on patients suspected to suffer from TSEs; autopsies are most important to have a definite diagnosis and are mandatory to identify the new variant of CJD recently recognized in the UK and France. A subsequent similarly designed EU Biomed-2 CA “Human transmissible spongiform encephalopathies: neuropathology and phenotypic variation” started on June 1, 1997, and finished May 31, 2000 (Budka, 1999a). Another following EU QoL CA “Human Transmissible Spongiform Encephalopathies: The Neuropathology Network (PRIONET)” started on Oct 1, 2000. It continues and expands the previously established European networking, with special emphasis on research into clinical, pathological, and molecular phenotypes of TSEs in Europe, including assessment of the distribution of the CJD variant in which an origin from BSE (“mad cow disease”) has become probable. Most interest was in atypical clinico-neuropathological presentations of dementing conditions especially in younger age groups. Neuropathologists have been invited to submit cases for further evaluation, including assessment of an eventual TSE basis; so far autopsy samples from more than 900 cases have been analysed and entered the ENDAPRID database.

In addition, Prof. Budka has participated in three EU BIOMED-2 Shared Cost Actions on the molecular biology and pathology of prion diseases (Project Leader: Prof. J. Collinge) which

started in July 1996, in Oct. 1997, and in Aug. 1998, respectively. The “European Centralized FACility for Human Transmissible Spongiform Encephalopathies (TSECFAC)”, coordinated by Prof. Budka, was established in August 1998. Prof. Budka has actively contributed to several WHO consultations on human and animal TSEs since 1996 (WHO, 1996a; WHO, 1996b; WHO, 1996c; WHO, 1997; WHO, 1998a; WHO, 1998b); he has been involved in WHO efforts to implement TSE surveillance systems in various parts of the world. The Institute of Neurology, University of Vienna, was proposed as a WHO Collaborating Centre for human TSEs. Moreover, Prof. Budka serves as a member of the TSE/BSE AdHoc Group of the EU Scientific Steering Committee.

Finally, the Austrian Reference Center for Human Prion Diseases (Österreichisches Referenzzentrum zur Erfassung und Dokumentation menschlicher Prionen-Erkrankungen / ÖRPE; head: Prof. H. Budka) was established from July 1, 1996, at the Institute of Neurology, University of Vienna (Budka, 1996b). It coordinates surveillance of human TSEs in Austria. As first successes, we have identified the first Austrian case of CJD in a dura transplant recipient (Radbauer et al., 1998a), one CJD family with a yet undescribed *PRNP* and molecular constellation (Hainfellner et al., 1996e), and the first Austrian family with familial fatal insomnia (FFI) (Almer et al., 1999; Budka et al., 1998; Budka et al., 1997). Moreover, Austrian cases of familial CJD with the codon 200 *PRNP* mutation have been shown as unrelated to other worldwide CJD200 families (Lee et al., 1999). CJD surveillance in Austria has been networked in the BIOMED-2 and QoL EU Cas on surveillance of CJD in Europe (Project Leader: Prof. R.G. Will, Edinburgh, UK (Will, 1995).

At the University of Vienna, Departments of Pathology and Dermatology, another group has addressed the distribution of the normal cellular prion protein (PrP<sup>c</sup>) in the body, especially in the skin and intestines (Pammer et al., 2000; Pammer et al., 1999; Pammer et al., 1998). This work is important when considering the possibilities for entry and spread of prions.

In Vienna, the company Baxter (especially Prof. H.P. Schwarz) is involved in development of testing methods especially on blood, partly in collaboration with Prof. Adriano Aguzzi in Zurich, Switzerland. Unfortunately they have not replied so far on my request for details, but some publications are on record (Fischer et al., 2000; Politopoulou et al., 2000; Zimmermann et al., 1999).

Another Austrian group, Dr. Reichl at Haemosan, an SME in Graz, focuses on decontamination procedures for industrial processes of biological materials, especially blood products. Dr. Reichl leads 3 EU BIOMED-2, BIOTECH and FAIR Shared Cost RTD Projects on “Assessment and improvement of selected technologies to remove or inactivate TSE-causing agents“, “Low levels of TSE infectivity in blood: Determination of titre and evaluation of

removal“, and “TSE spiking experiments for process validations: evaluation of different sources of infectivity and spiking approaches“ in collaboration with international partners.

Specific research topics investigated by Prof. Budka’s group include the following:

### **1. CJD (human and experimental)**

A retrospective epidemiological study of human TSEs in Austria (Hainfellner et al., 1996b), based on the nationwide collection of neuropathologically diagnosed, i.e. definite cases, showed a gradual increase in recent years; in 1995, the mortality rate was 1.25 per million inhabitants (Hainfellner et al., 1996c), and in 1996 1,41 (Radbauer et al., 1998b) which are the highest rates of definite human TSEs observed in Europe and the world. These data are important because Austria is a BSE-free country without a BSE risk scenario; the high CJD incidence is attributed to improved surveillance due to generally increased awareness of neurologists and the high autopsy rate in Austria (Bignall, 1996; Boulton, 1996). A detailed assessment did not reveal any profession at higher risk than the general population (Radbauer et al., 1998b). However, diagnosed cases were not evenly distributed throughout the country; cases were diagnosed more than three times as frequently in Vienna and surrounding Lower Austria (2,07 cases per million per year in the last 4 years) than in the rest of the country (0,63 cases per million per year in the last 4 years), indicating regionally differing quality of case retrieval and possibly double as high “true” incidence rates than generally assumed (Radbauer et al., 1998b).

Investigations on clinical laboratory diagnosis of CJD started at the Institute of Neurology by the group of Prof. H. Bernheimer, in close cooperation with Prof. Budka’s group and ÖRPE. This project was originally supported by a Research Grant from the Bundesministerium für Wissenschaft und Verkehr. The occurrence of the 14-3-3 protein has been studied in cerebrospinal fluid (CSF) samples from probable and possible CJD patients, respectively, and from controls (Bernheimer et al., 1998). Further investigations were directed to comparative studies on 14-3-3 and other diagnostic parameters in CSF and blood and published as a large European collaborative study (Zerr et al., 2000), confirming the usefulness of this diagnostic marker.

Some time ago, we described the first observation of “unilateral” CJD (Yamanouchi et al., 1986); this is important for recognition of the clinical variability of CJD. The previously described conjugal occurrence of CJD in an Austrian couple (Jellinger et al., 1972) was not confirmed by modern immunocytochemical detection of the prion protein (PrP) (Hainfellner et al., 1996a). This is an important argument against speculations on a possibility of environmental human-to-human transmission of CJD.

Several other studies have addressed, or are in progress to elucidate, the development of tissue pathology in TSEs: clinico-neuropathological overviews (Budka, 1996a; Budka, 1997b; Budka, 1998a; Budka, 1998c; Budka, 2000a; Budka, 2000c); the presence of peculiar tubulo-vesicular structures which are specific to TSEs (Liberski et al., 1992); the type and pattern of PrP deposition in a large series (Budka et al., 1996b; Hainfellner and Budka, 1996; Hainfellner et al., 1996d) which is an important point of reference when considering comparison with the newly recognised CJD variant in the UK; the PrP deposition in the brain stem which might give some hints on spread from a possible infectious origin (Pietrini et al., 1996), and the presence of associated Alzheimer-type brain tissue pathology which is able to modify the pattern of PrP deposition (Hainfellner et al., 1998). The distribution of parvalbumin positive neurons in brain correlates with hippocampal and temporal cortical pathology in CJD; selective damage to this subset of inhibitory neurons (Belichenko et al., 1999) might be the substrate of prominent excitatory symptoms in CJD such as myoclonus and EEG periodic hyperactivity (Guentchev et al., 1997). Only FFI differs in terms of this specific neuronal vulnerability from other TSEs (Guentchev et al., 1999). We have successfully extended these studies to experimental TSEs, showing that damage to this specifically vulnerable neuronal subset is the earliest pathological event in the brain during incubation (Guentchev et al., 1998). Studies on different “strains” and “glycotypes” of PrP in Austrian TSE patients, as revealed by Western blotting, have been initiated in collaboration with Prof. Gambetti and Dr. Parchi, Cleveland, OH, USA. In addition, tissue material of Austrian TSE patients was sent to Prof. Collinge, London, for additional blotting and transmission experiments. In collaboration with a group from Slovakia, we started a detailed clinicopathological analysis of a large number of familial CJD200 cases; this study should allow for identification of neuropathological features which will assist in delineating this familial CJD form from other human TSEs. In collaboration with European and American groups, distinct subtypes of sporadic CJD were identified in a large series by the combined assessment of clinical, pathological, genetic, and molecular features (Parchi et al., 1999).

Studies on experimental CJD showed that neuroaxonal dystrophy is an important part of the neuropathology of TSEs (Liberski et al., 1995). Another report studied the sequential development of tissue pathology correlated with PrP deposition in experimental CJD (Kordek et al., 1999).

Deposition of disease-associated prion protein was found to prominently involve the peripheral nervous system in experimental scrapie (Groschup et al., 1999). Trigeminal and dorsal root ganglia, Ganglion coeliacum, thoracicum and nodosum contained ganglion cells and fewer satellite cells with prominent granular PrPsc deposition. As a novel deposition pattern, punctate deposits in adaxonal location were seen along nerve fibres of peripheral nerve adjacent to ganglia. Such prominent involvement of the PNS in two different experimental scrapie models

emphasizes the need to consider the PNS in natural scrapie and other TSEs including bovine spongiform encephalopathy as potential source of infectivity. In human TSEs, such deposits were very rare (Hainfellner and Budka, 1999). Most recently, we succeeded to identify, in experimental TSEs, oxidative stress as an important pathogenetic process leading to neuronal loss (Guentchev et al., 2000), similar to what is happening in other neurodegenerative conditions.

Finally, we reviewed the present state of some TSE problems (Budka, 1998b; Budka, 1999b), of iatrogenic CJD (Budka, 1998a), and the safety of human blood and blood products (Budka, 2000b).

## **2. Gerstmann-Sträussler-Scheinker disease (GSS)**

The original Gerstmann family of Austria has been rediscovered and submitted to pedigree updating and clinical, neuropathological, and molecular genetic studies (Hainfellner et al., 1995). The classical P102L mutation was confirmed in the PrP gene (*PRNP*) (Kretzschmar et al., 1991). A change in phenotype from the classic ataxic to a CJD-like dementing type was observed in a recent patient; comparative molecular analysis showed that the *PRNP* genotype was identical at codon 129 (M/M homozygosity) in both phenotypes (Hainfellner et al., 1995). This means that additional factors (other genes? environmental?) determine phenotypic expression in this inherited TSE.

Another study described the ultrastructural neuropathology of GSS (Liberski and Budka, 1995) which includes also tubulovesicular structures (Liberski and Budka, 1994). Microglia has been found as an important constituent of PrP plaques (Barcikowska et al., 1993). As in Alzheimer's disease, paired helical filaments dissociate from amyloid formation (Liberski et al., 1996).

In collaboration with European and American groups, two patterns of PrP fragments were found to codistribute with distinct phenotypes in Gerstmann-Sträussler-Scheinker (GSS) P102L disease, including the original Austrian GSS family (Parchi et al., 1998). The results indicate that the neuropathology of prion diseases largely depends on the type of PrP-res fragment that forms *in vivo*. Because the formation of PrP-res fragments of 7-8 kDa with ragged N and C termini is not a feature of Creutzfeldt-Jakob disease or fatal familial insomnia but appears to be shared by most GSS subtypes, it may represent a molecular marker for this disorder (Parchi et al., 1998).

### 3. Familial fatal insomnia (FFI)

After having discovered the first Austrian FFI family in collaboration with the Neurological University Clinic Vienna (Budka et al., 1998), presence and distribution of tissue pathology in 3 FFI brains was analysed and correlated with PrP deposition and the *PRNP* genotype; there is distinct dissociation between the prominent tissue pathology and absent or sparsely detectable PrP<sup>res</sup> (Almer et al., 1999; Budka et al., 1997). The occurrence of FFI around the world was recently reviewed (Budka, 1998d). Although two major disease types can be delineated according to homo- or heterozygosity at *PRNP* codon 129, there is still marked heterogeneity between patients with the very same *PRNP* genotype (Budka, 1998d).

Moreover, we have recently identified a prominent disturbance of the serotonergic nervous system as likely substrate of some symptoms in FFI which clearly deviate from other TSEs (Wanschitz et al., 2000).

### 4. Kuru

By cooperation with the NIH in Bethesda, MD, USA, one of the apparently last sets of archival Kuru brain specimens could be investigated with modern neuropathological methods including immunocytochemistry for PrP (Hainfellner et al., 1997). This is important because the new CJD variant in the UK and France has been considered to share clinicopathological characteristics with Kuru; although some neuropathological features are shared by both conditions, also major differences were delineated.

**Manuscripts currently submitted for publication** include: Inherited Prion Disease with A117V Mutation of the Prion Protein Gene: A Novel Hungarian Family; Marked increase of neuronal prion protein expression in neurodegeneration; Deposition patterns of disease-associated prion protein in captive mule deer brains with chronic wasting disease; Increased incidence of sporadic Creutzfeldt-Jakob disease on the island of Crete associated with a high rate of PRNP 129 meth/meth homozygosity in the local population; and Mutations of the prion protein gene: clinicopathological correlations.

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## BELGIUM

### 1. Description of Main TSE activities underway in Belgium including Public Health Aspects.

Until recently, research on TSE was not really supported nor stimulated in Belgium. The Federal Ministries of Health and of Agriculture and the Regional Ministries for Research did finance some activities related to epidemiological and basic science studies.

Best research activities on TSE conducted in Belgium are related to epidemiology within European networks. Seven academic centres ensure CJD concerted surveillance in Belgium. Collaborative research on prion diseases with other European laboratories are oriented towards fundamental research on prion propagation and to TSE surveillance systems.

### 2. How these activities fit into the principal areas of the European Action Plan on TSE research:

#### a) clinical, epidemiological and social research on human and animal SEs

Diagnosis of Creutzfeld-Jacob disease through clinical and neuropathological examination	Team 1
Case control study of sporadic, iatrogenic and familial CJD	Team 1
<p><b>Epidemiology of transmissible spongiform encephalopathies in ruminants in Belgium</b></p> <p>A longitudinal study is performed to measure the annual cumulative incidence of neurological cases suspected or not to be caused by BSE in domestic and wild ruminants in Belgium. The neurological cases will be classified according to etiological criteria. The aim of this study is to provide data needed for a proper evaluation of the epidemiosurveillance of TSE in countries with a low incidence and classified III for the geographical risk.</p>	Team 5
<p><b>Surveillance network for CJD in Belgium</b></p> <p>In collaboration with 7 Belgian academic centers there is a surveillance for CJD cases in Belgium. This epidemiological surveillance is completed by the analysis of public health issues (ex.: blood transfusion) for the country and this analysis can lead to some specific recommendations (ex.: prevention in hospital). [funded through extended European collaborative study group of CJD]</p>	Team 6
Case control study in collaboration with University of Antwerp (UIA).	Team 6

Collaboration with a BSE experts group in order to have a link between human and animal aspects.	Team 6
The establishment of a European network for the surveillance of ruminant TSE and the standardisation of the process and criteria for the identification of suspected cases [funded through FAIR PL 987021 1999-2002]	Team 7
A network for supply of BSE tissues and fluids for European collaborative research [funded through FAIR CT 98 3651 1999-2001]	Team 7
Research on the immunohistochemical TSE detection in the mandibular lymphnodes in sheep as an in vivo test method and the significance of genotypic sensitivity [funded through Ministry of Agriculture]	Team 7

**b) the infectious agent and its mechanisms of transmission**

Prion protein gene sequencing	Team 1
<p><b>Role of PrP in prion spread and establishment of central nervous system infection</b></p> <p>The goal of this project is to identify weak links in the chain of prion propagation which in the long term may be amendable to therapeutic intervention.</p> <p>Five steps in prion spread and replication are addressed:</p> <ul style="list-style-type: none"> <li>= passage through the intestinal barrier</li> <li>= passage of prions through the skin</li> <li>= the nature of the neuroimmune interface</li> <li>= propagation and replication in the CNS</li> <li>= propagation and species barrier</li> </ul> <p>[funded through FAIR project No PI 96022, 1999-2001]</p>	Team 3
<p><b>Sequencing the canine gene coding for prion protein</b></p> <p>The canine gene coding for prion protein will be sequenced in order to explore the molecular basis of the absence of susceptibility of the dog to BSE agent infection.</p>	Team 4

### c) Diagnosis of SEs

Diagnosis of Creutzfeld-Jacob disease through clinical and neuropathological examination [funded through EuroCJD group]	Team 1
Study of cerebrospinal fluid alterations (qualitative and quantitative determination of 14-3-3 and isoforms, amyloid- $\beta$ protein, microtubule-associated protein tau, cytokines, prion protein)	Team 1
Phosphorylation of tau protein as marker of disease progression	Team 1
<p><b>A new and sensitive immunodetection method for prion proteins</b></p> <p>The Laboratory of human Histology has developed a patented immunoquantitative PCR method (iqPCR) based on immunocapture, immunodetection and quantitative PCR to reveal and quantify antigens.</p> <p>This method enables detection of prion proteins. Current research defines parameters necessary for measurement of PrPres in different tissues of infected animals or humans.</p> <p>Funded through Convention Wallonne No 14531= iqPCR; 2000-2002</p>	Team 3
Research on the immunohistochemical TSE detection in the mandibular lymphnodes in sheep as an in vivo test method and the significance of genotypic sensitivity [see also a)]	Team 7

### d) Risk assessment of SEs

<p><b>Evaluation of the TSE risk in medicinal products authorised in Belgium</b></p> <p>A risk evaluation was made regarding the presence of animal-derived material in medicinal products on the Belgian market, either as an active ingredient, or as an excipient or used during the production of medicinal products. All the information is introduced in an Access-data-file, which is continuously updated.</p>	Team 2
Epidemiology of transmissible spongiform encephalopathies in ruminants in Belgium [see a)]	Team 5
Surveillance network for CJD in Belgium [see a)]	Team 6
The establishment of a European network for the surveillance of ruminant TSE and the standardisation of the process and criteria for the identification of suspected cases [see a)]	Team 7

### e) treatment and prevention of Ses

Surveillance network for CJD in Belgium [see a)]	Team 6
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### Sources for funding for TSE research in Belgium

EuroCJD Group
CMP Biotechnology Working party (BWP)
FAIR PI 96022 1999-2002
Convention wallonne 14531-iqPCR 2000-2002
Extended European collaborative Study Group of CJD
FAIR PL 987021 1999-2002
FAIR CT 983651 1999-2001
Ministry for Agriculture

### 3. Principal research teams and their areas of expertise: names, addresses, full details

Team	Details
1	Department of Neurology Laboratory of Neurobiology, Born Bunge Foundation, University of Antwerp Universiteitsplein 1 B-2610 Wilrijk <b>Patrick Cras, MD, PhD, director, Jean-Jacques Martin, MD, PhD, neuropathologist</b> Tel: +32 3 821 34 23 Fax: +32 3 825 54 67 Email: <a href="mailto:cras@uia.ua.ac.be">cras@uia.ua.ac.be</a>
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7	Veterinary and Agricultural Research Institute Groeselenberg B-1180 Brussels <b>Dr E. Vanoptenbosch, Dr S.Roels</b> Tel: +32 2 3754 455 Fax: 32 2 375 0979 E-mail: <a href="mailto:stefan.roels@var.fgov.be">stefan.roels@var.fgov.be</a>

#### **4. Collaboration with other countries and openness of the programme to collaboration:**

**Team 1:** Collaboration in the context of the EuroCJD group with other European centres. The programme is open to and depends on external collaborations.

**Team 2:** A. Maes attends CPMP Biotechnology Working Party meetings (specific attention to TSE problems) at the EMEA in London and is a member of the TSE certification expert group at the European Directorate for the Quality of Medicines (EDQM) in Strasbourg.

R. Dobbelaer is member of the CPMP Biotechnology Working Party (BWP) and E. Voets also attends the meetings of the BWP. She is involved in Creutzfeldt-Jakob disease and blood components and plasma derivatives.

**Team 3:** Collaborations in the framework of the EU-funded project Fair n° Pl 96022; 1999=2001

Collaboration with Eurogenetec, and Biocode, both in Liège in the Convention Wallonne n° 14531=iqPCR; 2000=2002 project

**Team 6:** Participation in the extended European collaborative study group of CJD (NEURO CJD) and also subgroup 'Public Health'. Openness to other collaborations.

#### **Team 7:**

FAIR PL 987021 project: Cooperations with M. Rogers, G. Wells, M. G. Doherr, B. E. C. Schreuder, M. Groschup, J. J .Badiola, A. Souza, H. A. Kretzschmar, L. Larry, A. J. Galo...

FAIR 98 3651 project: Collaborations with S. Done, M. Groschup, J. Sanchez-Vizcaino, A. Douglas, J. Agerhorn...

Research on immunohistochemical TSE detection: Collaboration with Dr M.Smits (NL).

## DENMARK

### 1. Description of main TSE research activities underway including Public Health aspects

#### HUMAN TSE :

Since May 1997, all suspected cases of human CJD has been mandatory re-reportable to the Department of Epidemiology, Statens Serum Institut, Also, these data are included in the European EUROCCJD-project.

In order to classify the notified CJD-cases according to accepted criteria; a national expert group has been set up. The plan is to review all cases by neuropathology, EEGs and scans if available. So far no cases of variant CJD have been diagnosed.

The number of annual diagnosed cases of CJD has varied from 5 to 8 with 2 to 8 cases being definite. Giving a mean annual incidence of 0.8 per million inhabitants.

#### ANIMAL TSE :

The principal goal is to develop improved diagnostic means to ensure early and sensitive detection of TSE-agents in a rapid, high-throughput format. New developments in assay specificity will also be pursued, most importantly the ability to discriminate between Scrapie agents and the BSE-agent.

Activities will focus on development of composite synthetic peptide prion mimics that will be used for production of specific antibodies and also used in seed assays as indicators for presence of structure-modulating misfolded prion proteins. Also the capillary electrophoresis method for detection of abnormal prion proteins in buffy coat cells will be implemented and applied to bovine and ovine samples and the basic assay principle will be applied to other immunoassay formats. The use of plasminogen for detection of misfolded prion protein or for its concentration will be investigated. Finally it will be attempted to develop conditioned cell lines that are sensitive to misfolded prion protein.

### 2. How these activities fit into the principal areas of the European Action Plan on TSE research:

#### a) Clinical, epidemiological and social research on human and animal SE

##### HUMAN TSE :

In Denmark, a national diagnostic centre for CJD will be established at the University Hospital in Copenhagen. We are planning to intensify the surveillance of human CJD including variant CJD. Especially, the neuropathology surveillance is planned to be centralized as well as centralized DNA-sequencing; screening for mutations and analysis for e.g. 14-3-3 in CSF is planned.

No national research programmes on vCJD as such are planned, but international collaboration is strongly supported.

## ANIMAL TSE :

Surveillance of BSE, Targeted screening of risks groups (clinical cases, fallen stock, emergency slaughter, feed cohorte to positive cases) 15.000 samples/year (Danish Veterinary Laboratory). Screening slaughter cattle > 30 month estimated to be 250.000 samples/year (private laboratories).

Danish Veterinary Laboratory is national reference laboratory for animal TSEs and participate in the following projects:

FAIR CT 98-6056 European Scrapie Network  
Larry Paisley, epidemiology [lpa@svs.dk](mailto:lpa@svs.dk)

FAIR CT 98-7021 Surveillance and diagnosis of ruminant TSEs  
Larry Paisley, epidemiology [lpa@svs.dk](mailto:lpa@svs.dk).

FAIR 98-3651 A network for the supply of BSE tissue and fluids for European Collaborative research  
Thomas Krogh Nielsen, pathology [tkn@svs.dk](mailto:tkn@svs.dk)

### **b) The infectious agent and its mechanisms of transmission**

#### **c) Diagnosis of Ses**

Development of improved diagnostic means to ensure early, sensitive, non-invasive detection of TSE-agents in a specific manner, i.e. achieving discrimination between at least Scrapie agents and the BSE-agent in a rapid, high-throughput format. To reach this goal, basic investigations on the infectious agent, its mechanism of transmission and its pathogenic mechanisms at the molecular level will be studies with the double aim of providing more insight into which entity should really be considered the "infectious" units and to utilise the knowledge obtained for the design of assays based on new principles, including assays for "seed" ability, and cell-line based assays. It is assumed that protease resistance will not be a parameter than can be included in such an assay, as it is likely that pathogenic and protease-sensitive forms of the prion protein exist and will be able to transmit disease.

#### **d) Risk assessment of SE's**

#### **e) Treatment of prevention of TSE**

### **3. Principal research teams and their areas of expertise: names, addresses, full details**

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**cell lines**

#### **4. Collaboration with other countries and openness of your programme to collaboration**

The 5<sup>th</sup> Framework Programme: Quality of Life and Management of Living Resources:  
HUMAN TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES: THE NEUROPATHOLOGY NETWORK (PRIONET).

Dr. Henning Laursen (Neuropathology) e-mail: [hlaursen@rh.dk](mailto:hlaursen@rh.dk)

Biomed-2 concerted Action: EUROPEAN CDJ COLLABORATIVE SURVEILLANCE  
(NeuroCJD)

Dr. Henning Laursen (Neuropathology) e-mail: [hlaursen@rh.dk](mailto:hlaursen@rh.dk)

Collaboration with other countries include Norway (prof. Ulvund), Switzerland (dr. Oesch), the US (dr. Schmerr), Iceland (dr. Sigurdarsson) and Ireland. The project is open for collaboration with others especially for localization of samples; contacts have been established with Frédéric Lantier, INRA, F-37380 Nouzilly.

# FINLAND

## 1. Description of main TSE research activities underway including public health aspects

### Human TSE

**Descriptive Epidemiological Research.** We have monitored the descriptive epidemiology of Creutzfeldt-Jakob disease (CJD) in Finland since 1974. After an initial increase - apparently due to increased awareness and better diagnostic facilities - the annual incidence has remained at the same stable level at approximately 1 case per million. Although familial occurrence has been noted (see below), no cases of variant CJD or iatrogenic CJD have been detected.

**Research on Familial CJD.** A large family with autosomal dominant CJD was described by us in 1979. The affected patients showed an earlier onset and longer duration than typical examples of sporadic CJD. Furthermore, they lacked the characteristic EEG changes. A molecular genetic analysis of this family in collaboration with Dr. Gajdusek's group revealed a novel mutation in the PRNP gene at codon 178. An identical mutation was later found at the same codon by an Italian-American group studying patients with fatal familial insomnia. A joint study of patients with the Finnish type familial CJD and patients with fatal familial insomnia demonstrated that the disease phenotype, caused by the mutation, is determined by a common polymorphism of the PRNP gene at codon 129, a novel genetic mechanism. In vitro studies of synthetic prion protein peptides showed that mutated peptides were much more fibrillogenic than wild type peptides.

**Studies of familial Alzheimer's disease clinically simulating CJD.** We have described a very early onset form of Alzheimer's disease with early and prominent myoclonus, clinically simulating CJD (M146V mutation of the presenilin-1 gene). Recently, we have described yet another novel Alzheimer phenotype, termed variant Alzheimer disease, clinically characterized by spastic paraparesis in addition to dementia, and caused by a deletion encompassing exon 9 of the presenilin-1 gene. Even this condition has to be taken into consideration in the clinical differential diagnosis of atypical forms of prion disease.

**Molecular epidemiological studies on polymorphisms of the PRNP gene.** We have carried out molecular epidemiological studies of Alzheimer's disease and other forms of dementia in a prospective population-based and autopsy-controlled sample of very elderly individuals since 1991. We are now analyzing the distribution of PRNP gene polymorphisms in this and other younger population-based samples.

**Surveillance of CJD in Finland.** We participate in two EU-BIOMED-funded Concerted Actions on the epidemiology and neuropathology of prion diseases NEURO-CJD and PRIONET.

### Animal TSE

Scrapie and BSE were, as are all the diseases which have not been diagnosed before in Finland, compulsory notifiable diseases until 1990 when they added to the list of compulsory notifiable diseases in veterinary legislation. Epidemiological survey of BSE/scrapie has been carried out since 1995 in National Veterinary and Food Research Institute. No cases of BSE or scrapie have been found in Finland. The activities of the Institute are mainly involved in the diagnosis and surveillance of animal TSEs.

**2. These activities belong to the following areas of TSE research :**

- (a) Clinical and epidemiological research on human and animal SE
- (b) The infectious agent
- (c) Diagnosis of SEs
- (d) Risk Assessment of SEs

**3. Principal research teams and their areas of expertise :**

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**Neuropathology of human TSEs**

**Surveillance of TSEs**

**Molecular pathology of TSEs**

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**Immunodiagnosis of animal TSEs**

**Epidemiology of animal TSEs**

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**Histopathological diagnosis of animal TSEs**

**Surveillance of animal TSEs**

#### **4. Collaboration with other countries and willingness for collaboration**

Professor Matti Haltia has collaboration with many other groups.

Finland is also interested in collaboration in the area of diagnosis of animal TSEs, especially immunodiagnosis. No cases of BSE or scrapie have yet been found in Finland.

# FRANCE

## 1. Description of main TSE research activities underway in France including Public Health aspects.

Research in France is organised into concerted actions (ACC) and finalised programmes for biological tools (PRR).

There are 4 concerted actions (ACC):

- Fundamental biology and yeast/fungus models
- Pathogenesis and transmission of TSEs
- Human and animal epidemiology, risk assessment, modelling and sociology
- Therapeutics and safety

There are 3 lab networks whose aim it is to provide biological tools for the scientific community (PRR):

- Proteins and antibodies
- Cellular models and transgenic animals
- Diagnostic tests

## 2. How these activities fit into the principal areas of the European Action plan on TSE research:

### a) Clinical, epidemiological and social research on human and animal SE

#### **ACC2: Transmission and pathogenesis of TSEs**

- Neuronal death in TSEs: *in vivo* studies in humans, sheep and mice, role of microglial cells and *in vitro* studies
- Pathogenesis of sheep scrapie
- Study of serial passage of BSE in sheep with different PrP alleles
- Study of scrapie and BSE strains in sheep and mice
- Perinatal transmission of BSE in sheep

#### **ACC3: Human and animal epidemiology, risk modelling and sociology**

- Epidemiological surveillance of CJD
- Epidemiology and genetic study of sheep scrapie
- Socio-political configurations for dealing with TSE at the national and European levels

## **b) The infectious agent and its mechanism of transmission**

### **ACC1: Fundamental biology and analogous models**

#### 1. PrP

- Conformational dynamics of ovine / murine PrP
  - Molecular origin of sCJD (ORF mutation of PrP mRNA)
  - Interactions of PrP with chaperones or other ligands
  - PrP expression and role during neuronal differentiation
  - PrP, copper, oxidative stress, role in synaptic transmission
  - Characterisation of partially folded forms of PrP and their role in fibril formation
- Analogous models (yeast, fungus)
    - pHET-s, a prion model in *Podospora anserina*
    - Genetic control of cellular degeneration of filamentous fungi
    - Mechanisms of conversion of Ure2p to [URE3]
    - Structure of Ure2p

### **ACC2: Transmission and pathogenesis of TSEs**

- Synthetic PrP peptides as models for understanding the neurotoxic mechanisms and to inhibit PrP conversion
- Role of cerebellar circuitry in prion propagation
  
- Study of TSE-responsive genes
  
- Role of PrP in sperm cells: location, conversion, and role
- Role of PrPc in the immune system
  
- Peripheral cellular targets, early steps of neuroinvasion
- Study of anti-PrP T cell response in mice
- Study of the gut barrier with special attention to the role of M cells

### **PRR: Production of tools**

#### 1. Proteins and antibodies

- Production of different allelic forms of the prion protein with special attention for stability, quality, and purity
  
- Production of monoclonal PrP antibodies
- IHC screening of PrP antibodies on human / sheep tissues
- EM screening of PrP antibodies
- Development of PrP immunolocalisation methods in experimental TSE models

#### 2. Transgenic animals, cellular models

- Generation of transgenic mice expressing the ovine/bovine/human prion protein
- Generation of transgenic rabbits overexpressing the ovine prion protein
- *Microcebus murinus* as a primate model for the study of TSEs
- Development of *in vitro* models of neuronal death
- Development of cells infectable with sheep scrapie/other TSEs

## **c) Diagnosis of SEs**

### **PRR: Production of tools**

#### **3. Diagnostic tests**

- Development of an immunodiagnostic test for CJD based on the detection of 14-3-3 protein in the CSF.
- Diagnosis of sheep scrapie based on the detection of cortisol metabolites
- Diagnosis of BSE by a sandwich immunoassay

Several brain and tissue banks are set up:

- Human brain and tissue banks (Pitié-Sapétrière and Lariboisière Hospitals)
- Sheep and cattle tissue banks (AFSSA, INRA)

## **d) Risk assessment of SEs**

### **ACC2: Transmission and pathogenesis of TSEs**

- Assessment of suitability of transgenic mice expressing ovine PrP for strain typing of ruminant TSEs
- Risk linked to blood transfusion and BSE in humans

### **ACC3: Human and animal epidemiology, risk modelling and sociology**

- Modelling of TSE-specific risk of meat products to provide a basis for TSE regulation and prevention
- Risk perception of TSEs/BSE: determinants and variability

### **ACC4: Therapeutics and safety**

- Validation of a method of identification and labelling of specified risk material
- Methods of safe removal of ruminant spinal cord at the slaughterhouse

## **e) Treatment and prevention of SEs**

### **ACC4: Therapeutics and safety**

- Therapeutic perspectives by using synthetic peptides interfering with PrP accumulation
- Development of cellular models and analysis systems for a rapid evaluation of new approaches in TSE therapeutics.

**Appel d'offres ESST-PRIONS 1999**

**Projets ACC1 proposés pour financement après évaluation des 22 projets soumis**

<b>Project Leader</b>	<b>Institution</b>	<b>Project</b>
BEGUERET Joël	CNRS	Hets protein, a model for prions in <i>Podospora anserina</i>
CULLIN Christophe and MELKI Ronald	CNRS	Genetic analysis of [URE3+], a yeast prions in <i>Saccharomyces cerevisiae</i> , and Conversion mechanisms of ure2p into Ure2p[URE3]
DEBEY Pascale	INRA	Conformational dynamic of ovine PrP: comparative study of two allelic variants that determine susceptibility to scrapie
DRON Michel	CNRS	Search for PrP mRNA that harbour an open reading frame mutation in sporadic cases of Creutzfeldt-Jakob disease
DUBUISSON Jean	CNRS	Interactions between Chaperones and PrP
KELLERMANN Odile	CNRS	PrP expression during neuronal differentiation
LEHMANN Sylvain	CNRS	PrP cleavage, Cu <sup>++</sup> , oxidative burst and TSEs
LLEDO Pierre-Marie	CNRS	Electrophysiological aprocach of the function of PrP-c in hippocampus
NERI Christian	C.E.P.H.	Cellular and molecular modelisation and biological effects of normal and mutated human PrP expressed in <i>Caenorhabditis elegans</i>
SANSON Alain	CEA	Murine PrP stability: structural propension to form alpha helices and its relationships with glycosylation.
SILAR Philippe	University of Paris VII	Genetic control and unconventional agents that cause cellular degenerative processes in <i>Podospora anserina</i>
AUCOUTURIER Pierre and CARNAUD Claude	INSERM	Molecular and cellular characteristics of T-cell mediated immune response against PrP in mice
BAILLY Yannick	CNRS	Route of neuroinvasion of the cerebellum and brain in mouse: role and dysfunctions of cerebellum during TSEs.

CESBRON Jean-Yves	University of Grenoble	PrP-c function in immune system
DANDOY-DRON Françoise	CNRS	Host gene expression during TSE: implication of Scrapie responsive gene 1 (Scrg-1), of Spi 2, an alpha-1 antichymotrypsin analogue, and other genes.
DORMONT Dominique	CEA/CRSSA	Risk associated with blood in humans exposed to BSE agent: a non-human primate model."
FOURNIER Jean-Guy	CEA/CRSSA	Identification of the target cells of TSE agents after peripheral infection.
GRAY Françoise	University of Paris	Neuronal death during human TSEs
PILLOT Thierry	INSERM	Neurotoxicity mechanisms of a PrP-derived peptide (PrP 118-135): fusigenic properties and pathogenic consequences
SALES Nicole	INSERM	Neuroinvasion and axonal transport of PrP-res
SCHELCHER François	INRA/ENV	Scrapie agents and BSE agent dissemination as a function of species, host genotype and route of inoculation: experimental infection and serial passages in sheep and transgenic mice.
CHABRY Joëlle and VINCENT Jean-Pierre	CNRS	Synthetic PrP-derived peptides as conversion inhibitors: therapeutic tools
SIRAMI Jean	ADIV	Study of non-contaminant procedures for removing bovine and ovine spinal cord in slaughterhouses
BERTHON Patricia	INRA	Immunohistochemistry screening of polyclonal and monoclonal antibodies directed against PrP: use of sheep organ slices."
FONTAINE Jean-Jacques	National Veterinary School (Alfort)	Antibody screening: immunohistochemistry detection of PrP in TSE affected ruminants.
FOURNIER Jean-Guy	INSERM	AntiPrP antibody screening for electron microscopy
FREYSSINET Jean-Marie	INSERM	Neuronal death diagnosis in CNS
GAGNON Jean	CNRS	PrP purification
GRASSI Jacques	CEA	Anti-PrP antibody production: generation of antibodies that could differentiate PrP-c and PrP-res
HOUEBINE Louis-Marie	INRA	Ovine PrP-transgenic rabbits
LABONNARDI ÈRE Claude	INRA	Monoclonal antibodies directed against natural isoforms of ovine PrP
LAUDE Hubert	INRA	Development of cellular models for in vitro scrapie propagation.
LEHMANN Sylvain	CNRS	In vitro propagation of TSE agents.

LEMAIRE Catherine	CNRS	Development of highly sensitive transgenic animals to human and bovine TSE infection
ROSSI Bernard	Inserm	Production of monoclonal antibodies by injection of PrP-Fc or PrP-DNA
TOUTAIN Pierre-Louis	INRA	Diagnosis of scrapie: use of cortisol metabolite, sensitivity, specificity and predictive values.
VILOTTE Jean-Luc	INRA	Transgenic mice expressing ovine PrP-VRQ allele as a model of scrapie and establishment of immortalized cell lines.
CHATEURAYN AUD Francis	CNRS	Sociology, public policy, and alert networks: differences between TSE risk evaluation and perception between national and european levels.
EYNARD Pascal	Cemagref	TSE associated risk modelisation: a tool for decision and prevention.
LAUDE Hubert	INRA in collaboration with FRCVDA-Tubingen (M. Groshup)	Infectivity in ELISA positive Western blot negative cattle brain samples: use of transgenic mice harbouring the bovine PrP gene.

# GERMANY

## 1. Description of main TSE research activities underway in your country including Public Health aspects.

Research on TSE is jointly supported by the Federal Ministry of Education and Research (BMBF), the Federal Ministry of Health (BMG) and the Federal Ministry of Consumer Protection, Food and Agriculture (BMVEL). In addition the German Research Organisation (DFG) and other public and private funding agencies support research on these subjects. Research activities focus on the analysis of the nature of the infectious TSE-agent, its transmission mechanisms, clinical and epidemiological questions and diagnostics for TSEs. The table below includes activities in TSE-research in Germany, which were pursued during the year 2000 and onwards.

Germany has a national concerted plan for TSE research which is currently updated. Future funding initiatives of the BMBF will focus on research in the field of new diagnostics for TSEs and the development of therapies for CJD/nvCJD. Especially with regard to the development of new therapies progress in the understanding of the pathology of other neurodegenerative diseases like Alzheimer's may provide helpful insights into human TSEs. Therefore interdisciplinary research crosslinking groups working on therapeutic approaches for other neurodegenerative diseases with CJD/nvCJD research will be funded. A second funding initiative centers on the development of new diagnostics tools for human and animal TSEs that are more sensitive or have a higher specificity than present tests or allow TSE-strain typing. Clinical research on CJD will be a focus of a national research network on human dementia (KNM Demenz) which BMBF plans to establish this year. Furthermore BMBF supports the "BrainNet", a research infrastructure initiative for human neurodegenerative diseases. The "BrainNet" collects and stores tissue and brain samples of patients, including CJD-patients, for future research. Finally a TSE-research platform will be established, which will serve as a means to inform on and co-ordinate the divers national TSE-research activities.

The activities of the BMG focus on the surveillance of Creutzfeldt-Jakob-Disease, the safety of blood and medical products (Paul-Ehrlich-Institute, Langen) and the spread of the infectious agent within the organism (Robert-Koch-Institute, Berlin). In the case of nvCJD the epidemiology is done at the Universities of Göttingen and Munich and the Robert-Koch-Institute.

The BMVEL has funded the National Veterinary Reference Laboratory for BSE and Scrapie at the National Institute for Virus Diseases in Animals in Tübingen (BFAV) since 1992. Research activities focus on animal TSEs, especially on diagnosis, epidemiology,

the infectious agent and its mechanisms of transmission. Beginning January 2001 the institute for new and unknown animal diseases at the BFAV, with a strong focus on animal TSEs, was established.

The DFG has funded a number of projects dealing with basic mechanisms of neurodegenerative processes and especially with Alzheimer's disease, Morbus Parkinson a.s. contributing directly or indirectly to the understanding of prion diseases and BSE/TSE. Since 1994 about 30 Mio. DM have been invested in the research of this global subject by financing individual research projects and coordinated funding programmes. Since 1994 several projects have been funded with approximately 6.5 Mio. DM concerning the closer theme prion disease. Further applications will be decided during the next months by the grants committee of the DFG. Focus of most activities is the genesis, spreading, reproduction and pathogenesis of the prion protein.

## 2. **How these activities fit into the principal areas of the European Action Plan on TSE Research:**

The projects listed below represent a subset of TSE-research projects, which received funding in the 2000 or have started this since then.

### a) **Clinical, epidemiological and social research on human and animal SE**

<b>Projects</b>	<b>Project leader Institution</b>	<b>Time of funding</b>
Studies on diagnosis, epidemiology and molecular pathology of human SE	Prof. Dr. Kretzschmar (LMU München) Prof. Dr. Poser, (Univ. Göttingen)	05/ 1993 – 04/1999 05/ 1999 – 04/2004
National Reference Laboratory for BSE and Scrapie	Dr. Groschup (BFAV)	11/ 2000 - 05/2003
Studies on pathogenesis of SEs with hippocampus cell cultures of PrP <sup>0/0</sup> -mice and production of transgenic mice	Prof. Kretzschmar (LMU München)	09/ 1994 - 08/1997 09/ 1997 - 08/2000
Fibril induced domains of the prion protein	Dr. Groschup, B. Teufel BFAV, Tübingen	09/ 1994 - 08/1997 09/ 1997 - 02/2001
brain net: German Reference-Centre for CNS	Prof. Kretzschmar (LMU München)	1999 - 2003
CJD in the European Union – incidence and risk factors	Prof. Poser (Uni. Göttingen)	05/1997-04/2000
Human transmissible spongiforme encephalopathies: neuropathology and phenotypic variation	Prof. Poser, Dr. Zerr (Univ. Göttingen)	05/1997-05/2000
An EU-wide “TSE-laboratory with no walls”	Prof. Kretzschmar (LMU München)	08/1998-07/2001

The establishment of a European network for surveillance of ruminant TSE and the standardisation of the process and criteria for identification of suspect cases	Dr. Groschup (BFAV), Prof. Kretzschmar (LMU München)	1998–2001 05/1999–05/2002
Therapy-study CJD	DR Otto, Prof. Prange (Univ. Göttingen)	1997-2001
Population based genetic study on scrapie resistance in sheep breeding in Germany	Prof. Brenig	2000-2005

**b) The infectious agent and its mechanisms of transmission**

<b>Projects</b>	<b>Project leader Institution</b>	<b>Time of funding</b>
Studies on pathogenesis of SEs with hippocampus cell cultures of PrP <sup>0/0</sup> -mice and production of transgenic mice	Prof. Kretzschmar LMU München	09/ 1994 - 08/1997 09/ 1997 - 08/2000
Structure and structural changes of PrP <sup>Sc</sup>	Prof. Riesner University of Düsseldorf	09/ 1994 - 08/1997 09/ 1997 - 08/2000
Studies on the spread of prions during gastrointestinal induced TSE-infections	Dr. Beekes, RKI	since 1994
Studies on oral transmission of the scrapie agent in hamsters and dynamics of scrapie pathogenesis	Prof. Dr. Diringer (RKI, Berlin)	01/ 1995 - 08/1997 07/ 1997 - 08/2000
CJD and BSE: an integrated molecular and experimental neuropathological analysis of prion neurodegradation, strain variation and transmission risks	Prof. Hunsmann/Prof. Bodemer (DPZ), Prof. Kretzschmar, DR. Windl (LMU München), Dr. Groschup (BFAV)	1997-2000
Functions of the prion protein in synaptic interactions	Prof. Herms, Prof. Kretzschmar (LMU München)	1998-2000
Role of the cellular prion-protein for microglial cell function	Dr. Prinz (MDC)	1998-2000
Relationship between conformation of PrP, infectivity and pathogenicity of BSE as a basis for diagnosis	Dr. Weiss, Prof. Kretzschmar (LMU München), Prof. Riesner (Univ. Düsseldorf)	01/1998-06/2001
Improving aspects for scrapie control in sheep and goats by study of host genotypes, TSE isolates and their in vivo interaction	Dr. Groschup (BFAV)	01/1998-06/2001
Cellular pathogenesis of prion diseases	Prof. Kretzschmar (LMU München)	06/1998-05/2001

Prion diseases: mechanisms of transmission and identification of targets for potential therapeutics	Dr. Stuke (DPZ), Dr. Groschup (BFAV), Prof. Kretzschmar, Dr. Schätzl (LMU München), Dr. Bürkle (Univ. Newcastle)	08/1998-07/2001
The bovine PrP: From structure analysis to the molecular mechanisms of conformational transitions	Dr. Harthl, Dr. Tatzelt (MPI Martinsried)	06/1998-05/2001
Investigation of putative signal transduction processes of normal prion proteins and their role in spongiforme encephalopathy	Dr. Ohm, (Charité Berlin)	06/1998-12/2001
Structure, function and interactions of PrP and PrPdomains	Dr. Bürkle (Univ. Newcastle), Prof. Kretzschmar (LMU München)	07/1998-06/2001
PrP oligomerisation and dimerisation as a model for the evaluation of TSE transmission modalities and as a target for therapeutic intervention against TSE	Dr. Weiss (LMU München) Dr. Schätzl (Max Pettenk.) Prof. Riesner (Univ. Düsseldorf)	1998-2002
Establishing the resources for and examination of transcription changes occurring during BSE infection	Dr. O'Brian, Dr. Schalkwyck (MPI Berlin)	1998-2003
Membrane insertion and glycosylation of PrP	Prof. Dobberstein (Univ. Heidelberg)	05/1998 02/2000
Functional characterisation of the prion protein	Dr. Tatzelt (MPI Martinsried)	07/1999-06/2002
Neurodegenerative diseases (The phenomenon of protein aggregation in different diseases)	Prof. Kretzschmar, Dr. Schätzl (LMU München), Dr. Tatzelt (MPI Martinsried)	05/2001-04-2004
Studies of prion proteins in cultured cells	Dr. Schätzl LMU München	09/ 1997 - 08/2000
Analysis of the structural change of the prion protein using the ER-method	Stöckel (MPI, Martinsried)	11/ 1998 - 10/2000
Investigation and localisation of disease associated changes in the CNS of scrapie by using the FT-IR-spectroscopy	Prof. Naumann, Dr. Beekes, RKI	06/ 1998 - 05/2001
Fibril induced domains of the prion protein	Dr. Groschup BFAV, Tübingen	09/ 1994 - 08/1997 09/ 1997 - 02/2001
Characterisation of RNA aptamers against fragments of the prion protein	Dr. Weiss (LMU München)	09/ 1994 - 08/1997 09/ 1997 - 08/2001
Mass spectroscopic methods for the analysis of the structure	Krause (Berlin)	09/ 1998 - 09/2000 10/ 2000 -

Studies on the localisation of the PrP in the membrane and the glycosilation of the protein	Prof. Dobberstein (Univ. Heidelberg)	05/ 1998 - 04/2000 02/ 2000 - 09/2001
The impact of microglia cells on the pathogenesis of prion diseases	Dr. Heppner (Zürich)	04/ 1999 - 03/2001
Functional characterisation of the prion protein	Dr. Tatzelt (MPI, Martinsried)	07/ 1999 - 06/2002
Analysis of the infectious prion protein in cultured cells	Dr. Schätzl (Max Pettenkofer Inst.)	09/ 1999 - 08/2001
Kinetic mechanism of the aggregation of peptides	Dr. Schuler (Bethesda)	07/ 1999 - 06/2001
Development of chemical and structural attributes of infectious aggregates	Dr. Hoffmann / Prof. Riesner (Univ. Düsseldorf)	08/ 1999 - 07/2002
Differential gene expression: Search for relevant genes for pathogenesis	Dr. Simon, Dr. Baier, RKI	since 1999
Neuroinflammation during TSE-infection	Dr. Baier, Neidhold, Schultz	since 2000
Prion Gene, other genes associated with neurogeneration and their gene products involved in sporadic inclusion body myositis	Lampe (TU Dresden)	07/ 2000 - 06/2002
Characterisation of the structural changes of hamster PrP and errors	PD Dr. Naumann / Dr. Beekes (RKI, Berlin)	2000 - 2003
Prion proteins	Prof. Günther (FZMB, Langensalza)	02/ 2001 - 12/2002

**c) Diagnosis of SEs**

<b>Projects</b>	<b>Project leader Institution</b>	<b>Time of funding</b>
Application of methods of fluorescence spectroscopy to the investigation and diagnosis of prion diseases	Prof. Eigen (MPI Göttingen)	09/ 1997 - 08/2000
Studies for the detection of PrP <sup>RES</sup> in different tissues and fluids of humans	Dr. Beekes, RKI	since 1998
Histopathological and immuno-histochemical diagnosis of BSE and Scrapie	Dr. Groschup, Dr. Hardt BFAV	07/ 1997 - 06/2000
Characterisation of RNA aptamers directed against fragments of the prion protein	Dr. Weiss (LMU München)	09/ 1994 - 08/1997 09/ 1997 - 08/2001
Methods for the diagnosis of human SE	Prof. Dr. Poser (Univ. Göttingen)	07/ 1997 - 06/1999 07/ 1999 - 06/2004
Development of a genetic profile on RNA level and proteins of mice infected with Scrapie	Dr. Schröder (PEI)	08/ 1998 - 08/2001

TSE-diagnosis in tissue and serum using FT-IR-spectroscopy	Prof. Naumann, Dr. Beekes, RKI	since 1998
Development of a diagnostic system for the detection of neurodegenerative prion diseases	Boehringer Ingelheim Vetmedica GmbH	01/ 1999 – 12/2001
Sensitive raman spectroscopy for the analysis of the conformation of prion proteins in membranes	Univ. Düsseldorf	07/ 1999 – 06/2002

<b>Projects</b>	<b>Project leader Institution</b>	<b>Time of funding</b>
Early diagnostics for TSE (Scrapie; hamster model)	RKI / PEI	11/ 1999 – 10/2002
Early diagnostics for transmissible spongiforme encephalopathies	Dr. Beekes, RKI	11/ 1999 – 11/2002
Early diagnostics for TSE: Role of the prion protein-look-alike in TSE	Dr. Schröder (PEI)	03/ 2000 - 03/2003
National Reference Laboratory for BSE and Scrapie	Dr. Groschup (BFAV)	11/ 2000 – 5/2003
Detection of risk material in meat products	- Not reviewed yet – (see also d)	2001 – 2002
Characterisation and differentiation of German BSE- and Scrapie-isolats	Dr. Groschup (BFAV)	03/ 2001 - 02/2004
Generation of ovine and bovine PrP transgenic mice for the development of improved bioassays for BSE and scrapie agent detection	Dr. Groschup (BFAV)	1997 - 2001
Development of novel diagnostics to assist quality assurance procedures in EU meat production	Prof. Schröder (Univ. Mainz)	1997-2001
Laboratory supported diagnosis of CJD	Prof. Poser (Univ. Göttingen)	1998-2001
Analysis and function of 14-3-3 isoforms: early diagnosis of CJD	Dr. Otto, Dr. Jens (Univ. Göttingen)	07/1998-06/2001
Diagnosis of TSE using PrPsc/PrPc-specific antibodies	Prof. Kretzschmar (LMU München), Miltenburger (CCR Cytotest Cell Research GmbH)	07/1998-06/2001
Quantity analysis of MR scans in CJD	Dr. Zerr (Univ. Göttingen)	11/1998-10/2001
Development and control of PrPsc-based test in humans and animals using cerebrospinal fluid and brain tissue	Prof. Kretzschmar (LMU München), Prof. Riesner (Univ. Düsseldorf)	10/1999-10/2002

Development of a rapid high throughput assay for sensitive and specific detection and strain typing of Creutzfeldt-Jakob-disease based on fluorescence correlation spectroscopy	Prof. Kretzschmar (LMU München)	2001-2004
A network for supply of BSE tissues and fluids for European collaborative research	Dr. Groschup (BFAV)	12/1998-11/2001

**d) Risk assessment of SEs**

<b>Projects</b>	<b>Project leader Institution</b>	<b>Time of funding</b>
Structural effects of public communication onto regulations in the context of the BSE conflict	Japp (Bielefeld)	01/ 2000
Trans-national public and structuring of political communication in Europe	Eder (Berlin)	11/ 2000 - 10/2002
Preventive soil protection	BMU	2001 - 2003
Detection of risk material in meat products	(see also c)	2001 - 2002
Risk assessment in primates of TSE transmission to humans through food and blood products	Prof. Hunsmann (DPZ). Prof. Löwer (PEI)	10/1999-09/2009
BSE transmission through food and blood products: A study to assess the risk for humans	Dr. Hahmann, Prof. Hunsmann (DPZ). Prof. Löwer (PEI)	1998-2001
Public perceptions of BSE and CJD risk in Europe	Dr. von Alversleben (Univ. Kiel)	07/1999-04/2002

**e) Treatment of prevention of SEs**

<b>Projects</b>	<b>Project leader Institution</b>	<b>Time of funding</b>
Chemoprophylaxis and chemotherapy for prion-infections	Prof. Müller University of Mainz	See above
Cell culture-investigation of prion-proteins	Dr. Schätzl LMU München	See above
Immunotherapy and immunoprophylaxis of Scrapie/BSE-infections	Dr. Baier, Dr. Beekes, RKI	since 2000

TSE agent inactivation, product quality evaluation and sterilization process simulation in rendering processes for the production of feed grade animal protein	Oberthür GmbH (Bawinkel)	1998-2001
Development of TSE treatment based on prion protein-binding oligosaccharides	Dr. Weiss (LMU München)	08/1999-07/2002
Prevention of protein aggregation by competitive $\beta$ -sheet binders	Prof. Schrader (Univ. Marburg), Prof. Riesner (Univ. Düsseldorf)	06/2000-05/2003
Inactivation of the causative agents of TSE by thermophilic proteases	Antranikian (Univ. Hamburg)	1997-2000

### 3. Principal research teams and their areas of expertise: names, addresses, full details

Addresses of groups involved in TSE research	Areas of expertise
<p><b>Prof. Dr. H. A. Kretzschmar</b>            LMU München            Inst. für Neuropathologie            Marchioninstr. 17            D-81377 München</p> <p>Phone: 089-7095-4900            Fax: 089-7095-4905            email: hans.kretzschmar@inp.med.uni-muenchen.de</p>	<p>The infectious agent and its mechanisms of transmission            Diagnosis of SEs</p>
<p><b>Prof. Dr. S. Poser</b>  <b>Dr. M. Otto</b>  <b>Dr. I. Zerr</b>            Universität Göttingen            Poliklinik, Zentrum für neurolog. Medizin, Abt. Neurologie            Robert-Koch-Str. 40            D-37075 Göttingen</p> <p>Phone: 0551-396636            Fax: 0551-397020            email: 106004.1022@compuserve.com</p>	<p>Clinical research on human SE            Epidemiological surveillance on CJD            Clinical study on CJD-therapy</p>
<p><b>Prof. Dr. D. Riesner</b>            Universität Düsseldorf            Inst. für Physikalische Biologie            Universitätsstr. 1            D-40225 Düsseldorf</p> <p>Phone: 0211-811-4840</p>	<p>The infection agent and its mechanisms of transmission            Diagnosis of SEs</p>

<p>Fax: 0211-811 5167 email: riesner@biophys.uni-duesseldorf.de</p>	
<p><b>Dr. H. Schätzl</b> LMU München Genzentrum/Max-von-Pettenkofer Institut Feodor-Lynen-Str. 25 D-81377 München</p> <p>Phone: 089-2180-6862 Fax: 089-2180-6898 email: schaeztl@lmb.uni-muenchen.de</p>	<p>Intracellular trafficking of prion proteins, development of therapeutic measures against human TSE</p>
<p><b>Prof. Dr. T.C. Mettenleiter</b> <b>Dr. M. Groschup</b> Bundesforschungsanstalt für Viruskrankheiten der Tiere (BFAV) Hauptsitz Insel Riems Boddenblick 5a D- 17498 Insel Riems</p> <p>Phone: 07071-967-257 Fax: 07071-967-105 email: martin.groschup@tue.bfav.de</p>	<p>Clinical and epidemiological research on animal SE, animal models</p>
<p><b>Prof. Dr. D. Arnold</b> Bundesinstitut für den gesundheitlichen Verbraucherschutz (BgVV) Thielallee 88-92 14195 Berlin</p>	<p>TSE in live stock, TSE in fish?, diagnostic tests</p>
<p><b>Dr. M. Baier / Dr. M. Beekes</b> Robert-Koch-Institut Fachgebiet 123 Nordufer 20 13353 Berlin</p> <p>Phone: 030-4547-2230 Fax: 030-4547-2609 email: baierm@rki.de</p>	<p>The spread of the infectious prion in the organism, routes of infection</p>
<p><b>Prof. Dr. J. Löwer</b> <b>Dr. Björn Schröder</b> Paul-Ehrlich-Institut Paul-Ehrlich-Str. 51-59 63225 Langen</p> <p>Phone: 06103-77-2000 Fax: 06103-77-1252 email: loejo@pei.de</p>	<p>Transcriptional and post-transcriptional mechanisms involved in the expression of the prion protein, differential gene expression in nervous tissue in TSE-infected individuals</p>
<p><b>Prof. Dr. G. Hunsmann</b> Deutsches Primatenzentrum GmbH Kellnerweg 4 37077 Göttingen</p>	<p>Model organisms, transgenic mouse-models</p>

Phone: 0551-3851-115/150 Fax: 0551-3851 184	
<b>Dr. S. Weiss</b> Genzentrum LMU München Feodor-Lynen-Str. 25 81377 München  Phone: 089-74017-400 Fax: 089-74017-448 email: weiss@lmb.uni-muenchen.de	Development of new diagnostics and therapies against TSEs, animal models
<b>Dr. J. Tatzelt</b> MPI für Biochemie Am Klopferspitz 18 82152 Martinsried  Phone: 089-8578-2208 Fax: 089-8578-2211 email: tatzelt@biochem.mpg.de	Molecular biology of TSE, aggregation mechanisms, development of therapeutic measures against prion-aggregation
<b>Prof. Dr. Bodemer</b> Bundesforschungsanstalt für Viruskrankheiten der Tiere Paul-Ehrlich-Str. 28 72076 Tübingen  Phone: 07071-967-300 Fax: 07071-967-303 email: walter.bodemer@tue.bfav.de	Transgenic mouse models, development of monoclonal antibodies against PrPsc
<b>Prof. Dr. C. Frömmel</b> Institut für Biochemie Humboldt-Universität, Charité Monbijou-Str. 10117 Berlin  Phone: (0)30 2802-6440 Fax: (0)30 2802-6615 Email: cornelius.froemmel@charite.de	Therapeutics, protein chemistry
<b>Prof. Dr. K.-O. Habermehl</b> Otto Kuhn-Stiftung Potsdamer Chaussee 14129 Berlin  Phone: 030-8036463 email: habermehl.gbd@snaflu.de	Diagnostics
<b>Prof. Dr. W. E. G. Müller</b> Institut für Physiologische Chemie Universität Mainz Düsbergweg 6 55099 Mainz  Phone: 06131-39-25910 Fax: 06131-39-25243 email: wmueller@mail.uni-mainz.de	Diagnostics

<p><b>Prof. Dr. M. Eigen</b>  Max-Planck-Institut für biophysikalische Chemie  Am Fassberg 11  37077 Göttingen</p> <p>Phone: 0551-201-1432  Fax: 0551-201-1435  email: manfred.eigen@gwdg.de</p>	Diagnostics using FCS-methodology
<p><b>PD Dr. Alexander Bürkle</b>  University of Newcastle  Dept. of Gerontology, IHE, Wolfson Reserach Centre, Newcastle General Hospital  Westgate Road  GB Newcastle upon Tyne, NE4 6BE</p> <p>Phone: +44-191-256-3324  Fax: +44-191-219-5074  email: alexander.buerkle@ncl.ac.uk</p>	Development of new diagnostics and therapies against TSEs,
<p><b>Prof. Dr. T. Schrader</b>  Dept.of Chemistry  Philipps-Universität-Marburg  Hans-Meerwein-Str.  35043 Marburg / Germany</p> <p>Phone: 06421 28-25544  Fax: 06421 28-28917  email:schradt@mail.uni-marburg.de</p>	Development of therapies, molecular tools for blocking prion protein aggregation
<p><b>Prof. Dr. B. Dobberstein,</b>  ZMBH  Im Neuenheimer Feld 282  69120 Heidelberg</p> <p>Phone: 06221-54 6820  Fax: 06221-54 6809  email: dobberstein@zmbh.uni-heidelberg.de</p>	cell biology, basic research on protein-protein-interactions
<p><b>Dr. J. Lampe</b>  Neurologische Universitätsklinik  TU Dresden  Fetscherstr. 74  01307 Dresden</p> <p>Phone: 0351-458-2524  Fax: 0351-458-4365  email: <a href="mailto:jlampe@aol.com">jlampe@aol.com</a></p>	Inclusion body myositis, genetic epidemiology

#### **4. Collaboration with other countries and openness of your programme to collaboration**

German scientists are already involved in a number of TSE-research activities funded within the European Research Programmes (4<sup>th</sup> and 5<sup>th</sup> FP; projects included under 2.). Participation of German research groups in the relevant calls of the ongoing “Quality of Life and Management of Living Resources” programme are encouraged, since this is seen as the most efficient manner in which competent scientists from different European countries can jointly pursue their research goals.

Co-operative bi- and multilateral research projects can be envisaged as complementing the European Research Programme and can provide additional impetus for networking of the most competent groups. The national funding initiatives for research into therapies and diagnostics for TSE are open to co-operation with scientists from other European countries on a matching funds basis.

# HELLENIC REPUBLIC

## **1. Description of main TSE research activities underway including Public Health aspects**

During the last ten years research in the field of TSEs is developed within the following areas:

- Characterisation of TSE infectious agent
- Improvement of TSE diagnosis and study of scrapie infectious agent.
- Comparative studies of carbohydrate composition in TSEs.
- Development of therapeutic reagents against TSE

All human cases are referred to the two National Centers for TSEs one for clinical (Prof M. Dalakas) and one for neuropathological evaluation (Prof P. Davaris, and E. Patsouris). Results are reported bi annually to the EU meetings organized by Prof. H. Budka and Prof. R. Will.

A National Scientific Committee has been established by the Hellenic Ministry of Health that is responsible for monitoring CJD.

All animal TSE cases are reported to the Department of Pathology and the Department of Infectious diseases, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki. Results are reported annually to the EU meetings organized by Dr F. Lantier.

A National Scientific Committee has been established by the Hellenic Ministry of Agriculture that is responsible for monitoring animal TSEs.

Finally a National Scientific Committee for TSE risk assessment has been established by the Hellenic Food Authority- EFET Ministry of Development with a particular interest in food hygiene.

## **2. How these activities fit into the principal areas of the European Action Plan on TSE Research**

### **a) Clinical, epidemiological and social research on human and animal SE**

2. BIOMED-I CA, The Human Prion Diseases: From Neuropathology to Pathobiology and Molecular Genetics. PL 921484 (H. Budka coordinator, T. Sklaviadis partner 1994-1997).

7. FAIR CA. A network for the supply of BSE tissues and fluids for European collaborative research. (S. Done coordinator, T. Sklaviadis partner 1998-2001).

13. Programme for surveillance of transmissible spongiform encephalopathies (TSEs). Proposed by Hellenic Republic Ministry of Agriculture for the year 2001 (S. Leontidis, O. Papadopoulos)

**b) The infectious agent and its mechanisms of transmission**

1. FAIR Programme, Directorate General VI, Commission of the European Communities Characterization of the Bovine Spongiform Encephalopathy( BSE) infectious agent. PL 920859. (T. Sklaviadis coordinator) 1994-1998)

3. Greek Ministry of Health. Expression of proteins that are related with Alzheimer Creutzfeldt-Jakob disease. A2γ/1440/8-4-92 (T. Sklaviadis coordinator 1992-1993)

4. BIOMED II Molecular biology of prion disease. The study of prion propagation, pathogenesis and intermammalian species barriers in transgenic and other models of prion disease. PL 961185 (J. Collinge coordinator, T. Sklaviadis partner 1996-2000)

6. JOINT CALL Prion diseases: mechanism of transmission and identification of targets for potential therapeutics. PL 976040. (J. Collinge coordinator, T. Sklaviadis partner 1997-2001)

**c) Diagnosis of SEs**

Greek laboratories participate in several projects that are related with TSE diagnosis with a particular interest in optimization and standarization of Western blotting and immuno detection protocols.

5. BIOMED II CJD and BSE: an integrated molecular and experimental neuropathological analysis of prion neurodegeneration, strain variation and transmission risks. PL962679 (J. Collinge coordinator, T. Sklaviadis partner 1997-2001)

8. FAIR SC Surveillance and Diagnosis of Ruminant TSE PL 98-7021 (M. Rogers coordinator, T. Sklaviadis partner, 1998-2001).

9. FAIR CA Concerted action for the setting up of multicentric epidemiological databases and biological sample banks for small ruminant scrapie. PL976056 (F. Lantier coordinator, O. Papadopoulos, T. Sklaviadis, P. Economidis partners1997-2000)

10. FAIR SC Breeding programmes improve prospects for scrapie control in sheep and goats PL 973305 (J. Michel Elsen coordinator, O. Papadopoulos, T. Sklaviadis, P. Economidis partners 1997-2001)

**d) Risk assessment of SEs**

Greek and Cypriot researchers are involved in two EU projects where risk assessment is one of the main tasks. Genotypic screening of healthy and infected sheep and goats is in progress.

9. FAIR CA Concerted action for the setting up of multicentric epidemiological databases and biological sample banks for small ruminant scrapie. PL976056 (F. Lantier coordinator, O. Papadopoulos, T. Sklaviadis, P. Economidis partners 1997-2000)

10. FAIR SC Breeding programmes improve prospects for scrapie control in sheep and goats PL 973305 (J. Michel Elsen coordinator, O. Papadopoulos, T. Sklaviadis, P. Economidis partners 1997-2001)

13. Programme for surveillance of transmissible spongiform encephalopathies (TSEs). Proposed by Hellenic Republic Ministry of Agriculture for the year 2001 (S. Leontidis, O. Papadopoulos)

**e) Treatment and prevention of SEs**

Current activities include isolation and characterization of peptides with potential therapeutic and diagnostic value against prion diseases.

6. JOINT CALL Prion diseases: mechanism of transmission and identification of targets for potential therapeutics. PL 976040. (J. Collinge coordinator, T. Sklaviadis partner 1997-2001)

11. Peptide identification with potential diagnostic and therapeutic use of neurodegenerative disorders Greek ministry of Development 97EKBAN2-1.2-35 (T. Sklaviadis coordinator 1998-2001)

12. Programme for eradication of transmissible encephalopathies (scrapie). Proposed by Hellenic Republic Ministry of Agriculture for the year 2001

**Sponsoring bodies for these activities are:**

The European Union: Programmes 1, 2, 4, 5, 6, 7, 8, 9, 10, 12 (50%), 13(50%)

General Secretariat of Research and Development: Programme 11.

For the coming years, research projects in the above mentioned fields, could be funded within the frame of the Operational Programme launched by the General Secretariat for R&T. Hellenic Republic, Ministry of Development.

Hellenic Republic, Ministry of Health: Programme 3. It will also announce a call for proposals with a budget of 0.6 M €.

Ministry of Agriculture: It has announced two programmes for eradication of TSEs (scrapie) 0.5 million ECUs and surveillance 0.8 million €.

### **3. Principal research teams and their areas of expertise: Names, addresses, full details**

The laboratory of Pharmacology at Aristotle University of Thessaloniki is involved in basic and applied research projects, concerning the molecular nature of the pathogens that cause Creutzfeldt-Jakob disease, scrapie and bovine spongiform encephalopathy. It currently employs three research scientists, one technician, two graduate students and several undergraduate students.

Prof Theodoros Sklaviadis, Lab of pharmacology, Dpt of Pharmaceutical Sciences, Aristotle Univ. of Thessaloniki, Thessaloniki, 54006 tel 30 31 997615, fax 30 31 997645

#### **Clinical contacts for human SEs**

Prof. M Dalakas(Neurology) Eginitio Hospital, E. Venizelou 72, 11527 Athens tel 30 1 7211682

Prof. P. Davaris (Pathology) Lab of Pathology-Anatomy, School of Medicine, Univ. of Athens Mikras Asias 75, 11527 Goudi, 30 1 7781487

Prof. E. Patsouris Lab of Pathology-Anatomy, School of Medicine, Univ. of Athens, Mikras Asias 75, 11527 Goudi, 30 1 7771206

Prof. A. Plaitakis (Neurology) Dept. of Neurology, School of Medicine, Univ. of Kriti, Iraklio Kriti tel 30 81 394648

#### **Clinical contacts for animal SEs**

Dept of infectious diseases, Lab. of Microbiology is involved in genotypic analysis of healthy and affected animal populations.

Prof O. Papadopoulos Dept of infectious diseases, Veterinary School, Aristotle Univ. of Thessaloniki, Thessaloniki, 54006 tel 30 31 999951, fax 999953

Prof. S Leontidis Dpt of Pathology, Faculty of Veterinary Medicine, Aristotle Univ. of Thessaloniki, Giannitson & Boutyra St., 54627 Thessaloniki tel 30 31 994531, fax 30 31 994403

### **4. Collaboration with other countries and openness of the programmes to collaboration**

Within the specific aims of the above programmes, Greek scientists are in contact with most of the participating laboratories in Europe. That includes exchange of personnel and reagents as well as training of young scientists.

# ICELAND

## Description of the main TSE Research activities underway in Iceland in 2001

### Animal TSEs

Sheep:

1. *Scrapie susceptibility*: We have done a breed survey of the prion gene (PrP) genotypes of Icelandic sheep. Healthy (n= 600) and scrapie affected sheep (n=100) have been analysed for polymorphism at codons 136, 154 and 171. We used density gradient gel electrophoresis and DNA sequencing to characterize novel polymorphisms at other codons. High- and low-risk genotypes were determined and this information is the basis of an annual genotyping service to sheep breeders and insemination centers. (Funded by 1)
2. *Pre-clinical scrapie*: Five whole herds have been PrP genotyped. The testing of PrP<sup>sc</sup> using IHC (immunohistochemistry) and WB (Western blots) has been finished for one flock and is in progress for the others. So far, sheep with low-risk genotypes have not had any signs of pre-clinical scrapie infection. . (Funded by 1).
3. *Scrapie free regions*: 500 sheep, from three quarantined regions where scrapie has never been found, were genotyped for the Sheep Breeders' Associations. Only a small difference in the frequency of genotypes was found in this regions compared to "scrapie affected regions". Sheep from these regions are used for restocking farms after scrapie culling. . (Funded by Icelandic science funds).
4. *Improving TSE diagnosis*. Different pre-treatments and antibodies for IHC are being tested in collaboration with other European groups in addition to standardizing of WB methods. Special emphasis placed on detection of pre-clinical cases. (Funded by 4).
5. *Scrapie transmission*: The possible role of vectors such as hay mites in scrapie transmission is being studied. (Funded by 6).

*PrP genes in Icelandic goats*:

The small (n=400) goat population in Iceland dates back to 9<sup>th</sup> century and has never been found to have scrapie. We investigated the PrP genes of 30 goats at codons 42, 136, 142, 143, 154 and 171. Silent polymorphism was found at codon 42. (Funded by 1).

*PrP genes in Icelandic cattle*:

The single bovine breed in Iceland dates back to the 9<sup>th</sup> century and has never had BSE. We are in the process of sequencing the prion gene of several animals of this old breed to investigate whether it differs from the uniform genotype found in the rest of Europe. (Funded by Icelandic science funds).

### Human TSEs

*CJD surveillance in Iceland*:

1. Search for vCJD. Patient files from the Neurology Clinic at the National Hospital, dating 20 years back, have been studied with regards to dementia in patients under 50 years of age. No patients with symptoms corresponding to vCJD were found. (Funded by 3).
2. Sporadic CJD. One sporadic case of CJD has been found in Iceland since this project started. I all 4 cases in the last 40 years were diagnosed, giving an annual case incidence of 0.4/million. (Funded by 5).

## **The infectious agent**

### *Investigation into the role of the prion protein:*

We are currently using the yeast-two hybrid system to analyze proteins able to bind to the prion protein expressed in yeast. Three genes coding for proteins binding to the normal prion protein *in vivo* and *in vitro* are under investigation. One is of unknown function and the other two are involved in the control of apoptosis. (Funded by Icelandic science funds)

## **European collaborations:**

- 1) FAIR CT 97-3305. Improving prospects for scrapie control in sheep and goats by study of host genotypes, TSE isolates and their in-vivo and in-vitro interactions (shared cost). Coordinator J-M Elsen.
- 2) FAIR 98-6056. Concerted Action for setting up multicentric epidemiological databases and biological sample banks for small ruminant scrapie. Coordinator F. Lantier.
- 3) Biomed 2. PL 963698. Concerted action. Co-ordination of national surveillance programs for CJD in the European Union. Coordinator: R. Will.
- 4) FAIR 98-7021. The establishment of a European network for the surveillance of ruminant TSE and the standardization and harmonization of the process and criteria for identification of suspect cases. Coordinator: E. Weavers.
- 5) QLK2-CT-2000-00837. Human transmissible spongiform encephalopathies The neuropathology network (PRIONET)". Coordinator: H. Budka.
- 6) FAIR-CT98-7023. Sigurdur Sigurdarson: "Role of environmental and host factors on the horizontal and vertical transmission of scrapie in naturally infected sheep flock."

## TEAMS

<p>Astridur Palsdottir Molecular Biology Laboratory Institute for Experimental Pathology Keldur University of Iceland v/Vesturlandsveg IS-112 Reykjavik Iceland Tel : 354/567.47.00. Fax : 354/567.39.79. <a href="mailto:Astripal@rhi.hi.is">Astripal@rhi.hi.is</a></p>	<p>G. Georgsson / S. Sigurdsson Veterinary Laboratory Ministry of Agriculture Institute for Experimental Pathology University of Iceland v/Vesturlandsveg IS-112 Reykjavik Iceland Tel : 354/567.47.00 Fax : 354/567.39.79. <a href="mailto:Ggeorgs@rhi.hi.is">Ggeorgs@rhi.hi.is</a></p>
<p>I. Jonsdottir Dept of Immunology Landspítali – University Hospital Hringbraut 101 Reykjavik Iceland Tel : 354/560.19.62. Fax : 354/560.19.43. <a href="mailto:Ingileif@landspitali.is">Ingileif@landspitali.is</a></p>	

## REPUBLIC OF IRELAND

### 2. How these activities fit into the principal areas of the European Action Plan on TSE Research

#### (a) Clinical, Epidemiological and Social Research on Human and Animal SE (12 projects)

##### **A study of the epidemiology of scrapie in Irish sheep flocks (1998- ongoing).**

The overall objective of this project was to elucidate the nature and extent of scrapie in Irish Sheep flocks with a view to its eradication. This was achieved through the following studies:

A detailed postal questionnaire survey was mailed to 6,000 sheep farmers in an effort to establish the national prevalence of the disease, farmer knowledge of scrapie and characteristics of affected and unaffected farms.

Epidemiological data was collected from 68 flocks which had a confirmed case of scrapie. Similar data was collected from 90 control farms (non-scrapie). This allowed the identification of risk factors for the introduction and maintenance of scrapie in a flock.

A longitudinal study of 12 flocks with active scrapie provided data on within-flock incidence, together with age, breed, sex and genotype profiles of confirmed cases of scrapie. The information will be used in the development of a model of disease dynamics.

Irish Research Teams Involved:	Teams 1,2,3,
Number of Other Countries Collaborating:	1
Source of Funding:	A
<b>Contact:</b>	<b>Dr. Anne Healy, Team 3</b>

##### **A study of the clinical neurology of naturally-occurring scrapie in Irish sheep flocks (1998- ongoing).**

Detailed neurological examination of 129 cases of scrapie (with repeated examination of 47 of these) provided information allowing categorisation of the principal clinical manifestations of the disease in Irish sheep and the progression of clinical signs. An observational study, comparing scrapie-affected and clinically normal sheep, looked at many behaviours including feeding, rumination, drinking, social interaction and movement.

Irish Research Teams Involved:	Teams 1,3
Number of Other Countries Collaborating:	1
Source of Funding:	A
<b>Contact:</b>	<b>Dr. Anne Healy, Team 3</b>

**The establishment of a European network for the surveillance of ruminant TSE and the standardisation and harmonisation of the process and criteria for the identification of suspect cases (1998-2002)**

The object of this project is to establish a network of laboratories in Europe capable of evaluating, adapting and implementing surveillance schemes which will identify ruminant TSE. Such schemes will employ standardised methods of diagnosis of known sensitivity which have been validated for use in the diagnosis of early clinical and pre-clinical cases of bovine, ovine and caprine TSE.

In order to achieve this the project will fulfil the following objectives:

- Establish a surveillance system which will suit the identification of a low incidence disease which, at present, cannot be diagnosed in the live animal. This system will take account of all pertinent risk factors and quality control procedures and will be sufficiently flexible to suit the particular requirements of any individual country.
- Examine those methods currently used for the immunodiagnosis of TSE in order to standardise the protocols and to establish a uniform interpretation of results.
- Establish the sensitivity of the standardised protocols and develop a standard model for the evaluation of novel TSE diagnostic protocols which might, in the future, be presented for validation.
- Carry out a detailed clinical investigation into bovine, ovine and caprine neurological diseases in order to identify those populations which are the most suitable candidates for detailed surveillance.

Irish Research Teams Involved:	Teams 1,4
Number of Other Countries Collaborating:	14
Source of Funding:	D
<b>Contact:</b>	<b>Dr. Eddie Weavers, Team 1</b>

### **Clinical presentation of bovine spongiform encephalopathy in the Republic of Ireland (Complete)**

On-farm clinical examinations of 40 cases of BSE in the Republic of Ireland were performed. Although there was considerable individual variation in clinical presentation, all cows had a combination of neurological signs in each of two categories (changes in behaviour and deficits in posture and movement), together with non-specific signs (weight loss, decreased milk yield, decreased appetite). Thorough clinical examination and detailed history taking are important in reaching a tentative diagnosis of BSE. The study confirms that BSE is a significant differential diagnosis for recumbency in adult cattle. A uniform and systematic approach to the clinical diagnosis of BSE is required to enable accurate comparisons of cases between countries and over time.

Irish Research Teams Involved:	Team 1
Number of Other Countries Collaborating:	–
Source of Funding:	G
<b>Contact:</b>	<b>Dr. Eddie Weavers, Team 1</b>

### **Human Transmissible Spongiform Encephalopathy: The Neuropathology Network (Prionet) (October 2000 – ongoing)**

This project includes:

- (a) the continuation and expansion of the European Neuropathological Data base for Prion Diseases (ENDAPRID) which requires the registration of cases and the submission of blocks.
- (b) retrospective and prospective examination of atypical dementias and neurodegenerative disorders, especially in the young
- (c) the collection and registration in the data base of tissues and body fluids from clinical and autopsy cases.

Irish Research Teams Involved:	Team 8
Number of Other Countries Collaborating:	
Source of Funding:	M
<b>Contact:</b>	<b>Dr. Michael Farrell, Team 8</b>

**National survey of PrP genotypes at codons 136, 154 and 171 in the main sheep breeds in Ireland (January 1998-December 1999)**

The objective of this study was to investigate genetic variation at codons 136, 154 and 171 in native (Belclare, Galway, Wicklow Cheviot, Donegal Blackface Mountain and Mayo Blackface Mountain) and imported (Texel, Bleu du Maine, Rouge de l'Ouest, Vendéen, Suffolk and Charollais) sheep breeds in Ireland. A total of 13 genotypes were found. The percentage of the most resistant genotype, AA<sub>136</sub>RR<sub>154</sub>RR<sub>171</sub>, varied from 1.8% in the Vendéen breed, 3.1% in Donegal Blackface Mountain, 10.0% in Texel, 11.1% in Wicklow Cheviot, 12.9% in Belclare, 22.0% in Charollais, 25.6% in Mayo Blackface Mountain, 33.3% in Galway, 46.4% in Bleu du Maine to 62.5% in Rouge de l'Ouest. (This data is published in O'Doherty *et al.*, 2000 Veterinary Record 146: 335-338; O'Doherty *et al.*, 2001 Research in Veterinary Science 00: 1-6)

Irish Research Teams Involved:	Team 2
Number of Other Countries Collaborating:	8
Source of Funding:	B
<b>Contact:</b>	<b>Dr. Torres Sweeney, Team 2</b>

**Study of the PrP genotype of scrapie affected sheep and their flock cohorts (January 1998 - to date)**

The objective of this study was, firstly, to genotype animals that were showing clinical signs of scrapie, and secondly, to compare the genotypes of these scrapie-infected animals with the healthy animals in the respective flocks. 154 sheep histopathologically confirmed with scrapie were genotyped (Table 1).

Table 1: Frequency of PrP genotypes in Irish scrapie-infected sheep

<b>Genotype</b>	<b>No. cases (n = 154)</b>	<b>%</b>
AA <sub>136</sub> RR <sub>154</sub> HH <sub>171</sub>	1	0.65
VA <sub>136</sub> RR <sub>154</sub> QR <sub>171</sub>	1	0.65
VV <sub>136</sub> RR <sub>154</sub> QQ <sub>171</sub>	2	1.30
VA <sub>136</sub> RR <sub>154</sub> QH <sub>171</sub>	4	2.60
AA <sub>136</sub> RR <sub>154</sub> QH <sub>171</sub>	25	16.23
VA <sub>136</sub> RR <sub>154</sub> QQ <sub>171</sub>	56	36.36
AA <sub>136</sub> RR <sub>154</sub> QQ <sub>171</sub>	65	42.21

Of these, 111 were animals from 11 flocks whose flock-mates were also genotyped. A total of 2,555 healthy flockmates were genotyped. This data is currently being statistically analysed.

Irish Research Teams Involved:	Teams 1, 2, 3
Number of Other Countries Collaborating:	8
Source of Funding:	B
Contact:	Dr. Torres Sweeney, Team 2

**“Breeding for resistance” programme (February 2000 – ongoing)**

The objective of this project is to determine if it is possible to eliminate PrP<sup>Sc</sup> from scrapie infected flocks using a “two year breeding for resistance” programme. Six entire flocks with a history of scrapie were genotyped for variation at codons 136, 154 and 171. A breeding programme was established in September 2000, where only rams of the genotype AA<sub>136</sub> RR<sub>154</sub> RR<sub>171</sub> were used as breeding sires. Only ewes and ewe lambs of the genotype AA<sub>136</sub> RR<sub>154</sub> RR<sub>171</sub> will be maintained on the farm as breeding stock. We predict that following the second year breeding season the original flock will be completely replaced with AA<sub>136</sub> RR<sub>154</sub> RR<sub>171</sub> animals. Lymph node and spinal cord samples will be analysed for PrP<sup>Sc</sup> from all cull animals.

Irish Research Teams Involved:	Teams 1,2
Number of Other Countries Collaborating:	–
Source of Funding:	G
Contact:	Dr. Torres Sweeney, Team 2

**Determination of genes other than the prion protein gene that could be influencing susceptibility to scrapie (January 1999–December 2002)**

The objective of this study is to identify if genes, other than the prion protein gene, are influencing susceptibility to scrapie. A flock of 70 VA<sub>136</sub> RR<sub>154</sub> QQ<sub>171</sub> half-siblings has been generated using one VV<sub>136</sub> RR<sub>154</sub> QQ<sub>171</sub> ram and 60 AA<sub>136</sub> RR<sub>154</sub> QQ<sub>171</sub> ewes. These animals have been maintained in the presence of scrapie infected sheep. These animals will be maintained until they show the clinical signs of scrapie. A genome scan will subsequently be performed on the DNA from these animals if differences in incubation time period are observed.

Irish Research Teams Involved:	Teams 1, 2
Number of Other Countries Collaborating:	4
Source of Funding:	F
Contact:	Dr. Torres Sweeney, Team 2

### **Is the black face mountain breed of sheep resistant to scrapie?**

**(January 2001 – December 2003)**

The objective of this project is to orally infect black face mountain sheep of susceptible PrP genotypes (VA<sub>136</sub> RR<sub>154</sub> QQ<sub>171</sub>) with scrapie to identify if these animals are resistant to scrapie regardless of their PrP genotype. Fourteen blackface mountain lambs will be selected based on a susceptible genotype (VA<sub>136</sub> RR<sub>154</sub> QQ<sub>171</sub>; n=7) and a resistant genotype (AA<sub>136</sub> RR<sub>154</sub> RR<sub>171</sub>; n=7). Animals will be orally challenged with brain from scrapie infected sheep. The resistant genotype group will be maintained for one year after all of the susceptible genotype animals have displayed the clinical signs of scrapie or until animals from both groups die of natural causes.

Irish Research Teams Involved:	Teams 1, 2
Number of Other Countries Collaborating:	–
Source of Funding:	E
Contact:	<b>Dr. Torres Sweeney, Team 2</b>

### **Spatial and Temporal Distribution of Bovine Spongiform Encephalopathy (BSE) in the Republic of Ireland (1998-ongoing)**

- (a) Thematic presentation of the distribution of cases and birthplaces of cases and their relationship to density of cattle by age.
- (b) Statistical analysis of data directed towards cluster analysis and case-control studies.

Irish Research Teams Involved:	Team 5
Number of Other Countries Collaborating:	This project is open to collaboration
Source of Funding:	G
Contact:	<b>Prof. John D. Collins, Team 5</b>

### **A spatial analysis of the distribution of Bovine Spongiform Encephalopathy (BSE) in Ireland.**

Analysis of the geographic distribution of disease can be useful in generating hypotheses regarding underlying risk factors. In the case of BSE in Ireland, a standardised questionnaire, which includes National Grid Co-ordinates, is completed for all BSE suspect animals notified to the Department of Agriculture, Food and Rural Development. This allows both cases and controls to be mapped using Geographic Information Systems technology. A visual assessment of the distribution of BSE cases in Ireland seems to indicate clustering of cases in certain areas. The objective of this study is to carry out a statistical analysis of the distribution of BSE cases in Ireland.

Irish Research Teams Involved:	Teams 11, 5
Number of Other Countries Collaborating:	This project is open to collaboration
Source of Funding:	G
Contact:	<b>Dr. Hazel Sheridan, Team 11</b>

## **(b) The Infectious Agent and its Mechanisms of Transmission**

### **(3 projects)**

#### **PrP glycosylation profile and proteinase K resistance of Irish scrapie cases**

**(September 1999 – May 2000)**

Variation in the biochemical structure of PrP<sup>Sc</sup> is associated with specific strains of Transmissible Spongiform Encephalopathies. This variation can be detected by the analysis of the banding patterns of PrP<sup>Sc</sup> following proteinase K digestion, SDS-PAGE and immunoblotting. The first objective was to determine if an individual animal could harbour more than one strain of scrapie by identifying the PrP glycotyping patterns from tissues from different regions of the Central nervous System. The second objective was to identify the range of PrP glycotyping patterns in the Irish sheep population. The final objective was to determine if the glycotyping pattern identified in sheep in Ireland is similar to the pattern of BSE infected sheep. Sixteen sheep were selected based on divergence in genotype, brain lesion profile, age of onset of clinical signs and clinical behaviour. PrP glycosylation profile was determined in tissues collected from the thoracic cord, thalamus, Basal ganglia, mediobasal hypothalamus, medulla and cortex. A single glycosylation profile was identified in all CNS regions from animals examined: mean  $\pm$  SD was di-glycosylated 48.7%  $\pm$  2.84%; mono-glycosylated 31.1%  $\pm$  1.64%; non-glycosylated 20.1%  $\pm$  2.66%. This profile is dissimilar from the reported profile of BSE inoculated sheep. This study suggests that only a single strain of scrapie exists in Ireland which is not BSE. (This data is published in Sweeney *et al.*, 2000 Journal of General Virology 81: 1621-1627).

Irish Research Teams Involved:	Teams 1, 2, 3
Number of Other Countries Collaborating:	8
Source of Funding:	B
Contact:	<b>Dr. Torres Sweeney, Team 2</b>

#### **Strain typing using the mouse bioassay system (September 1999 – ongoing)**

The objective of this project is to determine the strains of scrapie present in Irish sheep using the mouse bioassay system. Currently, four samples are inoculated into mice in Weybridge and one

sample in is inoculated in mice in the FRCVA, Germany. A further 16 samples are due to be infected into mice into the Institute of Psychiatry, London.

Irish Research Teams Involved:	Teams 1, 2, 3
Number of Other Countries Collaborating:	8
Source of Funding:	B
Contact:	<b>Dr. Torres Sweeney, Team 2</b>

**Defining the interaction between PrP<sup>C</sup> and caveolin-1 $\alpha$  and its role in the conversion of PrP<sup>C</sup> to PrP<sup>Sc</sup> in chronically scrapie infected mouse neuroblastoma cells.**

Scrapie is a fatal neurodegenerative disease affecting sheep and goats and experimentally infected small mammals. The disease is caused by the prion, a novel infectious agent composed of an aberrantly folded protein PrP<sup>Sc</sup>. PrP<sup>Sc</sup> is an isoform of a normal cell surface GPI anchored glycoprotein PrP<sup>C</sup>, whose normal cellular function remains unclear but may involve copper binding. The conversion of PrP<sup>C</sup> to PrP<sup>Sc</sup> occurs in an unidentified cellular compartment after PrP<sup>C</sup> has been glycosylated, the GPI anchor attached and the protein transported to the cell surface. Furthermore, there is genetic evidence for the involvement of a second factor in the conversion process, which has been, termed ‘protein X’. There is conflicting evidence with regard to the localisation of PrP<sup>C</sup> on the cell surface. We and others have shown that PrP<sup>C</sup> is associated with cell surface structures called caveolae or with cholesterol rich rafts though others have suggested that PrP<sup>C</sup> associates with clathrin coated pits. Recently we have demonstrated that the specific immunoprecipitation of PrP<sup>C</sup>, co-precipitates caveolin-1  $\alpha$  (the structural protein of caveolae). This raises the possibility that caveolin-1  $\alpha$  may be protein X. In this proposal we wish to investigate this possibility by comparing the binding of caveolin-1  $\alpha$  with wildtype PrP<sup>C</sup> and a series of mutated PrP molecules disrupted in their putative protein X binding site. If our hypothesis is proved correct we will have greatly furthered our understanding of the cellular events involved in the conversion of PrP<sup>C</sup> to PrP<sup>Sc</sup>. Secondly, we wish to investigate the effect of copper on the caveolin-1  $\alpha$ / PrP<sup>C</sup> complex given the observed effects of copper on the rate of PrP<sup>C</sup> endocytosis.

Irish Research Teams Involved:	Team 4
Number of Other Countries Collaborating:	–
Source of Funding:	I
Contact:	<b>Dr. Mark Rogers, Team 4</b>

### **(c) Diagnosis of Spongiform Encephalopathies (14 projects)**

#### **Establishment and management of a biological sample bank of tissues from scrapie affected sheep (1998- ongoing)**

In order to facilitate a number of TSE research projects a biological sample bank of frozen and formalin-fixed tissues, including central nervous tissue and various lymphoid tissues, from scrapie-affected sheep was established at the Central Veterinary Research Laboratory, Abbotstown. Details such as genotype, farm of origin, clinical neurology and age at time of euthanasia are included in a comprehensive data base. Consideration will be given to any research team interested in procuring tissue from this tissue bank.

Irish Research Teams Involved:	Teams 1, 2, 3
Number of Other Countries Collaborating:	15
Source of Funding:	C
Contact:	<b>Dr. E. Weavers, Team 1.</b>

#### Development of a novel antigen retrieval protocol for PrP immunohistochemistry (Complete)

A novel PrP antigen retrieval protocol has been developed as an alternative to the standard formic acid/autoclaving method. It involves permanganate oxidation of the section followed by a denaturing step. Simple to perform, it takes less than 30 minutes and all incubations are at room temperature. Excellent PrP<sup>Sc</sup> immunostaining is achieved with this method using PrP MAb F89/160.1.5 (O'Rourke *et al.*: Monoclonal antibody F89/160.1.5 defines a conserved epitope on the ruminant prion protein. Journal of Clinical Microbiology. 1998; 36(6):1750-1755)

*Current status:* Manuscript in progress.

Irish Research Teams Involved:	Team 1
Number of Other Countries Collaborating:	–
Source of Funding:	G
Contact:	<b>Dr. E. Weavers, Team 1.</b>

### **Determine the brain lesion profile of scrapie cases using histopathology (1998- ongoing)**

In rodent models, determining the pattern of vacuolar distribution and severity in specific neuroanatomical locations (the lesion profile) is an integral part of the TSE agent strain typing process. The aim of this project was to adapt this lesion profiling technique for use in sheep scrapie and to determine if the lesion profile is associated with specific strains of the infectious agent. A draft protocol for scoring specific areas representative of each brain region has been developed based on 44 naturally-occurring scrapie cases submitted to the Veterinary Laboratories Agency, Weybridge. This is currently being applied by the research partners involved in the project.

Irish Research Teams Involved:	Team 1
Number of Other Countries Collaborating:	5
Source of Funding:	B,C
Contact:	<b>Dr. E. Weavers, Team 1.</b>

### **Standardisation of immunohistochemistry (IHC) protocols to be used in the diagnosis ruminant TSEs. (1998-2002)**

This is a thirteen country collaborative project having the following objectives:

- To establish a standard reference IHC protocol for the diagnosis of BSE and scrapie.
- To establish approved PrP IHC methodologies in EU laboratories which are involved in TSE diagnosis.
- To set up a PrP IHC quality assurance scheme and to contribute to the construction of a model which will be used as a basis for the validation of novel TSE diagnostic methods.

Irish Research Teams Involved:	Team 1
Number of Other Countries Collaborating:	12
Source of Funding:	D
Contact:	<b>Dr. E. Weavers, Team 1.</b>

### **Anatomical distribution of PrP<sup>Sc</sup> in peripheral lymph nodes from scrapie-infected animals. (Jan 1998 – Sept 2000)**

The objective of this study was to examine the distribution of PrP<sup>Sc</sup> in the peripheral lymphoid tissue of sheep clinically affected with scrapie. Submandibular, pre-femoral and pre-scapular lymph nodes, as well as tonsil, were collected at necropsy from 45 sheep of the following PrP genotypes: AA<sub>136</sub> RR<sub>154</sub> QQ<sub>171</sub>, VA<sub>136</sub> RR<sub>154</sub> QQ<sub>171</sub> and AA<sub>136</sub> RR<sub>154</sub> QH<sub>171</sub>. Tissues were formalin fixed and processed to paraffin. Serial sections were taken from these tissues at 100µm intervals. These sections were immunohistochemically stained using Va82/98, a rabbit

polyclonal antiserum to a synthetic peptide representing residues 32-57 of the sheep PrP sequence. The results indicated that PrP<sup>Sc</sup> was uniformly distributed in lymphoid follicles throughout these tissues. PrP<sup>Sc</sup> was found in these four lymphoid tissues from all of the scrapie affected sheep studied. In conclusion, this study has demonstrated that PrP<sup>Sc</sup> is evenly distributed within the pre-femoral, pre-scapular, sub-mandibular and tonsillar lymphoid tissues.

Irish Research Teams Involved:	Teams 1,2
Number of Other Countries Collaborating:	–
Source of Funding:	A
Contact:	<b>Dr. Torres Sweeney, Team 2</b>

### **Identification of a suitable biopsy technique to collect lymphoid tissue for the preclinical diagnosis of scrapie (June 1999-Sept 2000)**

The objectives of the studies described were to identify a suitable biopsy site and technique to collect lymphoid follicles and to characterise any clinical side effects associated with these techniques. Two experiments were performed. In experiment 1, tissue samples were collected post mortem from 126 sheep on 5 lymphoreticular sites using various techniques post mortem. The three most successful combinations of sites and techniques were: third eyelid sample using a forceps and scissors ( $5.32 \pm 0.70$  lymphoid follicles per  $5 \mu\text{m}$  tissue section), mandibular lymph node sample using a Biopty™ gun ( $1.19 \pm 0.26$  lymphoid follicles per  $5 \mu\text{m}$  tissue section) and tonsil sample using a biopsy forceps ( $1.14 \pm 0.27$  lymphoid follicles per  $5 \mu\text{m}$  tissue section). In experiment 2, these three techniques were performed repeatedly, once every month, for 5 months, on live animals (n=5) under general anaesthesia. Several aspects of clinical effects in these animals were compared with controls (n=5) restrained and anaesthetised in the same way except biopsies were not taken. In the live animal, most lymphoid follicles ( $3.47 \pm 0.58$  per  $5 \mu\text{m}$  tissue section) were collected using the third eyelid biopsy technique. No measurable clinical side effects were associated with biopsying. Disruption of homeostasis as indicated by increased plasma cortisol was observed in all animals, and was related to general restraint rather than biopsying peripheral lymphoid tissue. We conclude that there were no clinical side effects associated with sequential biopsying of the mandibular lymph node, third eyelid and tonsil.

Irish Research Teams Involved:	Teams 1,2
Number of Other Countries Collaborating:	1
Source of Funding:	A
Contact:	<b>Dr. Torres Sweeney, Team 2</b>

**To determine the spatio-temporal distribution of PrP<sup>sc</sup> through the peripheral lymph nodes throughout the pre-clinical stage of scrapie (May 1998 – ongoing)**

A flock of 20 lambs born to clinically affected ewes has been established at the CVRL, Abbotstown. These animals were born in the spring of 1999 and 2000 and are of the following genotypes VV<sub>136</sub>RR<sub>154</sub>QQ<sub>171</sub>, VA<sub>136</sub>RR<sub>154</sub>QQ<sub>171</sub>, AA<sub>136</sub>RR<sub>154</sub>QQ<sub>171</sub>, AA<sub>136</sub>RR<sub>154</sub>QR<sub>171</sub> and AA<sub>136</sub>RR<sub>154</sub>RR<sub>171</sub>.

Biopsies are collected from the tonsil, third eyelid and submandibular lymph node every two months. The abnormal form of the prion protein has been detected in the peripheral lymphoid system of three animals between 6 and 12 months of age.

Irish Research Teams Involved:	Teams 1,2
Number of Other Countries Collaborating:	–
Source of Funding:	A
Contact:	<b>Dr. Torres Sweeney, Team 2</b>

**An examination of lymphoid tissue in the third eye lid of sheep (June 2000 – June 2000)**

The third eye lid contains lymphoid tissue which can be sampled and analysed for PrP<sup>sc</sup>. The objective of this project was to describe the anatomical distribution of lymphoid tissue within the third eyelid and identify the most suitable sampling site. Twenty clinically normal animals maintained at Lyons Research farm were used. All superficial parts of the tissue were collected including the bulbar, palpebral sides, the medial and lateral aspects, the areas at the base and at the free edge. Lymphoid follicles were consistently present in protuberances on the palpebral side of the third eyelid, both on the lateral and medial side of the T-shaped cartilage (4.4±1.24 follicles per 5?m tissue section). In contrast, tissue samples taken from the smooth palpebral areas had a lower number of follicles (0.3±0.21 follicles per 5?m tissue section). Lymphoid follicles could not be detected on the bulbar side in 6 of 9 animals examined. (This data is published in Thuring *et al.*, 2000 Veterinary Record 147: 631-632)

Irish Research Teams Involved:	Teams 1,2
Number of Other Countries Collaborating:	–
Source of Funding:	A
Contact:	<b>Dr. Torres Sweeney, Team 2</b>

### **Real Time Analysis of Antibody – PrP Reaction Kinetics (January 2000 – on going)**

The Biacore system is a sensor which works on an optical phenomenon of surface plasmon resonance i.e. detection of changes in refractive index on a surface, which evaluates the dissociation/association rate and affinity constants. These changes in refractive index are directly translated into mass, which means that interactions can be measured in real time, without modifying any of the participating molecules. The objective of this study was to examine the interaction of three monoclonal antibodies (P4, L42 and 6H4) with recombinant prion protein (Prionics) using the BIAcore biosensor system (Pharmacia Biosensor A B, Uppsala, Sweden). The recombinant prion protein was immobilised on to the surface of the sensor chip (CM5 sensor chip). Antibody binding results in response units (RU) showed that the recombinant prion protein had the greatest binding capacity with the antibody 6H4 (6H4 = 1036.1 RU, 6H4 and NaCl = 551 RU). Whereas the binding capacity of P4 and L42 were negligible (P4 = 2.5, P4 and NaCl = 2.1, L42 = 4.6 and L42 and NaCl = 4.5). It can be concluded that the P4 and the L42 antibody are not adequate enough to use, as their binding response is too low. However, 6H4 is the antibody that can be used for further kinetic studies.

Irish Research Teams Involved:	Team 2
Number of Other Countries Collaborating:	–
Source of Funding:	A
Contact:	<b>Dr. Torres Sweeney, Team 2</b>

### **Tissue distribution and molecular characteristics of sheep with an oral inoculation of BSE in comparison to sheep with an oral inoculation of the scrapie strain endemic to Ireland. (January 2001 – December 2003)**

The objective of this experiment is to compare the clinical signs, pathological profile and molecular characteristics of PrP<sup>Sc</sup> in sheep with an oral inoculation of BSE in comparison to sheep with an oral inoculation of the scrapie strain endemic to Ireland. Genetically susceptible (AA<sub>136</sub> RR<sub>154</sub> QQ<sub>171</sub>) pedigree texel sheep will be orally challenged with brain from scrapie infected sheep in Ireland. Simultaneously, genetically susceptible (AA<sub>136</sub> RR<sub>154</sub> QQ<sub>171</sub>) texel sheep will be orally challenged with brain from BSE infected cattle in the ID-DLO, Netherlands. A range of tissues will

be collected from all relevant part of the body and the tissue distribution of PrP<sup>Sc</sup> will be compared between the two treatment groups. The molecular characteristics of the PrP<sup>Sc</sup> will be compared in terms of glycosylation profile and proteinase K digestion.

Irish Research Teams Involved:	Teams 1,2
Number of Other Countries Collaborating:	2
Source of Funding:	E
Contact:	<b>Dr. Torres Sweeney, Team 2</b>

### **Study of neuronal loss in selected brainstem nuclei in BSE affected cattle (January 1998-Sept. 2000)**

The aim of this project was to assess neuronal numbers in the olivary nuclear complex, the dorsal vagal nucleus and the hypoglossal nucleus in clinically normal cattle of different ages and in BSE-affected cattle. Computerised stereological techniques for accurately counting cells in three dimensions within tissues were successfully developed. In our study we found, somewhat suprisingly, that there was no evidence of neuronal loss in cattle as between juvenile and adult animals. In BSE-affected animals, there was a significant loss of neuronal numbers in the olivary nuclear complex. This was the only nucleus of the three studied where extensive PrP deposition occurs in BSE-affected animals. Although neither the dorsal vagal nucleus nor the hypoglossal nucleus exhibited significant neuron loss, both these nuclei together with the olivary nuclear complex exhibited increased numbers of glial cells. However, the proportions of glial cells which were astrocytes did not significantly differ in any of the three nuclei.

These studies have refuted a number of previously published findings based on outdated older morphometric methods. Our studies suggest that neuronal loss is not a function of age when clinically normal juvenile and adult cattle are compared. Its occurrence in BSE appears to be associated with PrP<sup>Sc</sup> deposition rather than with neuronal vacuolation..

Irish Research Teams Involved:	Teams 1, 4, 7
Number of Other Countries Collaborating:	–
Source of Funding:	A
Contact:	<b>Dr. Hugh Bassett, Team 7</b>

## **Rapid automated diagnosis of BSE and scrapie for the purposes of correct and efficient monitoring of such diseases in Ireland.**

This project set out to develop automated processes for the diagnosis of TSE from immunostained tissue sections. Quantification of PrP-specific immunostain is difficult for the human operator to gauge. In addition, low levels of staining can also be difficult to detect. The advantages in using computer vision to analyse immunostain deposition will be gained from the objective, consistent and thorough nature of examination. An immunostain auto-recognition program was developed. The program can learn to recognise PrP-specific immunostains. The computer can thoroughly check every part of the tissue section for hundreds of shades of the immunostain. The pathologist can train the program by showing it a few positively and negatively stained sections. This can be done for any existing and new immunostains. The immunostain auto-recognition program can look for regions of positive stain that resemble blood vessels and tag them as blood vessels. Regions of low-level staining can be weighted by their amount of stain and location on the tissue section.

Programs were also developed for histopathological examination. One such programs can be taught, by the pathologist, to recognise vacuolation. Vacuoles once recognised, can be counted, graded by shape, and analysed for spatial distribution. Another program developed can be used to identify and count neurons. Another can identify and count glial cells. Finally the system can be used by the pathologist to perform combinations of immuno- and histopathological analyses as the location of features can be measured and cross-referenced. Images and records of analysis can be maintained indefinitely.

Irish Research Teams Involved:	Teams 1, 4
Number of Other Countries Collaborating:	–
Source of Funding:	A
Contact:	<b>Dr. Mark Rogers, Team 4</b>

## **Studies on the expression of metallothionein in BSE -affected cattle**

The distribution and levels of expression of metallothionein was assessed using immunohistology in normal, suspect and BSE positive animals. The level of expression of metallothionein was demonstrated to be higher in brains of BSE infected animals compared with controls. The expression was demonstrated to be, at least in part, with astrocytes.

Irish Research Teams Involved:	Teams 1, 4
Number of Other Countries Collaborating:	–
Source of Funding:	A
Contact:	<b>Dr. Mark Rogers, Team 4</b>

#### **(d) Risk Assessment of Spongiform Encephalopathies (7 projects)**

##### **Is PrP<sup>Sc</sup> present in cull sheep? (Nov 1999- Sept 2000)**

The objective of this project was to determine if cull sheep contain PrP<sup>Sc</sup> in either the peripheral lymphoid system or the spinal cord when slaughtered at the factory for human consumption. 600 sheep were sampled at random from three abattoirs (Ballymun, Slaney and Camolin) to identify if cull animals entering the abattoir have PrP<sup>Sc</sup> in the spinal cord or submandibular lymph node. All sheep were crossbred animals of mainly suffolk, texel, cheviot, blackface mountain, and Belclare descent. Approximate equal numbers from each ‘breed category’ were collected. Spinal cord samples were analysed using the Enfer test for PrP<sup>Sc</sup> detection. Spinal cord from three Suffolk ewes were PrP<sup>Sc</sup> positive in the Enfer test. From the 600 mandibular lymph nodes immunostained for the detection of PrP<sup>Sc</sup> six nodes were positive. These consisted of two black face mountain ewes and four suffolk ewes (including the three that were positive in the Enfer test).

Irish Research Teams Involved:	Teams 1, 2
Number of Other Countries Collaborating:	–
Source of Funding:	A
Contact:	<b>Dr. Torres Sweeney, Team 2</b>

##### **Disposal/utilisation options for meat and bone meal and tallow in the context of minimising risk from BSE**

The project investigated the disposal of meat and bone meal (MBM), tallow, and specified risk material (SRM), leading to the development of possible options for a National disposal plan. In addition, a risk assessment model, based on HACCP, of the disposal options for such material, and a traceability system capable of identifying the species from which MBM is produced were developed. The main findings were that both MBM and tallow can be used as co-fuels in peat fired and stationary engines for electricity production. Their use presents minimal risk to humans, in terms of unburnt infective residues and discharge to the atmosphere. When tallow is

burned as a fuel blend with diesel it can significantly and beneficially alter the composition of the combustion gases evolved.

Irish Research Teams Involved:	Team 6
Number of Other Countries Collaborating:	3
Source of Funding:	A
Contact:	<b>Prof. Shane Ward, Team 6</b>

**Measures to reduce contamination of meat and the environment with CNS tissue during slaughter and processing of cattle and sheep (1998-2001)**

The objective of this project is to identify critical points and procedures in existing slaughter practices which pose the greatest risk from cross contamination by CNS material and to propose solutions by the development of alternative procedures to those causing the greatest risks.

Irish Research Teams Involved:	Team 10
Number of Other Countries Collaborating:	2
Source of Funding:	K
Contact:	<b>Dr. J.J. Sheridan, Team 10</b>

**Contamination of meat and exposure of abattoir workers to CNS material during standard butchery practices prevalent in the member states of the EU (1999-2001)**

The objectives of this study are concerned with the exposure of abattoir workers to the BSE agent during standard butchery practices in different slaughter plants in EU member states. Information will be obtained on the levels of faecal contamination on carcasses and on processes for contamination control.

Irish Research Teams Involved:	Team 10
Number of Other Countries Collaborating:	2
Source of Funding:	L
Contact:	<b>Dr. J.J. Sheridan, Team 10</b>

### **Feasibility study for the disposal of rendering plant products with energy recovery (1998)**

Irish Research Teams Involved:	Team 9
Number of Other Countries Collaborating:	1
Source of Funding:	N
Contact:	<b>Dr. Paul Johnston, Team 9</b>

### **DNA methods for the traceability of beef (Complete)**

The objectives of the project were to (1) evaluate the potential of DNA based identification systems in each stage of the beef product chain, and (2) to produce an integrated validation system for beef traceability which could serve as a high accuracy check-audit procedure to assure customers that claims of traceability are justified. The project was successful in formulating a new DNA based quality assurance system that had immediate commercial application. Some of the research was rapidly adopted by industry. It also assisted in developing critical mass and international reputation in the area of molecular genetic product traceability within Ireland such that there is now significant participation in the EU Framework Programmes as well as patent development arising from the research.

Irish Research Teams Involved:	Team 12
Number of Other Countries Collaborating:	
Source of Funding:	A
Contact:	Prof. P Cunningham, Team 12

### **Feasibility study involving the identification of consumer demand for traceability of meat within the food chain, evaluating traceability system and assessment of methods of verification. (Complete)**

The project aimed to better inform policy makers and commercial interests as to the consumer demand for traceability, the types of traceability systems available and the methods through which the verification of traceability could be achieved. In addition, the project aimed to inform policy makers and commercial interests as to the food safety concerns in the Irish food market with particular reference to perceived meat hazards, risk reduction strategies and private and public responsibilities. The outcome suggests that consumers have limited understanding of traceability systems, but once explained felt that such systems could help to alleviate their concerns. However many preferred the concept of a quality assurance scheme over that of traceability. The project also determined the factors influencing consumer choice for meat, plus

the primary food safety concerns of consumers relating to meat, which were primarily BSE, growth hormones and e coli. The project made a number of suggestions which were intended to assist policy makers and commercial interests in optimising traceability systems and identifying consumer concerns, expectations and requirements.

Irish Research Teams Involved:	Team 13
Number of Other Countries Collaborating:	
Source of Funding:	A
Contact:	Dr. M. Keane, Team 13

### 3. Principal research teams and their areas of expertise : Name, addresses, full details

Team	Details
1	Central Veterinary Research Laboratory, Abbotstown, Castleknock, Dublin 15. E. Weavers, M. McElroy, E. Monks, A. Fullam Church, A.M. Roche, D. Sammin. Pathology, immunohistochemistry, clinical. <b>Contact: Dr. E. Weavers.</b> Tel: +353-1-6072621, Fax: +353-1-6072604, Email: <a href="mailto:weaverse@eircom.net">weaverse@eircom.net</a>
2	Molecular Biology Unit, Faculty of Veterinary Medicine, University College Dublin, Ballsbridge, Dublin 4. T. Sweeney, M. Aherne, M. Flavin, E. O'Doherty, K. Thornton, C. Thuring, Molecular genetics, western blotting, glycosylation. <b>Contact: Dr. Torres Sweeney</b> Tel: +353-1-6687988, Fax:
3	Department of Large Animal Clinical Studies, Faculty of Veterinary Medicine, University College Dublin, Ballsbridge, Dublin 4 A. Healy, M. Doherty, J. D. Collins, Y. Pawitan Clinical neurology, epidemiology. <b>Contact: Dr. Anne Healy</b> Tel: -353-1-6687988, Fax: -353-1-6683515, Email: <a href="mailto:anne.healy@ucd.ie">anne.healy@ucd.ie</a>
4	Department of Zoology, University College Dublin, Belfield, Dublin 4. M. Rogers, D. Fitzgerald, J. Hanlon, Molecular biology <b>Contact: Dr. Mark Rogers.</b> Tel: +353-1-7062197, Fax: , E-mail: <a href="mailto:mark.rogers@ucd.ie">mark.rogers@ucd.ie</a>
5	Veterinary Epidemiology and Tuberculosis Investigation Unit, Department of Large Animal Clinical Studies, Faculty of Veterinary Medicine, University College Dublin, Ballsbridge, Dublin 4. J.D. Collins, J. Griffin, P. White, G. McGrath, R. Hammond, Y. Pawitan. <i>Geographic Information Systems (GIS), risk analysis, epidemiology</i> <b>Contact: Professor John D. Collins</b> Tel: +353-1-6687988, Fax: +353-1-6608946, E-mail: <a href="mailto:jcollins@vetmed.ucd.ie">jcollins@vetmed.ucd.ie</a>

<b>6</b>	SRM Research Group, Department of Agricultural and Food Engineering, University College Dublin, Earlsfort Terrace, Dublin 2. Prof. S. Ward, Dr. K. McDonnell, Ms. N. Healy (PhD Student), Mr. S. Colgan (PhD Student), Mr. E. Cummins (PhD Student) <b>Contact: Prof. Shane Ward</b> Tel: +353-1-7167351, Fax: +353-1-4752119, E-mail: <a href="mailto:shane.ward@ucd.ie">shane.ward@ucd.ie</a>
<b>7</b>	Department of Veterinary Pathology, Faculty of Veterinary Medicine, University College Dublin, Ballsbridge, Dublin 4 H. Bassett, Markey, P. Breslin. <i>Pathology, Stereology</i> <b>Contact: Dr. Hugh Bassett</b> Tel: +353-1-6687988, Fax: +353-1-6603422, e-mail: <a href="mailto:hugh.bassett@ucd.ie">hugh.bassett@ucd.ie</a>
<b>8</b>	Beaumont Hospital, P.O. Box 1297, Beaumont Road, Dublin 9. <i>Neuropathology</i> <b>Contact: Dr. Michael A. Farrell</b> Tel: +353-1-8092643, Fax: +353-1-8092955
<b>9</b>	Department of Civil, Structural and Environmental Engineering, Trinity College Dublin <i>Environmental engineering</i> <b>Contact: Dr. Paul Johnston</b> Tel: +353-1-6081372, e-mail: <a href="mailto:pjohnston@TCD.ie">pjohnston@TCD.ie</a>
<b>10</b>	Teagasc, National Food Centre, Dunsinea, Castleknock, Dublin 15 <i>Food safety microbiology</i> <b>Contact: Dr. J.J. Sheridan</b> Tel: +353-1-8059500, Fax: +353-1-8059550, E-mail: <a href="mailto:j.sheridan@nfc.teagasc.ie">j.sheridan@nfc.teagasc.ie</a>
<b>11</b>	Veterinary Division, Department of Agriculture, Food and Rural Development, Agriculture House, Kildare Street. Dublin 2. H. Sheridan, W. Martin <i>Epidemiology</i> <b>Contact: Dr. Hazel Sheridan</b> Tel: +353-1-6072975, Fax: +353-1-6619031. E-mail: <a href="mailto:hazel.sheridan@daff.irlgov.ie">hazel.sheridan@daff.irlgov.ie</a>
<b>12</b>	Dept. of Genetics, Trinity College, Dublin 2 <i>Genetics</i> <b>Contact: Prof. P Cunningham</b> Tel: +353-1-6081064, Fax: +353-1-6798558.
<b>13</b>	Dept. of Food Economics, University College Cork <b>Contact: Dr. M. Keane</b> Tel: +353-21-90257, Fax: +353-21-276929

## Sources of Funding for TSE Research in Ireland

Code	Details
A	Irish Department of Agriculture and Food– Non-Commissioned Food Research Programme
B	FAIR Shared cost research project no. PL973305
C	Concerted Action-Biotech 1 project no. CT976056
D	FAIR Shared cost project no. PL 987021
E	Irish Department of Agriculture, Food and Rural Development – Food Institutional Research Programme
F	FAIR Shared Cost project no: PL987017
G	Irish Department of Agriculture, Food and Rural Development
H	FAIR CT97-3306
I	Enterprise Ireland Basic Science Awards
J	FAIR CT98-3651
K	EU FAIR 97-3301
L	FAIR CT98-7004
M	QLK2-CT-2000-00837
N	Cavan County Council and UAOS

#### 4. Collaboration with Other Countries

As is obvious from the details presented with each project, the majority of TSE research projects in the Republic of Ireland involve collaboration with other countries. All researchers expressed an openness to collaboration.

# ISRAEL

## Prion research in Israel, February 2001

### Main TSE research activities underway including Public Health aspects

There is currently no national programme for research in TSEs in Israel. However, laboratories involved in research, epidemiology and diagnosis collaborate extensively with one another and maintain extensive links with laboratories and authorities abroad.

#### A. Basic research

Several aspects of prion structure, metabolism, and replication are being investigated.

- Additional structural molecular components within the infectious particles. Current biochemical definitions of the PrP isoforms. Protease-resistance of PrP as a marker for infectivity. Computer modelling of PrP conformation.
- Metabolic and subcellular pathways of the PrP isoforms in the host cell. Cellular biosynthetic compartment of PrP<sup>Sc</sup>. The role of cholesterol-rich membranal rafts in the formation of PrP<sup>Sc</sup>. Accessory proteins in the metabolism of PrP; PrP-binding proteins. The role of copper in PrP metabolism.
- The processing of pathogenic PrP mutants. Pathogenesis in E199K transgenic mice.
- Mode of action of antiprion reagents such as sulfated glycans and other polyanions.
- Alternative PrP species in peripheral cells such as sperm.
- Translational control of PrP mRNA.

#### B. Improved diagnostics

- Effort is being invested in improving existing diagnostic procedures based on the detection of PrP<sup>Sc</sup> in various tissues, as well as in finding additional, surrogate markers.

#### C. Epidemiology and diagnosis of human and animal TSEs

- The majority of CJD patients in the country are familial cases linked to the E200K mutation. All suspected CJD patients are examined for this and other mutations, and their familial history is recorded. Records of CJD and vCJD since the 1960s are the topic of constant epidemiologic scrutiny. DNA samples of all CJD patients and many of their close family members are collected and saved for future research and epidemiological studies.

**Principal research teams and their areas of expertise:**

Addresses of groups involved in TSE Research	Areas of expertise
<b>Dr. Ruth Gabizon Hadassah University Hospital, Jerusalem</b>	<b>Prion structure and metabolism. Diagnostics. fCJD</b>
<b>Dr. Albert Taraboulos Hebrew University, Jerusalem</b>	<b>Cell biology of prions. Prions and cholesterol rafts.</b>
<b>Prof. Esther Kahana Barzilai Hospital, Ashkelon</b>	<b>Epidemiology, especially of fCJD</b>
<b>Dr. Zeev Meiner Hadassah University Hospital, Jerusalem</b>	<b>E200K fCJD</b>

## ITALY

### INVENTORY OF ITALIAN LABORATORY INVOLVED IN PRION PROJECTS NOT FINANCIALLY SUPPORTED BY EU

#### **M. CASTAGNARO**

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Since 1995 we have been involved in TSE research, BSE and scrapie particularly, and more broadly in neuropathology of animal diseases.

In our laboratories routinely histopathological, immunohistochemical and ultrastructural techniques have been applied for TSE diagnosis. For the same purposes we have also developed polyclonal antibodies which react against BSE- and scrapie-infected tissues.

We are now testing the specificity and sensibility of BSE rapid screening test by using recombinant Prp on ELISA and Western blot procedures.

#### **F. GUARDA**

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Laboratory of neuropathology

Histological diagnosis on nervous tissue

Histopathology, immunohistochemistry and wester-blot for transmissible spongiform encephalopathies

Histological and immunohistochemical studies on old age brain lesions (Alzheimer like lesions).

Characterization of brain tumors whit histological, immunohistochemical and molecular biological techniques (PCR).

Researches of possible prognostic markers of scrapie into cerebro spinal fluid.

#### **Laboratory of molecular biology**

PCR and in situ hybridization for the main infectious agents

#### **Laboratory of cell cultures**

#### **Electron microscopy center**

SAF identification

Ultrastructural investigations for routine diagnosis of diseases

## **G. POLI**

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The experimental approach of our research unit is divided in two principal directions:

1) The first aims to the synthesis and the evaluation, both in vitro and in vivo of lipophile and with low/null toxicity molecules, all of them being Congo red derivatives, with potential therapeutic/preventive activity (antiamyloidogenic and/or amyloidolytic) against the production/deposition into the brain of PrPres, the major component of Transmissible Spongiform Encephalopathies (TSE) causative agent. Congo red has been shown to inhibit amyloid deposition both in vitro and in vivo.

In vitro assays consist on the evaluation of the antiamyloidogenic and/or amyloidolytic effects of the molecules under test through three different approaches:

- a) inhibition, by the molecules under test, of the generation of the prion protein scrapie isoform (PrPres) in persistently infected neuroblastoma cells;
- b) evaluation of antifibrillogenic effects of the molecules under test on syntetic peptide 106-126 by analytical methods (HPLC and electron microscopy)
- c) evaluation of molecules activity in reverting PrPres, extracted from Syrian hamsters' infected brains in PrPsen, by Western blotting;

In vivo evaluation consist on the administration, using four different administration routes (intracerebral, intraperitoneal, subcutaneous and oral) of the molecules resulted more effective, together with the intracerebral infection with 263K scrapie strain; the evaluation of the molecules efficacy will be performed by measuring the mean incubation time, the mean survival time and the detected lesions in comparison with positive controls.

2) In the second one we want to develop a hypothetical immunological approach to realize a vaccine for prion disease. PrPres and PrPsen are poorly immunogens and only immunological stimulation in different species can induce antibodies against epitopes common to both proteins. Taking in account the experimental evidences obtained in Alzheimer disease (the  $\beta$ -amyloid major component injected in Tg mice is able to trick the immune system of the mice and to recognize amyloid as a foreign substance that should be attacked), in this project we want to inoculate 6 groups of 15 Syrian Hamster with different peptides homologous to 101-116 and 106-141 regions of hamster, a couple of heterologous peptides corresponding to 106-126 and 82-146 regions of human PrP, the recombinant PrP (Prionics) and the purified PrPsc of hamster. Each epitome will be emulsionated with a strong adjuvant (able to induce an autoimmune response). In order to realize a possible vaccine, each putative immunogens will be cloned and expressed in a baculovirus system to produce virus-like particles (VLPs).

## **M. SALMONA**

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## **G. FORLONI**

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### **Molecular determinants of prion diseases**

#### **Identification of the molecular determinants responsible for PrP conformational plasticity**

Characterizing the structural determinants of the pathological conformation of prion proteins (PrP) is important for a better understanding of the pathogenetic process. We study the conformational properties of synthetic peptides encompassing critical PrP regions. Using of a variety of chemico-physical and biochemical techniques, we determined the secondary structure of PrP derived peptides and assessed the presence, nature and stability of tertiary structures by evaluating hydrogen-deuterium exchange, resistance to protease digestion, self-association capacity, fibrillogenic behaviour and amyloidogenic properties. This work provides a qualitative and quantitative description of the conformational plasticity of PrP fragments.

#### **Interaction of PrP fragments with model membranes**

The interaction between PrP and the plasma membrane of neuronal and glial cells is of paramount importance for the pathogenicity of these proteins. A simplified experimental system can be used to study how PrP peptides interact with model membranes and see what are the minimal structural domains of PrP necessary for the interaction with membrane bilayers of known lipid composition. It also provides clues on the molecular mechanisms underlying PrP cytotoxicity.

#### **Intracellular distribution and biological activity of PrP peptides**

Correlations between the chemico-physical properties of PrP peptides and their biological effects can be drawn only on the basis of cell culture models. We determined the sub-cellular distribution of PrP peptides in these experimental systems and are currently identifying the biologically relevant intra-cellular target molecules. In addition, we evaluated how PrP peptides influence calcium homeostasis, and are studying interactions with other known intracellular second-messengers.

#### **Design of molecules interfering with fibril formation**

There is a general correlation between PrP fibril formation and neuronal cell toxicity or astroglial reactive responses. Thus, compounds capable of interfering with fibril formation may be useful therapeutic candidates.

We have already shown that the region between amino acid 106-126 of PrP is necessary for the formation of fibrils in buffer solutions. Using a simple in vitro assay measuring fibrillogenesis from a peptide corresponding to amino acids 106-126, we set up a protocol for the rational screening of molecules that inhibit the formation of fibrils or dissolve fibrils once formed.

**Laboratory of Biology of Neurodegenerative Disease**  
**Department of Neuroscience**

## **Prion-related encephalopathy (PRE)**

In collaboration with Neuropathology Division of the Carlo Besta Institute in Milan and the Department of Biochemistry and Molecular Pharmacology of our Institute and in analogy with the approach used in AD, the biological activities of synthetic peptides homologous to fragments of prion protein (PrP) purified from cerebral amyloid deposits in Gerstmann-Sträussler-Senker (GSS, an example of PRE) patients is investigated. The biologically active fragment PrP 106-126 in vitro reproduces the pathological events associated with PRE. The conformational transition of PrP from random coil or a helix to a  $\beta$  sheet has been associated not only with neurotoxic activity but also with the alteration of PrP connected with infectivity. Physicochemical methods and EM examination have been employed to study the aggregation of various amyloidogenic peptides. To better approximate the neuropathological condition, PrP 82-146 has been synthesised and its physicochemical and biological characteristics are currently being investigated. The in vivo studies using experimental scrapie are utilized to test the neuroprotective activity of several drugs including new classes of anti-amyloidogenic drugs

## **Protein aggregation and neurodegenerative disorders**

Cerebral amyloid deposits in Alzheimer's disease (AD) and prion-related encephalopathy (PRE) contain different proteins: Ab (~ 4 kDa) and PrP (~ 30 kDa). Both proteins are normally soluble, but in pathological conditions are converted to forms that are fibrous and relatively resistant to chemical denaturation and proteolytic digestion. Recently protein aggregation at different levels (extracellular, cytoplasmic and nuclear) has also been implicated in Parkinson disease (PD), in the group of neurodegenerative diseases associated with polyglutamine repeats, including Huntington's disease (HD) and spinocerebellar ataxia type III (SCA 3) and in amyotrophic lateral sclerosis (ALS). These findings suggest a common mechanism triggering the pathogenetic cascade responsible for the main neurodegenerative disorders. We will develop experimental models in vitro to study not only the pathogenesis of AD and PRE, as described above, but also the biological bases of PD, HD and ALS. The chemico-physical investigations and the intra- and extracellular application of proteins and their synthetic fragments to neuronal and non-neuronal cells, together with the cell lines producing the wild-type and mutated forms of aggregating proteins, will be used to investigate the pathological mechanisms and to develop potential therapeutic strategies.

## **F. VALFRE'**

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## **LIST OF THE RESEARCH GROUPS INVOLVED IN THE STRATEGIC PROJECT OF CNR (ITALIAN NATIONAL RESEARCH COUNCIL)**

**COORDINATOR: F. VALFRE'**

## **G. DESTEFANIS**

Dipartimento di Scienze Zootecniche,  
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Raccolta e diffusione dell'informazione.

Titolo della ricerca: Difesa e valorizzazione delle razze bovine italiane specializzate per la produzione della carne o a duplice attitudine (latte carne).

### **M. CARAMELLI**

Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta

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Sorveglianza epidemiologica delle Encefalopatie spongiformi trasmissibili.

Titolo della ricerca: Elaborazione di protocolli di vigilanza per le encefalopatie spongiformi trasmissibili degli animali.

### **M.C. SORGATO**

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Caratterizzazione dell'agente trasmissibile e dei meccanismi patogenetici.

Titolo della ricerca: Biologia e biochimica della proteina prionica.

### **P. LEONE**

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Recettività e resistenza alle encefalopatie spongiformi.

Titolo della ricerca: Recettività e resistenza alle encefalopatie spongiformi. Analisi dei polimorfismi del gene PrP nei bovini di razza italiana

### **L. FERRARA**

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Recettività e resistenza alle encefalopatie spongiformi.

Titolo della ricerca: Encefalopatia Spongiforme Bovina: analisi dei polimorfismi della PrP negli ovini e nei caprini.

## U. AGRIMI

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The ISS is the national institute of public health in Italy. It coordinates a network represented by 10 regional veterinary laboratories (Istituti Zooprofilattici Sperimentali, IZS) which are involved in the diagnosis and surveillance of animal infectious diseases and of zoonosis. The ISS participates to the research, control and epidemiological surveillance of both human and animal TSEs. The animal TSEs section, which is located at the Laboratory of Veterinary Medicine, is directed by Dr. Agrimi, while the human TSEs section, located at Laboratory of Virology, is directed by Prof. M. Pocchiari who is also the responsible for the National Register of CJD.

The Laboratory of Veterinary Medicine strictly collaborates with the Laboratory of Virology of ISS and with the National Reference Laboratory for animal TSE at the IZS of Turin.

The expertise of the group concerns the pathology and molecular biology of prion diseases.

In particular, current studies deal with:

*Natural scrapie: 1) Genetic factors influencing the susceptibility of Italian sheep and goat breeds; 2) role of the lymphoreticular system in the pathogenesis of the disease*

An epidemic of scrapie in sheep and goats, probably due to the use of an accidentally-contaminated vaccine against *contagious agalactia* prepared with sheep brain omogenate, was recently reported in Italy. The ISS is currently working on a project aimed at studying the genetic susceptibility of the Italian sheep and goats breeds to scrapie. The PrP gene from about one hundred cases of scrapie and from a suitable number of healthy controls has been sequenced paralleling the results with those from the PrP immunohistochemical examination of both brain and lymphoid tissues of each animal. The data concerning the susceptibility of two sheep breeds and one goats breed were preliminary presented at international congresses and are going to be sent for publication on refereed journals.

The distribution of PrPSc has been investigated in several lymphoid districts of scrapie-affected sheep and goats. Since stamping out of scrapie affected flocks is applied in Italy, studies are performed, in some instances, on the whole flock. The PrPSc from the brain and lymphoid tissues of natural scrapie cases coming from different geographic origin and from different breeds of sheep and goats is being characterised through glycotyping and a panel of monoclonal antibodies.

The structure, nucleotide composition and the genetic comparison of the doppel gene of 32 scrapie-affected and 24 healthy sheep of Sarda breed has been studied in collaboration with the Department of Genetic and Microbiology of the University of Pavia, Italy.

A project for typing the scrapie strains circulating in the national sheep and goat populations is ongoing at ISS and the second passage of scrapie isolates in mice has been already obtained from different sources.

A large collection of cases of scrapie in sheep and goats is available at the Laboratory of Veterinary Medicine.

*Development of improved bioassays for TSE agents based on the bank vole, a highly susceptible rodent species to scrapie.*

Preliminary studies showed an unprecedented high susceptibility of a wild rodents (bank vole or *Clethrionomys glareolus*) to both natural and mouse-adapted scrapie. The results from the experimental transmission of six cases of natural scrapie in sheep and goats from different geographic origins (Italy, France and United Kingdom) and of a mouse-adapted scrapie strain, to bank voles indicate survival times very much shorter in this species are than in mice (Tables 1a and 1b). Clinical signs in voles were always clearly distinguishable, and much more pronounced

neuropathological changes and PrPsc accumulation than in mice were observed. All experiments concluded so far resulted in 100% transmission rate in bank voles, compared to no more than 30-40% or even 10% in mice, based on neuropathology or PrPsc Western-blot detection, respectively.

Worthy to be mentioned is the comparable easy transmission to voles of scrapie cases from both sheep and goats as well as from sheep carrying different PrP alleles (136<sup>AA</sup>154<sup>RR</sup>171<sup>QQ</sup>, cases n. 2, 3, 4, 6 and 136<sup>VV</sup>154<sup>RR</sup>171<sup>QQ</sup>, case n. 5 – Table 1). Of interest are also the similar survival times observed after the inoculation of voles with either the brain or the palatine tonsil from a scrapie affected sheep (cases 6 vs 7).

Comparative transmission studies between voles and OvTg-mice have been already initiated. Preliminary results from the inoculation of 4 cases of scrapie to voles, wild type and ovine PrP<sup>VRQ</sup> transgenic mice showed again the shortest incubation periods in voles (Table 1). This is an interesting finding which confirms that factors other than the PrP sequence homology rate between the donor and recipient species, contribute to the species barrier effect.

The laboratory mouse is the species traditionally used for transmission studies, but this model presents critical limitations, namely: very long incubation times, not-always-successful transmission, subtle clinical signs, mild neuropathological lesions, and not-always-detectable levels of PrPsc in the brain. Current studies at ISS aim to develop vole-based models for transmission of TSEs more susceptible than mice.

Specific objectives are: 1) to extend to other TSEs previous findings on the highly susceptibility of voles to scrapie; 2) to study if the strain typing system currently used in mice could be also applied to voles; 3) to study the susceptibility of voles to low infectious titres of the agent of scrapie (and, possibly, of other TSEs) through LD50 comparative studies; 4) to study the role of polymorphisms of the PrP gene (already sequenced) in conditioning the susceptibility to the disease; 5) to investigate the susceptibility of voles to scrapie by the oral route and to study the possible spread and long-term persistence of scrapie infection in a colony of voles kept in semi-naturalistic settings. This will help to elucidate if the high susceptibility of voles to scrapie could have implications for scrapie epidemiology.

## **G. PIVA**

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Procedure diagnostiche, profilattiche e di controllo.

Titolo della ricerca: Sistemi di trasformazione in farina degli scarti di macellazione e metodologie di controllo dell'efficacia dei trattamenti e dell'efficacia nutrizionale

## **COORDINATOR: F. VALFRE'**

### **V.M. MORETTI**

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Commission Decision 94/381/EC banned the use of mammalian protein in the feeding of ruminants. With the same Decision feeding of ruminants with non-ruminant proteins may be authorised where it is possible to distinguish between animal protein from ruminant and non-ruminant species in rendered animal material.

The high temperature used for risk materials during rendering process is the main drawback to the application of traditional methods based on the determination of specific bio-molecules (proteins, fatty acids). Recently PCR-based techniques have resulted the most suitable methods for critical samples in which most of the DNA has been degraded.

In our laboratory we have developed a methodological approach to identify species in meat meal and feedstuffs using a PCR method to amplify a polymorphic region of mitochondrial cytochrome b gene. Species differentiation has been determined by digestion of the PCR fragment with restriction endonucleases. Comparison of published sequences of amplified region showed that the Restriction Fragment Length Polymorphism analysis may be a suitable method of testing inter-specific mutations of cytochrome b segment. Although informative restriction enzymes used in this study are only few among endonucleases which could be used to characterise all species, the choice of these enzymes was found to be more convenient since with few digestions is possible to discriminate between ruminant and non-ruminant species and between mammalian and poultry.

## **INVENTORY OF ITALIAN LABORATORY INVOLVED IN EU PRION PROJECTS**

**From Catalogue of funded projects. In Transmissible Spongiform Encephalopathies: The European Initiative. September 2000.**

### **CT96 0856 “Clinico-pathological features and pathogenesis of fatal familial insomnia”**

#### **E. LUGARESI Coordinator**

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### **CT97 2216 “Creutzfeldt-Jacob disease in the European Union-Incidence and risk factors”**

#### **M. POCCHIARI Participant**

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### **CT97 3308 “Separation, identification and characterization of the normal and abnormal isoforms of prion protein from normal and experimentally infected fish”.**

#### **C.L. BOLIS Coordinator**

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**CT97 3311 “Analysis of molecular factors affecting variability in BSE and scrapie susceptibility”**

**L. FERRETTI Participant**

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**CT97 6003 “Transgenic mice expressing human prion protein use for characterization of human encephalopathies, and sensivity for detection of infectivity”.**

**O. BUGIANI Participant**

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**CT97 6011 “*In vitro* investigation of PrP-induced neurodegeneration: development of a system for testing potential therapeutic agents”**

**F. TAGLIAVINI Participant**

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**CT97 6015 “European centralised facility for human transmissible spongiform encephalopathy (prion diseases)”.**

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**CT97 6029 “Risk assessment in primates of TSE transmission to humans through food and blood products”**

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**CT97 6050 “The bovine prion protein: from structure analysis to the molecular mechanism of conformational transitions”**

**M.C. SORGATO Coordinator**

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**CT97 6051 “Structure, function and interactions of prion proteins and prion protein domains”**

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**CT97 6055 “Trafficking pathways of normal and pathogenical isoforms of the prion protein”**

**C. ZURZOLO Participant**

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**CT97 6056 “Consented action for the setting of multicentric epidemiological data bases and biological banks for small ruminant scrapie”**

**G. RU Participant**

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**CT97 6060 “Cerebellar network alterations in prion diseases”.**

**E. D’ANGELO Participant**

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**CT97 6065 “Inactivation of the causative agents of transmissible spongiform encefalopathies by thermophilic and hyperthermophilic proteases”**

**M. ROSSI Participant**

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**CT98 3651 “A network for the supply of BSE tissues and fluids for European collaborative research”.**

**M. POCCHIARI Participant**

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**CT98 3727 “Laboratory supported diagnosis of Creutzfeld–Jacob disease”**

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**CT98 7019 “TSE-agent inactivation, product quality evaluation and sterilisation process simulation in rendering processes for the production of feed grade animal protein”**

**G. PIVA Participant**

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**CT98 7021 “The establishment of european network for the surveillance of ruminant TSE and the standardization of the process and criteria for the identification of suspect cases”.**

**M. CAMELLI Participant**

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e-mail:cea@to.izs.it

**CT98 7028 “Public perception of BSE and CJD risk in Europe, their interplay with media, policy initiatives and surveillance issues. Drawing the lessons for information policy”.**

**P.P. GIGLIOLI Participant**

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## NETHERLANDS

### **1/2 Description of main TSE research activities underway including Public Health aspects and how these activities fit into the principal areas of the European Action Plan on TSE research**

#### **a) Clinical, epidemiological and social research on human spongiform encephalopathies**

\* Erasmus University of Rotterdam, Dept. of Epidemiology and Biostatistics

(Dr. C.M. van Duijn), PO BOX 1738, 3000 DR Rotterdam, The Netherlands.

- Epidemiology of human prion diseases. Monitoring of the occurrence of CJD and related disorders in the Netherlands and Europe. Within the Netherlands, Department of Epidemiology & Biostatistics co-ordinates the monitoring of the disease, in collaboration with the Academic Medical Centres Amsterdam and Utrecht. The research programme is part of the EU funded Collaborative Action “Epidemiological surveillance of CJD in the European Union” (EUROCID: Co-ordinator : Prof Dr. Will; NEUROCID: Co-ordinator: Dr R Knight). In the collaborative studies, the Department of Epidemiology & Biostatistics is responsible for data management of the statistical analysis.

\* Academic Medical Centre, Medical School Amsterdam, Dpt. of Neurology

(Drs. W.A. Van Gool and P. Meijerink)

- Genetic characterisation of two newly identified Dutch families with an inherited form of prion disease.
- Evaluation of clinical value of 14-3-3 CSF assay and MRI of the brain for making an antemortem diagnosis of CJD.

#### **b) The infectious agent and its mechanisms of transmission**

\* Utrecht University Medical School (AZU-1), Dept. of Pathology

(Dr. G. Jansen)

- The role of macrophages in transportation of PrP<sup>Sc</sup> to the brain.

- \* Netherlands Cancer Institute, Amsterdam
  - (Prof. P. Peters [Group Leader], Dr. A. Mironov [postdoctoral fellow], E. Bos, M.S. [research associate]; in collaboration with Prof. S. Prusiner, UCSF, USA)
  - Subcellular trafficking of PrP in cultured cells and tissues of mice (transgenic or inoculated) by means of cryo-immunogold Electron Microscopy.
  - Identification of sites in cells where the PrP<sup>c</sup> form is converted into PrP<sup>sc</sup>.
  - The subcellular trafficking of the BSE-causing agent (PrP<sup>sc</sup>) in tissues and white blood cells.

- \* Institute for Animal Science and Health (ID-Lelystad), Lelystad
  - BSE/Scrapie project group (Drs M. A. Smits, A. Bossers, J.P.M Langeveld, L.J.M. van Keulen, and B.E.C Schreuder).
  - Genetic resistance to scrapie, including large scale genotyping in connection with a national scrapie control programme
  - Antigenic studies of PrP in sheep, cattle and mice
  - Studies on the pathogenesis of natural scrapie in sheep (“from gut to brain”)

**c) Diagnosis of spongiform encephalopathies**

- \* Erasmus University of Rotterdam
  - As part of the monitoring of the occurrence of CJD and related disorders in Europe, the group participates in projects aiming to improve the clinical diagnosis of CJD with the help of EEG, CSF, MRI.
  - Definition of clinical subtypes of new variant CJD.
- \* Academic Medical Centre
  - Evaluation of clinical value of 14-3-3 CSF assay and MRI of the brain for making an ante mortem diagnosis of CJD.
  - Quantification of MRI-abnormalities and 14-3-3 protein CSF levels in CJD and control patients.

- \* ID-Lelystad
  - Development of tools for diagnostic methods (monoclonal antibodies, immunohistochemical detection) on scrapie, BSE and CJD, aiming at:
    - a preclinical diagnostic test for routine application
    - a differential diagnosis of BSE and scrapie in sheep
  - Immunohistochemical localisation of PrP<sup>sc</sup> on brain and lymphoid tissues of diseased and infected sheep
  - Development of a rapid diagnostic method (mainly western blotting) for scrapie and BSE
  
- \* Utrecht University Medical School
  - Monitoring changes in the amount of CJD cases and phenotypes.
  - Improvement of detection of prion protein by means of panel of anti PrP antibodies.

#### **d) Evaluation of the risk of contracting spongiform encephalopathies**

- \* Erasmus University of Rotterdam
  - As part on the epidemiologic investigation of human prion diseases, studies of putative risk factors for CJD (genetic factors, medical and occupational history, animal exposure, diet) are being performed
  - The group in the Netherlands is leading a project on the identification of new genetic susceptibility factors for CJD. The project is conducted in collaboration with Belgium and Bulgaria.
  
- \* ID-Lelystad
  - Development of in vitro systems for detection of prion infectivity in sheep,
  - Studies on species barrier between cattle, sheep and other species (including maternal transmission), also by:
    - Development of an in vitro conversion assay of PrP, to predict transmissibility of prions within and between various species
    - Analysis of risk factors for the occurrence of TSEs in the Netherlands

### **e) Treatment and prevention of spongiform encephalopathies**

- \* Erasmus University of Rotterdam.
  - As an expert centre, the group advises clinicians and laymen on the opportunities on treatment and prevention of SEs.
  
- \* Academic Medical Centre
  - Creation and study (behavioural and neuropathological) of a transgene model of the recently identified Pr NP insert mutation.
  
- \* Netherlands Cancer Institute, Amsterdam
  - Application of anti-PrP antibodies in the prevention and treatment of BSE, using cultured cells and transgenic animal model systems. New Research Initiative (Jan 2001 onwards)
  
- \* ID-Lelystad
  - Development of methods that neutralise prions or block the uptake of prions (including vaccination strategies)
  - Development of methods for gene therapy for the treatment of prion diseases

### **3. Principal research teams**

- \* Academic Medical Centre, Medical School Amsterdam, Dpt. of Neurology  
(Drs. W.A. Van Gool and P. Meijerink) P.O.Box 22660, 1100 DD Amsterdam  
Tel: 31-20-5663842; Fax: 31-20-6971438; e-mail: w.a.vangool@amc.uva.nl
  
- \* Utrecht University Medical School (AZU-1), Dept. of Pathology  
(Dr. G. Jansen)
  
- \* Erasmus University of Rotterdam, Dept. of Epidemiology and Biostatistics  
(Dr. C.M. van Duijn), PO BOX 1738, 3000 DR Rotterdam, The Netherlands;  
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\* Netherlands Cancer Institute, Plesmanlaan 121, 1066CX Amsterdam  
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\* ID-Lelystad (Institute for Animal Science and Health), Lelystad  
BSE/Scrapie project group (Drs M. A. Smits, A. Bossers, J.P.M Langeveld, L.J.M. van  
Keulen, and B.E.C Schreuder, rapporteur);  
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#### **4. Collaboration with Other Countries**

**All groups are open for (future) collaborations, the present contacts are only partially  
listed in the text.**

# NORWAY

## 1. Main TSE research activities underway including public Health aspects

### HUMAN TSE

#### **Team 1:**

#### **Epidemiological studies of Creutzfeldt-Jakob disease and other TSE in Norway**

Two main objectives:

- Develop surveillance system for Creutzfeldt-Jakob disease (CJD) and other human TSE in Norway
- Identify risk factors for developing CJD and other human TSE in Norway through European the European project "NEURO CJD" financed by the BIOMED2 programme. The participating countries are Belgium, Denmark, Finland, Greece, Ireland, Norway, Portugal and Sweden together with Iceland and Israel. The group works closely with the EURO CJD project consisting of other EU members and Australia, Canada and Switzerland. The objectives of the NEURO CJD project are:
  - To establish agreed clinicopathological criteria for the classification of classical CJD and variant CJD (vCJD).
  - To co-ordinate CJD surveillance programmes in the following countries: Belgium, Denmark, Finland, Greece, Iceland, Ireland, Israel, Norway, Portugal and Sweden.
  - To harmonise methods of data collection and analysis and agree a minimal data set to be obtained.
  - To analyse risk factors for nvCJD including past medical history, occupation and diet.
  - To provide information on the epidemiological characteristics of CJD in the European Union.

The project is financed by The Norwegian Research Council.

### ANIMAL TSE

#### **Team 2:**

#### **"Scrapie - occurrence, clinic, epidemiology, pathogenesis and diagnostics".**

Start: 1998-01-01 to 2001-12-31

The project is financed by The Norwegian Research Council, and represents a continuation and extension of an internal project which started in 1992, after diagnosing scrapie in the region (mainly Rogaland county). Materials from sheep with scrapie have been, and are being, collected, and are studied morphologically, and are also being used in experimental inoculation studies in lambs. The department has its own experimental sheep flock (200 wfs) and licensed facilities to work with infections in sheep (isolates for studying live sheep, well equipped laboratories).

The main aim of the project is to "Increase knowledge about scrapie, i.e. occurrence, symptoms, microscopic changes, epidemiology, pathogenesis, infectivity carriers and diagnosis". Sub-aims are to find the occurrence of all forms of scrapie in South Western Norway, receive "suspect" sheep for clinical following up (in closed isolate), including testing out recent diagnostic tests (blood, eyelids, tonsils, lymph nodes, other), perform postmortems and microscopy of suspect animals, including testing more recent supportive techniques (particularly immunohistochemistry, IHC), correlate findings with prionprotein genotypes, and focus on subclinical infectivity carriers.

The laboratory is performing the PrP genotyping, which is very important for scrapie research. Scrapie-material from own defined scrapie cases is being used in inoculation experiments in lambs, and these are followed up in closed isolates, killed and examined after various periods, and examined for PrP and changes in organs and tissues (particularly lymphoid

organs and nervous tissue). This is an important long-term project to gain knowledge about PrPSc uptake and dissemination, i.e. pathogenesis.

**Team 3:**

**“Breeding towards scrapie resistance – effects on health and production”.**

**Project no. 110 634/122 (Norwegian Research Council)**

From 1999 to 2003

The project is a continuation of an earlier project (Prion protein gene polymorphisms in sheep with natural scrapie and healthy controls in Norway, 1996-1998).

This project is a continuation of the “genotyping-project”. Sheep are bred towards scrapie resistance, using the rather new knowledge about variations in the PrP-gene in sheep. The project will run for 5 years, and includes around 1100 sheep from 11 flocks. The aim is to disclose whether there is a connection between resistance towards scrapie and health or productivity characteristics. The animals are followed with detailed individual health and productivity registrations, in cooperation with the Sheep Control System (Sauekontrollen). Variations in the PrP gene (codons 136, 154 and 171), which are characteristic for resistance/susceptibility for scrapie, are used as markers for selection of rams as well as selection of breeding ewes. The aim is to shift the flock as quick as possible towards AARRRR homozygous state. Examinations like these must precede further general recommendations for breeding towards resistance within the country, as a mean of eradicating scrapie.

**Team 4:**

**”Studies of the Ovine and Bovine Prion-Like Doppel Protein and its Gene”**

We are studying the genetic control of TSEs and specifically the genetics and biochemistry of the prion-like Doppel gene and its protein product. All data generated in this co-operation will be communicated to the public through scientific journals.

**Team 5:**

Financial support from the Norwegian Research Council and EU for **research on genetic factors and basic molecular biology influencing the development of scrapie in sheep.**

One objective is to study the early stages of prion protein (PrP) uptake during per os challenge experiments on young lambs and sheep by analysing the molecular events and gene expression profiles within the gut and gut-associated lymphoid tissue following exposure to the scrapie agent. Supplementary to the studies of scrapie agent uptake, the normal level of PrP gene expression in various tissues from the digestive channel and nervous system are under examination and will be compared to expression levels during challenge as well as in spontaneous disease cases. Another activity underway is a detailed study of the PrP locus in sheep in collaboration with European labs engaged in the study of the cattle PrP locus. Detected variability in both sheep and cattle PrP gene regulation signals as well as in other genes linked to the PrP locus will be addressed to identify elements associated with increased risk of disease, not only in terms of sequence differences, but also looking for the effect of differences in expression levels.

**Team 6:**

The research activities that are currently underway in the Section of Pathology at the Department of Morphology, Genetics and Aquatic Biology, Norwegian School of Veterinary Science are encompassed by 3 Norwegian Research Council funded projects addressing the pathogenesis of scrapie in sheep.

The projects are:

- NFR project no. 118091/122: Uptake and persistence of the scrapie agent in the gut and gut-associated lymphoid tissues
- NFR project no. 116073/122: Transport of prion infectivity from site of uptake to the central nervous system
- NFR project no. 128528/110: Development of beta-fibrillosis with emphasis on microenvironments in the spleen

These projects address various aspects of the uptake, early accumulation and spread of the scrapie-agent as indicated by the presence of the disease-associated isoform of prion protein, PrP<sup>Sc</sup>, in lymphoid and peripheral nervous tissues. In collaboration with the Department of Sheep and Goat Research at Norwegian School of Veterinary Science in Sandnes, these projects have worked with material derived from an experimental oral infection model in PrP-genotype defined lambs. These studies have investigated tissues from 1 week to 11 months after oral exposure using immunohistochemical and histoblot techniques. Studies of the deposition of amyloid fibrils particularly within microenvironment of the spleen have also been undertaken, using mink as a model species.

**Team 7:**

Simulation model for spread of scrapie between Norwegian sheep flocks.

**Team 8:**

Main research activities at the National Veterinary Institute in Norway are directed towards development of early diagnostic methods regarding scrapie, including morphology, PrP<sup>Sc</sup> immunohistochemistry, immunoblot methods and evaluation of one of the rapid tests (the CEA Elisa-test).

NFR project no. 133 449/110 (Norwegian Research Council), expiry date 2001-12-31

**2. How these activities fit into the principal areas of the European Action Plan on TSE research:**

**Team 1:**

Fits into point (a)

**Team 2:**

Fits into point (not given by the author)

**Team 3:**

Fits into point (not given by the author)

**Team 4:**

Fits into point (not given by the author)

**Team 5:**

These activities would fit into areas (b), (c) and (e)

**Team 6:**

The research activities would be relevant to the following two areas: (b) and (c)

**Team 7:**

Fits into point (d) and (e).

**Team 8:**

Fits into point (c).

### 3. Principal research teams and their areas of expertise:

#### Team 1:

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#### Team 2:

- Ulvund, Martha J. Project leader, Professor, Norwegian School of Veterinary Science (NVH), Department of Sheep and Goat Research, Kyrkjevegen 332-334, 4325 Sandnes, Norway.
- Ersdal, Cecilie, PhD student, same address

#### Team 3:

- Arve Osland, Project leader, Dr. philos. NVH, Department of Sheep and Goat Research, Sandnes.
- Martha J. Ulvund, Professor, Norwegian School of Veterinary Science (NVH), Department of Sheep and Goat Research, Kyrkjevegen 332-334, 4325 Sandnes, Norway.

#### Team 4:

Partners:

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Norwegian School of Veterinary Science, Department of Morphology, Genetics and Aquatic Biology, N-0033 Oslo, Norway. Areas of expertise: Molecular biology, genetics & morphology

#### Team 5:

- Grethe Skretting, expertise in molecular- and cell-biology
- Ingrid Olsaker, expertise in genetics (classical and molecular)
- Arild Espenes, expertise in pathology and expression analysis

Address for all three:

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#### Team 6:

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**Team 7:**

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Expertise: epidemiology

**Team 8:**

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Expertise: immunohistochemistry, immunoblot, morphology
- Bjørn Bratberg, senior researcher, National Veterinary Institute, Dept. of Pathology, Box 8156 Dep, 0033 Oslo. Tel. +47 22 96 46 80, fax +47 22 56 59 69, E-mail: [bjorn.bratberg@vetinst.no](mailto:bjorn.bratberg@vetinst.no)  
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Expertise: morphology

**4. Collaboration with other countries and openness of your programme to collaboration**

**Team 1:**

Through NEURO CJD cooperation with Australia, Canada and Switzerland.

**Team 2:**

- Institute for Animal Science and Health (ID-DLO), PO Box 65, NL-8200 AB Lelystad, Holland (Langeveld JPM. Polyclonal antibodies, IHC)
- Federal Research Centre for Virus Diseases of Animals, Tübingen, Germany (Groschup, M. Monoclonal antibodies, IHC)
- Veterinary Laboratories Agency, Lasswade, UK (Jeffrey, M. Visit and internship for stipendiate)

Also:

- Institute of Neurology, Bern, Switzerland (Meyer R)
- Department of Biochemistry & Immunology, Danish Veterinary Laboratory, Copenhagen V, Denmark (Heegard PMH)
- EU Task 4, Fair 98-7021, clinical symptoms (Berthelin-Baker C)
- EU CT97-3305. Lesion profiling (Simmons M)

**Team 3:**

No direct collaboration with other countries.

We have, and will have, openness of our programmes to collaboration, if indicated and possible.

**Team 4:**

- Sergio Comincini & Luca Ferretti: [sergio.c@ipvgen.unipv.it; lucaf@ipvgen.unipv.it]  
Dipartimento de Genetica e Microbiologica, Università di Pavia, via  
Abbiategrosso 207, 27100 Pavia, Italia
- David Hills & John L. Williams: [john.Williams@bbsrc.ac.uk]  
Roslin Institute, Roslin, Midlothian, UK

**Team 5:**

Collaboration within EU-project " Analysis of molecular factors affecting variability in BSE and scrapie susceptibility- MASSES

- John Williams (Coordinator), Division of Molecular Biology, Roslin Institute (Edinburgh), (UK)
- Luca Ferretti, Università di Pavia, Pavia (IT)
- Gaudenz Dolf, Institute of Animal Breeding, University of Berne, (CH)
- Marc Vandeveld, Institute of Animal Neurology, University of Berne, (CH)

Other collaboration:

- Nora Hunter and Wilfred Goldmann, Neuropathogenesis Unit, Institute for Animal Health, Edinburgh

**Team 6:**

The Section is currently involved in EU research application with partners from Scotland, The Netherlands & Germany entitled " Studies on the alimentary pathogenesis of BSE agent and natural scrapie in sheep and mice. Implications for diagnosis and control ". The EU proposal number is QLRT-2000-02332.

**Team 7:**

The project is a part of EU-FAIR CT97 3305.  
The project is open for collaboration.

**Team 8:**

The project is involved in EU-FAIR CT97 3305 (scrapie strain typing)  
We are collaborating with INRA Tours-Nouzilly, France (Pierre Sarradin).

# PORTUGAL

## **Description of main transmissible spongiform encephalopathies (TSE) research activities including Public Health aspects**

No basic/experimental research on TSEs is carried out in Portugal. Both the human and animal groups study mainly the epidemiology of TSEs, the characterisation and pathogenesis of tissue lesions and diagnostic tools.

Activities in human TSEs are carried out in the Laboratory of Neuropathology of the Hospital Santa Maria-Lisbon (Director: Dr. José G. Pimentel), supported by the Ministry of Health.

Competent Authority activities in animal TSEs are centralised in the Laboratório Nacional de Investigação Veterinária, in Lisbon and Oporto (Director: Dr. Alexandre Galo) supported by the Ministry of Agriculture.

Additionally, a research group at the Technical University of Lisbon (Dr Armando Louza) is developing epidemiological studies.

A national registry for CJD has been established as well as a national monitoring programme for scrapie.

Following the health measures implemented by the Ministry of Health and the Ministry of Agriculture and Fisheries, a national commission was established with leading experts in neurology, epidemiology, microbiology and veterinary medicine in order to provide scientific based and independent advice on the subject to the government and to the whole community. Such national commission finished its fulfilment in March 2000 and was not re-established

Most of the teams working in TSEs participate in EU shared cost project concerted actions.

## **2. How these activities fit into the principal areas of the European Action Plan on TSE research**

### **a) Clinical, epidemiological and social research on human and animal SEs**

BMH4-CT-96-3698 "Co-ordination of national surveillance programmes for CJD in the European Union" Coordinator. Prof. R. Will. Participants: Dr. Pimentel and Dr. Lima

FAIR-CT97-6056 (1999-2001) "Concerted action for the setting up of multicentric epidemiological databases and biological samples banks for small ruminant scrapie". Coordinator Dr. Lantier. Participants Dr. Louza.

FAIR-CT98-7021 (1999-2001) "The establishment of a European network for the surveillance of ruminant TSE and the standardisation and harmonisation of the process and criteria for the identification of suspect cases". Coordinator Dr Rogers. Participants Dr. Galo and Dr Louza.

## **b) The infectious agent and its mechanisms of transmission**

PhD Project “Strain typing of Portuguese BSE and genetic susceptibility to prion diseases” Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Oporto, Instituto Gulbenkian Ciência (IGC) and Laboratório Nacional de Investigação Veterinária (LNIV), Lisboa. M.L. Orge, Prof. Simas and Dr A. Galo.

PhD Project “Behaviour of prion and amyloid protein at polymers surfaces and interfaces” University of Porto – Max Planck Institute (D) S.C. Pinto Rocha, Prof. M.C. Pereira and Prof. M.A. Coello.

## **c) Diagnosis of SEs**

BMH4-CT97-2034 (1997-2000) “Human transmissible spongiform encephalopathies: neuropathology and phenotypic variation”. Coordinator Prof Budka. Participants Dr.Pimentel, Dr Lima and Dr. Bentes.

QLK2-1999-30837 (2000-2002) “ Human transmissible spongiform encephalopathies: the neuropathology network”. Coordinator Prof. Budka. Participants Dr. Pimentel and Dr. Lima.

## **e) Coordination**

BM-CT97-6057 “Building a common database on scientific research and public decision on TSE in Europe” Coordinator Dr P. Joly. Participants Dr Gonçalves (ISCTE) and Mr. L. Gonçalves (LNIV).

FAIR-CT98-3651 “A network for the supply of BSE tissues and fluids for European collaborative research” Coordinator: Dr. S. Done. Participant Dr Galo.

## **3. Principal research teams and their areas of expertise: names, addresses, full details**

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#### **4. Collaboration with other countries and openness of your programme to collaboration**

At this moment the main collaborations with other countries are in the framework of the projects funded by the European Union listed in 2.

# SPAIN

## **1. Description of main transmissible spongiform encephalopathies (TSE) research activities in Spain including Public Health aspects**

Research on TSE in Spain is supported in the framework of two I+D National Programmes: the Ministry of Science and Technology (MCYT-National Plan of R&D) and the Ministry of Health (MSC-FIS). Certain activities are also supported by the Autonomic Communities (CCAA) and other public institutions.

Due to recent events, a National Strategic Action on TSE research has been launched in February of 2001. This action was designed to fund research projects, to promote the co-ordination of research teams and to support banks of biological tissues and complementary biosafety measures in research laboratories. The research covers a wide number of areas (infectious agent and its mechanism of transmission, diagnosis, risk assessment, TSE surveillance, risks material removal and food safety) in order to mobilise new and complementary expertise to increase the critical mass.

In 1997 the National Plan for Surveillance of Animal Spongiform Encephalopathies was organised, creating a Reference Laboratory (Facultad de Veterinaria Universidad de Zaragoza) to ratify or to discard previous diagnosis.

Recently, (27/12/2000) a Royal Order (3454/2000) was adopted to establish and to regulate an Integrated Co-ordinated Programme of Surveillance and Control of Animal Spongiform Encephalopathies. This programme addresses the systems of detection of TSE, the control of materials used in animal nutrition, the inspection of the by-products and dead animals processing plants, and the control of the elimination of high risk materials.

Other two National Reference Laboratories of diagnosis are programmed to co-ordinate the analytical controls among the authorised laboratories of CCAAs.

With regard to Creutzfeldt-Jakob disease(CJD), the National Recording of the disease and a System of Surveillance was created in the Health Institute "Carlos III" (Madrid). Several CCAAs have established units of biodiagnosis of the CJD and the other prion-related diseases.

In addition several Spanish laboratories also participate in EU projects of the 4<sup>th</sup> and 5<sup>th</sup> Framework Programmes in both human and animal TSEs.

Finally a Scientific Multidisciplinary National Committee have been recently (February of 2001) established with experts in neurology, epidemiology, veterinary medicine and food safety in order to provide advice to National Plan of R&D for research strategies.

## **2. How these activities fit into the principal areas of the European Action Plan on TSE research**

### **a) Clinical, epidemiological and social research on human and animal SEs**

1-Creutzfeldt-Jakob disease in Spain: incidence and risk factors (MSC) (1998-2000) Dr. Jesús de Pedro Cuesta

2-Spanish population exposed to drugs based on blood derived products: identification, control and sanitary supervision (1999-2001) Dr. Jesús de Pedro Cuesta

3-Diagnosis and surveillance on BSE. Collaborative agreement between the Ministry of Agriculture, Fisheries and Food and the University Zaragoza (2000-2001) Prof Juan J. Badiola

#### **b) The infectious agent and its transmission mechanisms**

4-Role of PrP in prion spread and establishment of central nervous system infection (MCYT-EU) (1998-2001) Prof Juan J. Badiola

#### **c) Diagnosis of SEs**

5-Biological diagnosis of human transmissible spongiform encephalopathies (MSC) (1999-2002) Dr. Alberto Saiz.

6-Diagnosis of transmissible spongiform encephalopathies using PrP-Sc/PrPc specific antibodies (MCYT) (1998-2000). Dr. María Gasset.

7-Early diagnosis of transmissible spongiform encephalopathies: coupling of strategies of pathogen PrP amplification to affinity sensors (MCYT) (2000-2003) Dr. María Gasset

8-Development of a practical diagnostic method for the early detection and control of transmissible spongiform encephalopathies (TSEs) (MCYT) Dr Belén Pintado.

#### **d) Risk assessment of SEs**

-Separation, identification and characterization of the normal and abnormal isoforms of prion protein from normal and experimentally infected fish (MCYT-INIA -EU) (1998-2001) Dr. Antonio Figueras et Dr. José Manuel Sánchez-Vizcaíno.

9-Investigation transborder programme on genetic resistance to scrapie and transmission factors of prion (Co-operation Fund Aquitaine-Euskadi) (1998-2000) Dr. Ina Beltrán and Dr. Felisa Arrese.

10-Detection of susceptible to scrapie ovine genotypes in Laxta herds and its application to the prevention and development of genetically resistant herds in the C.A.P.V. (DAPGV) (1999-2001) Dr Arantza Sanz and Dr. Felisa Arrese.

### **3. Principal research teams and their areas of expertise: names, addresses, full details**

Pedro Cuesta, Jesús de  
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Diagnosis/epidemiological surveillance/risk factors of human TSE

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Nature of the agent, diagnosis

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Infectious agent/degenerative disorders in human TSE

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Biological diagnosis of human TSE

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Diagnosis, pathology

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Diagnosis

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Diagnosis, transgenic mice, transmission studies in pigs and fish

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Epidemiological surveillance, diagnosis

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Transmission studies in fish

Juste Jordán, Ramón, García-Perez, Ana, Beltrán Ina, Arrese Felisa and Sanz Arantza.  
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Epidemiological surveillance/genetic resistance

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Clinical diagnosis

#### **4. Collaboration with other countries and openness of your programme to collaboration**

At this moment the main collaborations with other countries are in the framework of the following projects funded by the European Union and specific bilateral co-operations.

1-BMH4-CT97- 2216 (1997-2000) “CJD in the European Union- incidence and risk factors and QLRT-2000-1999-31709 “ CJD : epidemiology, risk factors and diagnostic tests” EURO-CJD – Coordinator. Prof. R. Will. Participant: Dr. de Pedro Cuesta.

2-BMH4-CT98-3727 (1998-2000) “Laboratory supported diagnosis of CJD”. Coordinator. Prof. Poser. Participant: Dr. de Pedro Cuesta.

3-BIOTECH-CT97-6046 (1998-2000) “Diagnosis of TSEs using PrPsc/PrPc-specific antibodies”. Coordinator Prof. Kretzschmar. Participant Dr Gasset.

4-BIOTECH-CT97-6060 (1998-2000) “Cerebellar network alterations in in prion diseases”. Coordinator Dr Axelrad. Participant Dr Ferrer.

5-BMH4-CT97-2034 (1997-2000) “Human transmissible spongiform encephalopathies: neuropathology and phenotypic variation”. Coordinator Prof Budka. Participants Dr Cruz-Sánchez and Dr. Navarro.

6-QLK2-1999-30837 (2000-2002) “ Human transmissible spongiform encephalopathies : the neuropathology network”. Coordinator Prof. Budka. Participants Dr Cruz-Sánchez and Dr. Navarro.

5-FAIR-CT97-3308 (1997-2001) Separation, identification and characterization of the normal and abnormal isoforms of prion protein from normal and experimentally infected fish. Coordinator Prof. Bolis . Participants : Dr Sánchez-Vizcaíno and Dr. Figueras.

6-FAIR-CT97-3306 (1998-2001) New approaches to the diagnosis and control of TSEs. Coordinator Dr M. Rogers. Participant Dr Sánchez-Vizcaíno.

7-FAIR-CT97-6056 (1999-2001) Concerted action for the setting up of multicentric epidemiological databases and biological samples banks for small ruminant scrapie. Coordinator Dr. Lantier. Participant Prof. Badiola.

8-FAIR-CT98-7021 (1999-2001) The establishment of a European network for the surveillance of ruminant TSE and the standardisation and harmonisation of the process and criteria for the identification of suspect cases. Coordinator Dr Rogers. Participant Prof. Badiola.

9- FAIR-CT98-7023 (1999-2001) Role of environmental and host factors on the horizontal and vertical transmission of scrapie in naturally infected sheep flocks. Dr García Perez.

At present, the National Programmes allow the participation of non-national groups, but not direct funding. New schemes of co-operation based on exchange of information, reciprocity, etc. are being considered.

## SWEDEN

### 1. Description of main TSE research activities underway including public Health aspects :

Project	Responsible scientist	Supported by
<b>A.</b> ” In vitro studies of cytopathological changes in transmissible spongiform encephalopathy ”	<b>Katarina Bedecs, PhD</b> Department of Neurochemistry, Stockholm University <a href="mailto:Coco@neurochem.su.se">Coco@neurochem.su.se</a>	<b>SJFR/FOMAS</b> <b>2001-2002</b> <b>297 000</b> <b>SEK/year</b>
<b>B.</b> ” Synthesis, processing and uptake of the bovine prion protein in bovine and ovine cells ”	<b>Prof Tommy Linne,</b> Department of veterinary microbiology/virology, BMC, SLU, Box 585, S-751 23 Uppsala <a href="mailto:Linnet@bmc.uu.se">Linnet@bmc.uu.se</a>	<b>SJFR/FOMAS</b> <b>2001-2002</b> <b>382 000</b> <b>SEK/year</b>
<b>C.</b> ” Studies of the Prion protein”	<b>Prof Tommy Linne,</b> Department of veterinary microbiology/virology, BMC, SLU, Box 585, S-751 23 Uppsala <a href="mailto:Linnet@bmc.uu.se">Linnet@bmc.uu.se</a>	”Doktorandtjänst ” <b>2001-2004,</b> <b>Vet Faculty,</b> <b>SLU., 315000</b> <b>SEK/year</b>
<b>D.</b> ” Evaluation of PET in diagnosis of and differential diagnosis of different forms of CJDs ”,	<b>Prof PO Lundberg,</b> Dept Neuroscience and Neurology, University hospital S-751 23 Uppsala Sweden <a href="mailto:Po.lundberg@neurologi.uu.se">Po.lundberg@neurologi.uu.se</a>	<b>Research project recently finished</b>
<b>E.</b> ” BSE-testing programme ”	<b>Prof Berndt Klingeborn,</b> Department of Virology, National Veterinary Institute (SVA), Box 585, Uppsala <a href="mailto:Berndt.klingeborn@sva.se">Berndt.klingeborn@sva.se</a>	<b>Testing of fallen stock,</b> <b>20000</b> <b>animals/year</b>

**2. How these activities fit into the principal areas of the European Action Plan on TSE research :**

- **Clinical, epidemiological and social research on human and animal SE**  
Project D. and E. above
- **The infectious agent and its mechanisms of transmission**  
Project A., B. and C. above
- **Diagnosis of Ses**  
Project D. and E. above
- **Risk Assessment of Ses**  
Project E. above
- **Treatment of prevention of Ses**  
Project D. above

**3. Principal research teams and their areas of expertise: names, addresses, full details**

A. Research team: Katarina Bedecs, PhD, , P. Ostlund, H. Lindgren  
Areas of expertise: Galanin research, In vitro models of prion induced neurodegradation  
Department of Neurochemistry, Stockholm University  
[Coco@neurochem.su.se](mailto:Coco@neurochem.su.se)

B. C. E. Research team: Prof Tommy Linne, M. Klingeborn, MSc, M Simonsson, PhD ; Prof Berndt Klingeborn  
Areas of expertise: Virology, In vitro expression of PrP, studies of protein processing and degradation, PrP diagnosis,  
Department of veterinary microbiology/virology, BMC, SLU, Box 585, S-751 23 Uppsala  
[Linnet@bmc.uu.se](mailto:Linnet@bmc.uu.se)

D. Research team: Prof PO Lundberg, Dept Neuroscience, Neurology, University hospital, S-751 23 Uppsala Sweden, Dr Y. Olsson, Neuropathology, University hospital, S-751 23 Uppsala Sweden, I. Nennesmo, K. Ekbohm Huddinge hospital.  
Prof B. Langstrom, H. Engler, M. Bergstrom, PET Centre,, University hospital, S-751 23 Uppsala Sweden,  
Areas of expertise: Neurology, Diagnosis of CJD, Treatment of CJD  
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Research team: Profs Krister Kristensson, T. Hokfelt, Department of Neuroscience, Karolinska Institutet, S-171 77, Stockholm, Sweden  
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**4. Collaboration with other countries and openness of your programme to collaboration**

EU projects/actions:

NEUROCID (PO. Lundberg), PRIONET (PO. Lundberg), "The role of plasma membrane structure in prion propagation, transport and pathogenesis" (A : Taraboulos, K. Kristensson, BMH4-CT98-6027), " New approaches to diagnosis and control of TSE's" (M. Rogers, T. Linne, FAIR CT 97-3306)

## UNITED KINGDOM

### **1. A description of the main TSE research activities underway in the UK, including public health aspects.**

The main Government funders of research on BSE and the other TSEs of animal and public health significance are MAFF and the Biotechnology and Biological Sciences Research Council (BBSRC). The Department of Health (DH), the Medical Research Council (MRC) and the Scottish Office Department of Health all fund work on CJD and public health aspects of TSEs. In addition, the MRC funds research aimed at understanding the structure and function of the prion protein and its role in pathogenesis and disease progression. The Food Standards Agency (FSA), established in April 2000, has taken over the management of a portion of MAFF TSE research and will soon fund new work.

Research is carried out in a wide number of leading scientific institutions in the UK, including universities, hospital medical schools, Government Agencies and Institutions.

By the end of last financial year, 1999/2000, UK Government funders (MAFF, BBSRC, MRC and DH) had spent over £140 million on research into transmissible spongiform encephalopathies affecting animals and man. This is a large programme, which has been developed progressively since the beginning of the BSE epidemic in cattle. It is designed to provide a better understanding of prion disease. Information from the programme has formed the basis for policy decisions directed at the eradication of BSE from the national cattle herd and the protection of public and animal health. The research covers, amongst other areas, epidemiology, diagnostic procedures and test development, therapeutic development, transmission, pathogenesis of disease, studies on the nature of the causative agent and inactivation of the agent.

Co-ordination of research by the UK funding groups is achieved by liaison at several levels, most directly through the Transmissible Spongiform Encephalopathy Research and Development Funders Co-ordination Group chaired by the DH Director of R&D under a joint DH/MAFF secretariat. The Group is constituted with representatives of all funding bodies. Its aim is to ensure that the programme of R&D funded in this field addresses priority issues of national interest and constitute a coherent strategy when considered as a whole.

In addition, a High Level Committee on Research and Development into TSEs was set up in January 1997. This is chaired by the Cabinet Secretary who makes regular reports to the Prime Minister. Its purpose is to ensure that a research strategy which fully addresses UK Government's policy needs is in place and agreed by all funders, and to identify any barriers to progress and make recommendations for overcoming them.

## **Ministry of Agriculture, Fisheries and Food**

TSE Research and Surveillance Unit: Dr Hilary Gates

Email address: [Hilary.J.Gates@maff.gsi.gov.uk](mailto:Hilary.J.Gates@maff.gsi.gov.uk)

<http://www.maff.gov.uk/>

MAFF has a wide-ranging programme of research on TSEs, addressing important issues that affect both human and animal health. The research programme was established and has changed over time to address directly MAFF's policy aims to:

- Protect public health through the prevention of risk of infection with the BSE agent
- Eradicate BSE from UK cattle
- Prevent the recurrence of a BSE-like epidemic
- Eradicate TSEs from UK sheep

This is a large and comprehensive research programme, that has evolved since the identification of BSE in 1986, to take account of identification of vCJD in 1996 and the possibility that BSE may have infected sheep.

MAFF's current TSE programme can be divided broadly into 4 areas: transmission, epidemiology, pathogenesis and diagnostics.

### **Transmission**

#### a) Lateral and maternal transmission

The current programme is concentrating on transmission of TSEs in sheep. Two studies are in progress and three new proposals aimed at investigating natural routes of transmission are under consideration for funding. One of the experiments in progress is looking at vertical transmission of BSE in sheep of three different PrP genotypes. Another is investigating maternal transmission using embryos of a genotype known to be susceptible to a particular strain of scrapie.

The question of whether scrapie can be transmitted via the embryo is also being studied. Fifty embryos from donor ewes with naturally acquired scrapie infection have been transferred into scrapie-free Suffolk ewes imported from New Zealand.

b) Effective exposure

Studies investigating what constitutes effective oral exposure in cattle have shown that as little as 1 gram of BSE-affected material will transmit disease. A second study is now underway with oral dose levels between 1 gram and 1 milligram of BSE-affected brain.

c) Tissue sources of infection

Studies to determine which tissues carry infectivity have been extremely important in identifying high risk materials that should be kept out of the food chain. Most bioassays for tissue infectivity have been conducted in panels of mice. Comparative studies have shown that bioassay in cattle is approximately 500-fold more sensitive than in mice. At present work is underway to see whether cattle pick up lower levels of infection than mice. A wide range of tissues have been collected and inoculated into cattle. These have had their infectivity assessed by inoculation into mice in a former study.

A relatively new study started in 2000 is looking at the extent to which there is under-estimation of the infectivity of sheep BSE tissues when tested in mice.

Studies on abnormal prion protein (PrP<sup>Sc</sup>) distribution and tissue infectivity in sheep are also underway. These include studies on sheep of different PrP genotypes infected with natural scrapie and studies of PrP<sup>Sc</sup> distribution and infectivity in sheep experimentally infected with BSE.

The programme also includes work on sheep and bovine transgenic mice.

d) Genetic predisposition

Further information on the genetic influence on resistance and the course of disease development in sheep of different genotypes is being produced. Groups of scrapie free sheep of different breeds and genotype have been challenged with a characterised strain of scrapie or with cattle BSE.

## **Epidemiology**

From the start of the BSE epidemic, case data have been collected which have been subjected to extensive epidemiological analysis. This work is continuing in a collaborative project between VLA and Massey University, New Zealand.

Epidemiological studies with sheep aim to identify risk factors that are important in the introduction and maintenance of scrapie infection and disease in sheep flocks. The studies are being undertaken using commercial flocks which have been recruited for the purpose. Data from these studies will be used to construct a mathematical model that describes the dynamics of scrapie between flocks and how this is affected by genotype. This will allow the most likely routes of transmission to be identified. The model will also provide an estimate of the current prevalence of scrapie and the impact of different control policies.

## **Pathogenesis**

### **(a) BSE in sheep**

Two complementary projects involving sheep of different breeds and genotypes are exploring the pattern of tissue infectivity that develops when sheep are challenged orally with BSE infected cattle brain.

The question of whether BSE changes in appearance when passed from one sheep to another is also being examined.

### **(b) Scrapie in sheep**

Several studies are exploring the pathogenesis of natural scrapie, each focusing on different aspects. The effect of genotype on PrP distribution and vacuolation is being examined with emphasis on the identification of potential carrier states and sub-clinically infected sheep in flocks heavily infected with scrapie.

Because PrP<sup>sc</sup> accumulates in lymphoid tissue it has been suggested that the detection of PrP<sup>sc</sup> in tonsils could provide a useful means of monitoring the incidence of scrapie in flocks. However, more needs to be known about the time after infection at which PrP<sup>sc</sup> starts to accumulate in the

tonsils and whether this is consistent for different breeds and genotypes. Early evidence suggests that it is not. A study aimed at exploring this issue is underway.

### (c) Studies at the cellular level

Several studies at the cellular level are underway. One is exploring the cell types that sustain PrP accumulation in the intestinal mucosa, the lymphoreticular system and the peripheral nervous system in BSE in mice and cattle. A second is looking at whether lysosomes are involved in the first identifiable changes that occur in neuronal cell bodies during scrapie infection.

A further study is underway which is looking at the effect of the BSE agent on bovine cell lines.

## **Diagnostics**

This remains a high priority area as the tests in use currently have been validated only on CNS from animals at the late stage of disease development.

MAFF's current programme includes work in the following areas:

### **a) Production of tissues for test development**

Tissues and body fluids from many of MAFF's projects are banked for future use. However, in addition there are some projects with the specific aim of producing tissues for this purpose. Both cattle and sheep tissues are being produced. Tissue archives are referred to in section 3 of this document.

### **b) Evaluation of tests developed elsewhere**

A long term project at the VLA provides funding for the further development and evaluation of tests originally produced elsewhere. This part of the programme includes the evaluation of the ICE test.

### **c) Search for new markers**

### **d) Protein structure**

MAFF is funding one project where an assay dependent on the conformation of the prion protein is being developed and tested.

### **Strain typing**

Part of MAFF's programme has been aimed at characterising different strains of TSEs. Most of the strain typing studies with BSE have used British isolates. A new project which is due to begin shortly will determine whether Swiss BSE is different from British BSE.

Strain typing, both to characterise the strains of scrapie encountered in natural infection and to look for BSE in sheep in the national UK flock is a feature of many of the sheep projects supported.

### **Inactivation of the TSE agent**

MAFF is funding a number of projects to see whether inactivation can be achieved using new technology. These include the use of high pressure steam, a combination of autoclaving and exposure to alkali and highly thermostable proteolytic enzymes. In addition there is a study exploring how the process of tallow separation and solvent extraction affect PrPsc.

### **Department of Health**

TSE Research Programme contact: Dr John Stephenson [john.stephenson@doh.gsi.gov.uk](mailto:john.stephenson@doh.gsi.gov.uk)  
<http://www.doh.gov.uk/>

The Department's programme of research on vCJD was initiated in 1996, and to date over £12m have been committed to this programme, in addition to the core support for the CJD surveillance Unit in Edinburgh. The DH research budget for 2001/02 has been increased to £5.8m per annum, with an additional £1m per annum being allocated for diagnostic development in conjunction with MAFF. The research support is targeted at six main areas,

- epidemiology,
- strain typing,
- diagnostics,
- therapeutics,
- safety of blood and blood products
- decontamination of surgical instruments.

The Department of Health is supporting TSE research on the following topics:

- The provision of high level containment facilities at the Institute of Animal Health Compton and St Mary's Hospital Medical School, London for the production and use of transgenic mice for transmission studies of TSEs
- Strain typing and transmission studies using the new animal facilities to determine whether vCJD derives from oral or environmental exposure to BSE-contaminated food
- An extension of the existing surveillance for all cases of CJD, to include paediatric and young adults, and to confirm sporadic CJD variants
- Case control studies to ascertain whether certain lifestyles, diets or medical treatments increase the risk of developing CJD
- Retrospective analysis of previous deaths from dementia-like diseases and a current three year study of autopsies in the Oxford area, to ensure that no cases of CJD have been missed
- Studies on whether occupational, environmental exposure or physical contact with other CJD patients leads to a higher risk of developing CJD
- An analysis of data sets from the UK, US and France, relating to the development of CJD in some patients treated with human pituitary growth hormone.
- Surveillance of haemophiliacs to ascertain if they are at increased risk of contracting CJD or vCJD.
- Development of new clinical and laboratory diagnostic tests for CJD.
- Investigations of possible TSE infectivity in blood and whether infectivity can be transmitted by transfusion or the use of blood products
- Development of a sensitive and specific diagnostic test for the detection of TSEs in blood and blood products
- Surveillance of all cases of intellectual and neurological deterioration in children, as an “early warning system” to detect any future changes in the patterns of vCJD.
- Several research programmes have recently been initiated to evaluate novel mechanisms for inactivating TSEs and for decontaminating surgical instruments.
- Mathematical modelling to predict the future size of the epidemic.
- Development of therapeutic drugs to treat patients.
- Studies of detectable PrP<sup>Sc</sup> in tissues collected prospectively and retrospectively.

A budget of £3.5m per annum has been allocated to the Policy Research Programme to enable the commissioning of this research. In addition, there are further substantial contributions from other Funders such as MAFF, MRC, BBSRC, DETR, FSA and the Wellcome Trust.

## **Biotechnology and Biological Sciences Research Council**

TSE Research Programme contact: Mrs Meg Wilson

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<http://www.bbsrc.ac.uk/>

BBSRC have funded a programme of research on TSEs over many years. The fourth phase of the programme was launched in 1998 and builds on BBSRC's earlier investment to ensure that the skills and expertise that have been built up are fully exploited. The programme aims to encourage collaborations between centres with well-established expertise in TSE research and other groups with complementary expertise that are able to bring new approaches to bear on the important questions.

Current BBSRC objectives for TSE research are:

- the nature of the infectious agent;
- the molecular basis of strain variation in scrapie;
- the use of transgenic animal models for studies of the nature of the infectious agent, transmission and the species barrier;
- the molecular basis of pathogenesis of the TSE agents;
- the genetic control of host animal susceptibility.

It is intended that there will be further calls for proposals in 2001. A schedule will be published when available. In addition, BBSRC will participate in relevant joint calls by the Funders, which have been identified by the Joint Funders' Group, on specific aspects of TSE research.

## **Medical Research Council**

Contact details for MRC Programme Managers:

Dr Karen Finney Tel: 020 7636 5422

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The MRC has funded basic research into spongiform encephalopathies since the late 1970s, when it began studying the UK epidemiology of naturally occurring CJD. In May 1996, the MRC and the Department of Health responded to the announcement of a possible link between BSE and vCJD by issuing a joint call for high quality proposals. These would contribute through basic or applied research to the key issues relevant to TSEs and human health. These included

diagnosis in life, the aetiology and epidemiology of CJD, disease progression, and treatment and prevention.

The Council funds research into the aspects of TSE disease that relate to human health. Its TSE research budget has risen from £3.4M in 1998/99 to an estimated £6.5M in 1999/00. The broad MRC TSE research portfolio has been developed in partnership with DH, and together with BBSRC, MAFF, and the FSA as part of a co-ordinated programme to respond to public health and research needs in the field.

### **The MRC TSE research portfolio**

The MRC has committed £22 million between 1999 and 2004 to fund TSE research across the spectrum from basic biological studies, through to applied clinical research, epidemiology and risk assessment. A dedicated MRC Prion Unit, directed by Professor John Collinge, is the focus of MRC funded TSE research. The Unit was set up in 1998 to create an international centre of excellence. It plays a key role in linking basic science to clinical research and focuses principally on human prion diseases. Overall, the MRC supports the work of more than 50 expert scientists who are currently working on 26 projects in locations throughout the UK. Their aim is to gain a better understanding of TSE disease, to assess the danger it poses, and to work out how it might be tackled. Molecular genetic studies, animal models, and molecular and cellular functional analysis are among the tools being used to investigate:

- The relationship between vCJD and BSE and human prion disease susceptibility.
- Inter-mammalian species barriers to prion transmission
- The mechanism of TSE transmission, PrP replication, and disease progression.
- The molecular structure of normal and disease-causing prions and the biological function of PrP.
- Approaches to earlier diagnosis and therapy.

A complete list of all MRC's TSE research can be found on the MRC website at [http://www.mrc.ac.uk/tse\\_c.html#Current](http://www.mrc.ac.uk/tse_c.html#Current).

The Council encourages innovative research proposals; particularly those that involve collaboration between centres with well-established expertise in TSEs research, or between such centres and other groups with complementary expertise that are able to bring new approaches to bear on the important questions. Favourable consideration is given to proposals that involve

collaboration with centres of excellence outside the UK which would strengthen the UK's own capability in the field.

### **Research opportunities and needs**

TSE research remains an area of high priority for the MRC and new approaches are being considered to strengthen the UK's capability in the field. Two key goals are improved diagnostic tests and new treatments for TSEs, and in particular for vCJD. Priority areas include:

- the biological and epidemiological relationship between CJD and BSE;
- epidemiological modelling of CJD
- the analysis, perception and communication of risk in relation to CJD
- improving understanding of how TSEs cause disease, particularly during its very early stages
- early disease progression and diagnosis in life including *in vivo* imaging approaches
- integrated molecular, epidemiological and clinical approaches to understanding the cause(s) of sporadic CJD and the relationship with atypical dementias
- molecular, genetic, cellular and functional approaches to elucidating mechanisms of TSEs transmission, PrP replication, pathogenesis and clinical progression
- development and improvement of animal models and cell culture systems;
- the biological function of normal PrP
- the molecular structure of the prion proteins
- development of non-invasive pre-clinical tests for human TSE diseases, particularly vCJD e.g. a blood test or throat swab
- investigating contamination/decontamination of surgical instruments
- rational approaches to developing therapy including vaccine based treatment approaches.

### **Food Standards Agency**

Contact: Dr Stephen N. Dixon

Meat Hygiene Research Co-ordinator

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FSA Website: <http://www.foodstandards.gov.uk>

A major element of the Agency's overall objective is the protection of public health from TSE's in food. In carrying this objective forward the Agency co-ordinates its research with that of other Government departments, (particularly the Department of Health, and the Ministry of Agriculture Fisheries and Food).

The information generated by the research programme is used, in a risk based approach, to develop legislation. This, together with strict enforcement of controls, ensures that the consumer can have confidence in the safety of food.

The Agency's policy aims to:

- Ensure that BSE (TSE) controls reflect risk, are proportionate to the risk and are based on the latest scientific knowledge;
- that enables the efficient and effective enforcement of the BSE controls, and
- provides accurate, independent information on BSE (TSE) issues and the effectiveness of enforcement.

The Agency's current research priorities are:

- Risk assessment on BSE in Sheep.
- Studies to establish whether the BSE prion is present in the milk of dairy cows infected with BSE.
- Investigation of neural embolism and the potential for visceral dissemination following captive bolt stunning and slaughter of cattle and sheep.
- Large scale monitoring of material from slaughtered sheep to establish the prevalence of TSEs in the national flock
- Large scale monitoring of material from slaughtered sheep with a diagnostic test capable of distinguishing between BSE and Scrapie.
- Real time diagnostic tests of proven sensitivity, reproducibility, repeatability and reliability for the detection of TSEs at clinical and sub-clinical levels in sheep and cattle.
- Studies to test the validity of the assertion that the process employed in the production of sausage casings ensures that lymphoid tissue and, with it, any risk of infectivity is removed.
- Research to assess the risk to the consumer from the use of sheep intestines for sausage casings.
- The development of methods for the detection of the presence of Mechanically Recovered Meat (MRM), in meat products.
- Extend the ongoing programme of tissue infectivity studies to assess whether the most sensitive tests for material going into the food chain have been utilised and whether the sample sizes are large enough for the tests that have been done.

## Future calls for research

All funders are currently involved in producing a call for research proposals on the development of diagnostic tools for TSE.

## 2. How these activities fit into the principal areas of the European Action Plan on TSE Research

The list of abstracts of research projects funded by UK funders will be inserted here. These abstracts will be categorised according to the EU categories specified in the template on which the rest of this document is based.

A list of projects supported by MAFF, BBSRC, MRC, DH and the FSA can be found on the MRC website at [http://www.mrc.ac.uk/tse\\_2c.htm](http://www.mrc.ac.uk/tse_2c.htm). The list is regularly updated and contains links for abstracts on each project currently supported by the UK funders.

## 1. Clinical epidemiological and social research on human Ses

### 1.1. Compare agent strains recovered from vCJD patients with BSE and 'normal' CJD, GSSS and FFI strains

<b>Project Title: Phenotypic variation in CJD: a clinical, pathological and molecular biological study.</b>	<b>Contact: Dr J W Ironside, National CJD Surveillance Unit, Edinburgh</b>
<b>Abstract:</b> Sporadic Creutzfeldt-Jakob disease (CJD) is the commonest human spongiform encephalopathy. The CJD Surveillance Project in the UK has recently extended the phenotype of this disorder by the identification of (1) a new variant of CJD in young patients, and (2) a subset of older sporadic CJD patients with accumulation of protease-resistant prion protein (PrPRES) in the brainstem and spinal cord. Phenotypic variation is recognised in animal spongiform encephalopathies, and has been attributed to host (genetic) and agent (PrPRES) properties. In this study, factors which determine disease phenotype in CJD will be investigated in the unique collection of sporadic, new variant, familial and iatrogenic cases of CJD and control cases in the CJD Surveillance Project case control study; (2) regional localisation and quantitation of PrPRES deposits in the CNS (by immunocytochemistry and the histoblot technique); (3) PRNP genotype; (4) PrPC expression in the CNS and other organs (determined by in situ hybridisation). Agent factors will be investigated by Western blot analysis of PrPRES species in different brain regions; this will be supplemented by the results of experimental transmission studies in mice (which form a separate application). These studies will allow a fuller understanding of host and agent factors in relation to disease phenotype and pathogenesis and will provide new perspectives on postulated human CJD agent strains, an important consideration in relation to the potential effects of the BSE in man.	

<b>Project Title: Strain characterisation of the Creutzfeldt-Jakob disease agent by transmission to mice</b>	<b>Contact: Dr M Bruce IAH Edinburgh</b>
<b>Abstract: Abstract is currently unavailable</b>	

***1.2 The incidence (including re-evaluation of previously diagnosed CJD cases), geographical distribution and role of specific risk factors (genotype, diet exposure, environment)***

<b>Project Title: To undertake prospective multisource surveillance of all cases of PIND occurring in children in the UK</b>	<b>Contact: Professor C Verity, University of Cambridge</b>
<b>Abstract: Abstract is currently unavailable</b>	

<b>Project Title: A cohort study of risk of CJD in people in the UK treated with human pituitary growth hormone</b>	<b>Contact: Professor A Swerdlow, Institute for Cancer Research, Surrey</b>
<b>Abstract: Abstract is currently unavailable</b>	

<b>Project Title: Predicting future numbers of cases of vCJD</b>	<b>Contact: Professor P Smith, LSHTM, London</b>
<b>Abstract: Abstract is currently unavailable</b>	

<b>Project Title: A case control study of risk factors for sporadic, familial and new variant CJD.</b>	<b>Contact: Professor R.G. Will, NCJDSU, Edinburgh</b>
<b>Abstract: Abstract is currently unavailable</b>	

<b>Project Title: Survey of Oxford autopsy brain tissue for evidence of Creutzfeldt-Jakob disease</b>	<b>Contact: Dr. M. Esiri, University of Oxford</b>
<b>Abstract: Abstract is currently unavailable</b>	

<b>Project Title: Development of a model for neuropathological surveillance of the elderly population for CJD</b>	<b>Contact: Professor J. Lowe, University of Nottingham</b>
<b>Abstract: Abstract is currently unavailable</b>	

<b>Project Title: Possible under ascertainment of new variant CJD: a systematic retrospective study.</b>	<b>Contact: Dr. R. Salmon, University of Wales – Cardiff</b>
<b>Abstract: Abstract is currently unavailable</b>	

<b>Project Title: A comprehensive examination of the Corsellis brain collection to identify any possible unidentified cases of prion disease</b>	<b>Contact: Dr. S. Gentleman, Imperial College, London</b>
<b>Abstract: Abstract is currently unavailable</b>	

<b>Project Title: Study of the incidence of detectable PRP<sup>Sc</sup> in human tonsil tissue in the UK</b>	<b>Contact: Professor J. Collinge MRC Prion Unit</b>
<b>Abstract: Abstract is currently unavailable</b>	

<b>Project Title: An epidemiological investigation into exposure of the population of Devon and Cornwall to the bovine spongiform encephalopathy agent between 1986 and 1998</b>	<b>Contact: Dr. D. Hilton, Derriford Hospital, Plymouth</b>
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<b>Project Title: Immunocytochemical testing for disease-associated prion protein in lymphoid tissues (tonsil and appendix) from anonymised individuals: 1990-present</b>	<b>Contact: Professor J. Ironside, NCJDSU, Edinburgh</b>
<b>Abstract: Abstract is currently unavailable</b>	

<b>Project Title: Neuropathological spectrum of human TSE': relationship with atypical dementias: a national retrospective review.</b>	<b>Contact: Professor J. Ironside, NCJDSU, Edinburgh</b>
<b>Abstract: Abstract is currently unavailable</b>	

<b>Project Title: Neuropathological spectrum of human TSE': relationship with atypical dementias: a national retrospective review. (Leeds element</b>	<b>Contact: Dr. L. Bridges, General Infirmary, Leeds</b>
<b>Abstract: Abstract is currently unavailable</b>	

<b>Project Title: Neuropathological spectrum of human TSE': relationship with atypical dementias: a national retrospective review. (Oxford element)</b>	<b>Contact: Professor M. Esiri, University of Oxford</b>
<b>Abstract: Abstract is currently unavailable</b>	

<b>Project Title: Neuropathological spectrum of human TSE': relationship with atypical dementias: a national retrospective review. (Royal London element)</b>	<b>Contact: Dr. J. Geddes, Queen Mary and Westfield College</b>
<b>Abstract: Abstract is currently unavailable</b>	

<b>Project Title: Neuropathological spectrum of human TSE': relationship with atypical dementias: a national retrospective review. (Institute of Psychiatry element)</b>	<b>Contact: Professor P. Lantos, Kings College</b>
<b>Abstract: Abstract is currently unavailable</b>	

<b>Project Title: Neuropathological spectrum of human TSE': relationship with atypical dementias: a national retrospective review. (Nottingham element)</b>	<b>Contact: Professor J. Lowe, University of Nottingham</b>
<b>Abstract: Abstract is currently unavailable</b>	

<b>Project Title: Neuropathological spectrum of human TSE': relationship with atypical dementias: a national retrospective review. (Aberdeen element)</b>	<b>Contact: Dr. J. McKenzie, University of Aberdeen</b>
<b>Abstract: Abstract is currently unavailable</b>	

<b>Project Title: Neuropathological spectrum of human TSE': relationship with atypical dementias: a national retrospective review. (Manchester element)</b>	<b>Contact: Dr. D. Mann, University of Manchester</b>
<b>Abstract: Abstract is currently unavailable</b>	

<b>Project Title: Neuropathological spectrum of human TSE': relationship with atypical dementias: a national retrospective review. (Romford element)</b>	<b>Contact: Dr. A. Marshall, Oldchurch Hospital, Romford</b>
<b>Abstract: Abstract is currently unavailable</b>	

<b>Project Title: Neuropathological spectrum of human TSE': relationship with atypical dementias: a national retrospective review. (Royal Free London element)</b>	<b>Contact: Dr. J. McLaughlin, Royal Free Hospital</b>
<b>Abstract: Abstract is currently unavailable</b>	

<b>Project Title: Neuropathological spectrum of human TSE': relationship with atypical dementias: a national retrospective review. (Bristol element)</b>	<b>Contact: Dr. T. Moss, Frenchay Hospital, Bristol</b>
<b>Abstract: Abstract is currently unavailable</b>	

<b>Project Title: Neuropathological spectrum of human TSE': relationship with atypical dementias: a national retrospective review. (Cardiff element)</b>	<b>Contact: Dr. J. Neal, University of Wales, Cardiff</b>
<b>Abstract: Abstract is currently unavailable</b>	

<b>Project Title: Neuropathological spectrum of human TSE': relationship with atypical dementias: a national retrospective review. (Glasgow element)</b>	<b>Contact: Dr. J. Nicoll, University of Glasgow</b>
<b>Abstract: Abstract is currently unavailable</b>	

<b>Project Title: Neuropathological spectrum of human TSE': relationship with atypical dementias: a national retrospective review. (Newcastle element)</b>	<b>Contact: Dr. R. Perry, University of Newcastle</b>
<b>Abstract: Abstract is currently unavailable</b>	

<b>Project Title: Neuropathological spectrum of human TSE': relationship with atypical dementias: a national retrospective review. (Institute of Neurology element)</b>	<b>Contact: Professor F. Scaravilli, Institute of Neurology, UCL</b>
<b>Abstract: Abstract is currently unavailable</b>	

<b>Project Title: Neuropathological spectrum of human TSE': relationship with atypical dementias: a national retrospective review. (Southampton element)</b>	<b>Contact: Professor R. Weller, University of Southampton</b>
<b>Abstract: Abstract is currently unavailable</b>	

<b>Project Title: Neuropathological spectrum of human TSE': relationship with atypical dementias: a national retrospective review. (Cambridge element)</b>	<b>Contact: Dr. J. Xuereb, University of Cambridge</b>
<b>Abstract: Abstract is currently unavailable</b>	

<b>Project Title: Neuropathological spectrum of human TSE': relationship with atypical dementias: a national retrospective review. (Hull element)</b>	<b>Contact: Dr. D. Crooks, Hull Royal Hospital</b>
<b>Abstract: Abstract is currently unavailable</b>	

<b>Project Title: Neuropathological spectrum of human TSE': relationship with atypical dementias: a national retrospective review. (Belfast element)</b>	<b>Contact: Dr. I. Allen, Health and Personal Social Services, Northern Ireland, Belfast</b>
<b>Abstract: Abstract is currently unavailable</b>	

**1.3 The process of identification of suspected cases and the sensitivity of the surveillance system**

**1.4 The research on risk perception of the population in relation to prion disease**

## 2. The infectious agent and its mechanisms of transmission

### 2.1. The characterisation of the agent

<b>Project Title: Structural studies of recombinant disease-associated mutant prion proteins in lipid membranes</b>	<b>Contact: Dr T J T Pinheiro, University of Warwick</b>
<b>Abstract:</b> It is proposed to characterise the binding kinetics and equilibrium properties of recombinant prion proteins (PrP) to lipid membranes. Wild-type PrP and variant proteins containing single point mutations known to be associated with the development of prion diseases are purified from high-yield expression in <i>E. coli</i> . Binding of PrP will be monitored by surface plasmon resonance (SPR) spectroscopy using model membranes of biologically-relevant lipid composition, which will mimic the raft domains in the plasma membrane where PrP molecule are thought to accumulate. The structure of PrP and disease-associated variants in lipid membranes will be determined by attenuated total reflection Fourier transform infrared (ATR FTIR) spectroscopy. Several mutants associated with prion diseases are ready for these structural studies, and particular emphasis will be given to the GSS-mutant A117V associated with a transmembrane form of PrP. The role of membrane-associated conformations of PrP in the conversion process of PrP-C into PrP-Sc will be investigated by circular dichroism and FTIR spectroscopy, and PrP fibril formation will be monitored by polarised light microscopy and electron microscopy.	

<b>Project Title: Characterisation of the Molecular Processing of the Prion protein.</b>	<b>Contact: Dr N M Hooper, University of Leeds</b>
<b>Abstract:</b> Prion diseases of humans and animals, including transmissible spongiform encephalopathies (TSEs), are characterised by the conversion of the normal cellular form of the prion protein (PrP <sup>C</sup> ) into an insoluble, protease-resistant abnormal form (PrP <sup>Sc</sup> ). Cell lines stably expressing mouse PrP, including forms of the protein containing mutations associated with human prion disorders and TSEs, will be established. These cell lines will then be used to study the post-translational processing of wild type and mutant forms of PrP. This will include an examination of the intracellular targeting of PrP, in particular its association with glycosphingolipid/cholesterol-rich membrane microdomains and with proteins within these microdomains. The possibility of disrupting these associations as a way of preventing the formation of PrP <sup>Sc</sup> will be considered. We will also identify and characterise at the molecular level the protease that cleaves PrP within the neurotoxic region of the protein thereby preventing formation of PrP <sup>Sc</sup> . This novel protease will be purified and its cDNA cloned and sequenced. The distribution and activity of this enzyme in normal individuals and those susceptible to prion disorders will be examined, and the possibility of altering the activity of the enzyme investigated as a possible therapeutic approach. This project will not only provide valuable insight into the post-translational processing of PrP, but will also identify possible therapeutic targets with which to treat prion disorders of humans and animals.	

<b>Project Title: Structural investigations of the release factors eRF-1 and eRF-3 and the prion-like properties of Sup35 (yeast eRF-3)</b>	<b>Contact: Dr D Barford, Insitute of Cancer Research</b>
<p><b>Abstract:</b> The structures of eRF-1, eRF-3 and soluble Sup35 will be determined by X-ray crystallography and the Sup35 fibres will be examined using X-ray fibre diffraction. These structures will allow insight into the mechanism of translation termination, specifically tRNA mimicry and mRNA stop codon recognition by eRF-1 and its TP-dependent regulation mediated by eRF-3. Soluble Sup35 undergoes a conformational change to form fibres in an auto-catalytic fashion that is concentration dependent and seeded by preformed fibres, similar to human prion proteins. The structures of soluble Sup35 and its fibres will provide an understanding of this process and be relevant to prion proteins that are associated with transmissible spongiform encephalopathies.</p>	

***2.2. The mechanisms of propagation, transport and pathogenesis including elucidation of possible common links with neurodegenerative diseases***

<b>Project Title: SE1428</b> <b>Pathogenesis studies of experimental BSE in sheep</b>	<b>Contact: Dr M Bruce IAH, Edinburgh</b>
<p><b>Abstract:</b> An investigation into the tissue distribution of BSE infectivity in experimentally challenged sheep.</p> <p>Genetically selected sheep that are resistant to natural scrapie but susceptible to BSE will be challenged by the oral route. Groups of sheep will be sacrificed after 6, 12, 18 and 24 months and when showing clinical signs of BSE. A range of tissues will be collected and levels of infectivity will be estimated by mouse bioassay.</p> <p>The work will lead to an understanding of BSE pathogenesis in sheep and indicate what tissues contain high levels of infectivity.</p> <p>This information is essential to assess possible risks to humans, should BSE have been recycled into the national sheep flock.</p> <p>The project will be reviewed at the end of year 3.</p>	

<p><b>Project Title: SE1928</b>  <b>PrP<sup>Sc</sup> distribution and kinetics in lymphoid tissues of sheep with natural scrapie: effects of PrP genotype and strains</b></p>	<p><b>Contact: M Jeffrey</b>  <b>VLA, Lasswade</b></p>
<p><b>Abstract:</b> This study is part of a larger EU Fair-funded proposal. Recently, a new technique has been developed for the diagnosis of scrapie in sheep based on the immunohistochemical detection of PrP<sup>Sc</sup> in tonsil biopsies. With this technique, scrapie could be diagnosed in Texel sheep of highly susceptible PrP genotypes years before the onset of clinical signs. At present, no data are available on the time of onset of PrP<sup>Sc</sup> accumulation in the tonsils of sheep carrying other PrP genotypes or originating from other European flocks. Further studies are required to evaluate the potential of PrP<sup>Sc</sup> detection in tonsils for a surveillance system designed to monitor the incidence of scrapie-infected sheep and in identifying pre-clinically infected sheep. Such an improved surveillance system would be essential if breeding programmes for the control of scrapie are to be implemented in the EU member states.</p> <p>The purpose of the present proposal is to develop a standardised, sensitive immunohistochemical method for the demonstration of PrP in peripheral tissues of scrapie-infected sheep and to determine the onset of PrP accumulation in tonsil in individual Suffolk, Cheviot or Texel sheep carrying susceptible or resistant genotypes. We will also determine the cell types and sub-cellular sites in which PrP may accumulate in peripheral lymphoid tissue of 171QQ Suffolk sheep and to determine the extent and distribution of PrP<sup>Sc</sup> and PrP<sup>res</sup> accumulation in lymphoreticular tissues in Suffolk, Cheviot and Texel sheep each carrying highly susceptible PrP genes. To determine whether different scrapie strains are present in naturally diseased flocks of different breeds in Scotland, France and the Netherlands, mouse bioassay will be incorporated into the study design. However, the results of such strain-typing experiments will run longer than the planned life of this project.</p>	

<p><b>Project Title: SE1937</b>  <b>Sub-cellular studies of intestine, lymphoreticular tissues and peripheral nervous system in the murine and bovine BSE</b></p>	<p><b>Contact: Martin Jeffrey, VLA, Weybridge</b></p>
<p><b>Abstract:</b> The sub-cellular pathology features of the intestine and the possible cell types which may sustain PrP accumulation in the intestinal mucosa, lymphoreticular tissues and peripheral nervous system will be investigated in murine and bovine BSE. Previously developed methods for detection of PrP by electron microscopy will be further developed in order to detect PrP in peripheral tissues.</p> <p>Immunohistochemical techniques and electron microscopy will be used to determine the distribution of nerve endings in gut mucosa and lymphoid tissues. The distribution of PrP will be related to nerve endings and nerve cell bodies, and to functional and morphological alterations in lymphoid-series cells determined in project SE1909. These studies will aid understanding of the early cellular events and pathogenesis of the transmissible spongiform encephalopathies and will try to elucidate the route by which PrP (and infectivity) may gain access to the CNS.</p> <p>The studies are part of ongoing pathogenesis studies of BSE/scrapie performed at CVL.</p> <p>An understanding of the early events following BSE infection and, in particular, characterisation of the pathways involved in the infection gaining access to the CNS may help development of disease control and prevention strategies.</p>	

<p><b>Project Title: SE1940</b>  <b>Electron microscopic studies of CNS changes in sheep scrapie</b></p>	<p><b>Contact: Dr Martin Jeffrey, Dr Marion M Simmons</b>  <b>VLA, Weybridge/Lasswade</b></p>
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**Abstract:**

We wish to determine the sites of subcellular localisation of PrP in natural sheep scrapie, and identify any morphological changes which may be consistently associated with this protein accumulation. In part, it is necessary to perform such examinations because mouse models examined (even if they are representative as regards the changes they do show) do not show entirely the same range of changes as are seen in sheep. Any morphological changes will be compared with previous studies performed by us in BSE (SE 1906) and rodent models. We also wish to investigate lysosomal involvement in vacuolation (as indicated from observations of naturally occurring BSE (SE 1906)) and will develop enzyme and ultrastructural immunogold methods for the detection of lysosomal markers.

This proposal addresses the difficulty of diagnosing natural scrapie in the same way as BSE, i.e. using the presence of vacuolar pathology in a limited number of neuroanatomical sites as the principal diagnostic criterion, without recourse to more extensive neurohistological examination. A detailed study of the ultrastructural changes associated with natural disease will establish whether there are any consistent, non-vacuolar features which would be of potential diagnostic value

**Project Title: SE1943**  
**Studies of PrP localisation in murine spleen and vascular endothelium**

**Contact: M Jeffrey**  
**VLA, Lasswade**

**Abstract:** Expression of PrP<sup>C</sup> within peripheral viscera and the P.N.S. is necessary for scrapie infection to gain access to the CNS, and PrP accumulation is central to the pathogenesis of the TSEs. PrP accumulation and SAF are found in the spleen of scrapie-affected sheep or mice and infection is replicated in these tissues. Cerebrovascular amyloid formation in natural sheep scrapie follows primary endothelial accumulation of PrP, suggesting that systemic infection represents a part of the pathogenesis of the natural disease. We wish to determine the subcellular localisation of PrP in a murine scrapie model of cerebrovascular amyloidosis. We also wish to increase the sensitivity of previously developed immunogold methods to determine the cell types which contain PrP in excess in the spleen of mice, how these cells process the PrP (in particular, whether they produce the PrP in excess or merely accumulate it from the extracellular environment) and to determine how infection might be presented by the spleen to the P.N.S. We would also like to determine where PrP<sup>C</sup> is located on splenic cells. The methods that we have already developed for detection of PrP at sub-cellular levels in the CNS of scrapied mice are unlikely to be sufficiently sensitive to detect PrP within the spleen, tonsil or Peyer's patches. It is therefore important that we improve the sensitivity of detection of PrP in a well understood system in mice before we attempt to progress further our understanding of the natural disease. Ultimately, we would wish to determine for natural scrapie of sheep, how infectivity reaches the CNS. These studies will improve our understanding of the pathogenesis of the TSEs and may help inform on control strategies.

<b>Project Title: SE1944</b> <b>Studies of genotype, pathology, PrP and scrapie carrier status of sheep in flocks heavily infected with scrapie</b>	<b>Contact: M Jeffrey; AM Clark</b> <b>VLA, Lasswade</b>
<p><b>Abstract:</b></p> <p>The diagnostic hallmark of scrapie is vacuolation, yet the relationship between vacuolation profile and clinical disease, and strains in natural sheep scrapie, is ambiguous. Control strategies based on CNS vacuolation or genotype alone are unlikely to be successful. In the proposed study we will monitor genotype, vacuolation profile, the distribution patterns of PrPsen and PrPres in ileum, tonsil, spleen, mesenteric lymph node and in brain of cull sheep, sheep dying acutely without previous nervous signs (found dead) and in clinically diseased animals within heavily infected flocks of sheep. The principal objective is to recognise potential carriers and to provide a baseline for the histological or immunohistochemical recognition of such carriers which might be employed more generally within the sheep industry as part of a control or eradication programme. The relationship between vacuolation, and patterns of PrP accumulation and genotype will also be determined.</p>	

<b>Project Title: The genetic basis of cerebellar ataxia in mice lacking the prion protein</b>	<b>Contact: Dr D W Melton,</b> <b>University of Edinburgh</b>
<p><b>Abstract:</b> The prion protein (PrP) is a cell surface glycoprotein which is expressed at high levels in the mammalian brain. The first two PrP-deficient mouse strains produced did not display an overt phenotype. Subsequently, two further strains of PrP null mice were generated with adult-onset cerebellar ataxia. The aim of this project is to study the normal function of the prion protein by investigating the genetic basis for cerebellar ataxia. We will determine the time course of the development of ataxia through a linked series of behavioural, immunocytochemical and anatomical and anatomical studies, using co-isogenic wild-type and non-ataxic strains as controls. The structure of the Prnp locus in the two ataxic strains differs from that in the original non-ataxic lines in that the entire PrP coding region and part of the second intron has been removed and a different selectable marker has been inserted into the locus. Two complementary approaches will be used to determine the genetic basis for the ataxia. Firstly, we will carry out additional PrP gene targeting to determine whether the ataxic phenotype is due to the lack of the PrP coding region, or to the loss of a regulatory element from the Prnp locus, or to a direct effect of the selectable marker inserted into the Prnp locus on an adjacent gene. Secondly, the ability of different PrP transgenes to correct the ataxic phenotype will be assessed.</p>	

**Project Title: Roles of a transmembrane form of the prion protein and the prion-like protein, Doppel, in neurodegeneration**

**Contact: Dr N M Hooper ,  
University of Leeds**

**Abstract:** The infectious forms of the prion diseases are characterised by a conformational change in the cellular isoform of the prion protein (PrPC) to the scrapie isoform (PrPSc). Recently, certain inherited PrP mutations have been shown to cause neurodegeneration in the absence of PrPSc, utilising instead a novel transmembrane form CtmPrP. CtmPrP also influences the effectiveness of PrPSc in causing neurodegeneration in transmitted cases. Studies with PrP knockout mice have uncovered a PrP-like protein, termed Doppel (Dpl), which shares structural similarities with PrP and which may be involved in the neurodegeneration associated with prion diseases. The aims of this project are to determine how CtmPrP causes neurodegeneration, and to characterise Dpl at the molecular and cellular levels in order to assess its involvement in the neurodegeneration associated with prion diseases and/or other dementias. The objectives of this study are: (i) to express in mammalian cells mutants of murine PrP which predispose the protein to form CtmPrP, and compare their subcellular location, membrane anchorage and metabolism with PrPC; (ii) to determine the copper binding capacity and superoxide dismutase activity of the CtmPrP mutants in order to establish whether this form of the protein causes neurodegeneration by altering cellular copper homeostasis and/or antioxidant activity; (iii) to clone and express in mammalian cells the cDNAs encoding human and murine Dpl, and generate antibodies against the proteins; (iv) to determine the mode of membrane anchorage and subcellular location of Dpl, and whether the protein undergoes a conformational change or has an antioxidant function; (v) to investigate the effect of Dpl expression on the expression and properties of PrP, and to examine human brain samples for the presence of Dpl. The results from this study will lead to a better understanding of the molecular and cellular processing of PrP, and the role of CtmPrP and Dpl in neurodegeneration.

<b>Project Title: Studies on the expression of prion protein in the megakaryocytic/platelet lineage</b>	<b>Contact: Dr P Harrison, UCL, London</b>
<p><b>Abstract:</b> There is increasing support for the role of prions in the pathogenesis of transmissible spongiform encephalopathies (TSEs) e.g. BSE and nvCJD. The infectious prion (PrP<sup>Sc</sup>) is a modified isoform of an endogenous CNS protein termed prion protein (PrP<sup>C</sup>). Remarkably, blood platelets share a number of biochemical properties with neurones including PrP<sup>C</sup> expression. Platelets express PrP<sup>C</sup> as a GPI anchored membrane protein which is predominantly found within an internal granular pool. It is hypothesized that megakaryocytes and platelets could also carry PrP<sup>Sc</sup> particularly as the rogue protein has been detected within both bone marrow and spleen within infected individuals or animals. The detection of PrP<sup>Sc</sup> could have important implications not only for the potential transmission of TSEs via blood transfusion and blood derived products but could provide a unique possibility of developing a simple non-invasive screening test. It is proposed to study in detail the expression of prion protein within bone marrow stem cells, megakaryocytes and blood platelets. Preliminary data utilising antibodies to native PrP<sup>C</sup> suggests that blood platelets express significant levels of PrP<sup>C</sup> not only on the plasma membrane but within intracellular stores (i.e. the alpha granules) which can be detected either via Western blotting of platelet lysates/releasates or by a rapid flow cytometric assay. By application of an antibody that detects the rogue protein PrP<sup>Sc</sup> (from Prionics) it will be possible to determine whether stem cells, megakaryocytes and even platelets express detectable levels of PrP<sup>Sc</sup> within blood samples from individuals with CJD, nvCJD, mouse models of disease and even BSE infected animals (e.g. cattle). These studies will not only ascertain whether platelets can carry PrP<sup>Sc</sup> but at which stage of the disease the protein may be detectable and whether application of a simple flow cytometric test will be sensitive, reliable and reproducible enough to apply as a general screen.</p>	
<b>Project Title: Factors influencing the non-nutritive transit of proteins and particles across the human gut epithelium</b>	<b>Contact: Dr S Ghosh, Western General Hospital, Edinburgh</b>
<p><b>Abstract:</b> Current knowledge of protein and particle uptake by the gut indicates that there are two candidate routes by which TSE agents, prions, in food might cross the human gut mucosa. Prions may exploit the normal route for particle ingress, via M cells of the follicle-associated epithelium, as do many other infectious agents. Alternatively, in diseases where there is gross para-cellular leakage, this can provide a route for ingress of particles of the size range of the TSEs. There are few data for these cells and mechanisms in man. We will use post-mortem intestine and targeted endoscopic biopsies to define the normal features of human follicles and M cells, by a combination of light, dissecting and scanning electron microscopy. By studies in patients with celiac disease, ulcerative colitis and Crohn's disease (in the first instance) we will establish the extent of variation in M cell numbers in disease, and assess if any abnormalities correlate with clinical tests of gut permeability. Clinical tests of M cell function, and non-FAE particle uptake, will be devised, using fluorescent microparticles of 59nm and 1micrometers diameter, in-vitro and in-vivo approaches; these will be complemented by studies responses to enterally applied Keyhole limpet hemocyanin.</p>	

<b>Project Title: Protein Complexes That Regulate Intracellular Signal Transduction: Structure and Regulation By Phosphorylation</b>	<b>Contact: Professor A Aitken, University of Edinburgh</b>
<p><b>Abstract:</b> My programme of research involves elucidation of the role of 14-3-3 protein complexes in signal transduction, in particular the structure/function relationships of the interaction of 14-3-3 and other novel “adapter proteins” with signalling proteins. This involves a novel phosphopeptide motif, RSX<sub>12</sub>SpXP (where Sp is phosphoserine). We are investigating the distinct kinases that phosphorylate specific 14-3-3 isoforms and the interacting proteins and thus regulate interaction. We have raised isoform- and phospho- form specific antisera against 14-3-3.</p> <p>14-3-3 proteins also participate in protein complexes that are implicated in major diseases, including Creutzfeldt-Jacob disease (CJD) and Alzheimer’s disease. We are also analysing neuronal functions of particular 14-3-3 isoforms involved in trafficking, which may have important implications for the detection of early clinical stages of CJD, BSE and Alzheimer’s disease. We have developed a two-site ELISA for the rapid and sensitive screening of CSF using our specific monoclonal antibodies against 14-3-3. We have shown that distinct sub-sets of 14-3-3 isoforms are present in CSF from patients with CJD and Alzheimer’s and this can be used for differential diagnosis. Immunocytochemistry of each isoform of 14-3-3 in normal and scrapie brain mouse has been carried out. We are correlating this with the development of the known pathology and time of appearance of 14-3-3 isoforms in CSF.</p>	

<b>Project Title: Brain pathophysiology in experimental models of human prion diseases</b>	<b>Contact: Professor J G R Jefferys, University of Birmingham</b>
<p><b>Abstract:</b> Mice transgenic for the human prion protein gene (PRNP) will be infected with the human prion diseases Creutzfeldt-Jakob disease (CJD) and the new variant of CJD (nvCJD), which is potentially related to BSE. Cortical slices will be prepared from these mice at stages after inoculation in order to study the cellular and network electrophysiological abnormalities which could cause neurological symptoms, and which may contribute to the progression of these diseases. We will test whether late after hyperpolarisations are depressed in the way they were in hamster scrapie. We will also measure : other intrinsic neuronal properties, excitatory and inhibitory synaptic transmission, emergent network activities related to cognitive function, and anatomical changes. Deeper understanding of the basic mechanisms of the pathophysiology of human prion diseases will provide a rational basis for the development of: diagnostic electrophysiological tests, treatments for amelioration of symptoms, and perhaps for slowing the progression of the disease.</p>	

<b>Project Title: Do scrapie and Creutzfeldt-Jakob disease develop normally in mice with targeted deletion of serum amyloid P component</b>	<b>Contact: Professor M B Pepys, Royal Free and University College London</b>
<b>Abstract:</b> Cerebral amyloid deposits are a neuropathological feature of transmissible spongiform encephalopathy. In common with all other known types of amyloid they contain serum amyloid P component (SAP). We have lately confirmed our hypothesis that SAP contributes to the pathogenesis of amyloidosis by finding that experimental indication of reactive AA amyloidosis is markedly delayed in mice with targeted deletion of the SAP gene (SAP <sup>0</sup> ). Here we propose to investigate the incubation period, pathology and outcome of experimental prion infection in the absence of SAP. The results will indicate whether SAP contributes significantly to PrP amyloidosis and, if it does, whether cerebral amyloidosis is important for pathogenesis of scrapie and CJD. Evidence for participation of SAP will support the application to prion diseases generally of the SAP inhibition strategy we are currently pursuing with respect to systemic amyloidosis and Alzheimer's disease.	

<b>Project Title: Magnetic Resonance Investigation of the Pathogenesis of Spongiform Encephalopathy</b>	<b>Contact: Dr J D Bell, ICSM at Hammersmith, London</b>
<b>Abstract:</b> At present, post-mortem neuropathology and detection of PrP <sup>Sc</sup> are the only methods available to confirm clinical diagnosis of transmissible spongiform encephalopathy (TSE). There are no non-invasive methods to detect and follow disease progression in either man or animals. We intend to use <i>in vivo</i> magnetic resonance (MR) imaging and spectroscopy techniques to non-invasively determine the anatomical and biochemical changes in the brain associated with TSE. This project will follow three related lines of investigation to understand the contribution of anatomical and biochemical changes, and blood-brain barrier disruption, in the pathogenesis of the disease. Studies will be conducted in murine models of scrapie, one with widespread vacuolation (ME7) and another with more restricted vacuolation and conspicuous amyloid plaque formation (87V). We will examine whether there is a consistent relationship between severity of pathology (extent of PrP <sup>Sc</sup> accumulation; gliosis; vacuolation) in a given brain area and detectable changes in both conventional and contrast-enhanced MR imaging. We will also determine in these murine models, using a combination of <i>in vitro</i> MRS and biochemical techniques, whether neuronal loss is a fundamental lesion in TSE, which gives rise to clinical disease. Neuronal cell death will be correlated with other indices, including infectivity and the presence of PrP <sup>Sc</sup> . This comprehensive approach will provide fundamental data for evaluating MR imaging and spectroscopy as a diagnostic tool for Creutzfeldt-Jakob disease (CJD).	

<b>Project Title: A transgenic transmission research programme for human prion disease</b>	<b>Contact: Professor J Collinge, Imperial College</b>
<b>Abstract: <i>Abstract is currently unavailable</i></b>	

<b>Project Title: Bioassay of infectivity from patients with CJD</b>	<b>Contact: Dr M Bruce, IAH Edinburgh</b>
<b>Abstract : <i>Abstract is currently unavailable</i></b>	

<b>Project Title: Mechanisms of transmission of TSE agents from the intestine to lymphoid tissues</b>	<b>Contact: Dr G Macpherson, Oxford University</b>
<b>Abstract: <i>Abstract is currently unavailable</i></b>	

<b>Project Title: The role of the lymphoreticular system (LRS) in the replication of scrapie in genetically susceptible and resistant sheep</b>	<b>Contact: Professor I McConnell, Cambridge University</b>
<b>Abstract:</b> This project will analyse immunological aspects of the lymphoreticular systems (LRS) in the response to scrapie in sheep of different PrP genotypes. The project aims to establish the relative importance of cells of the LRS in the transport and spread of scrapie infectivity in genetically susceptible and resistant sheep.	

<b>Project Title: Molecular analysis of neuropathological changes in a mouse model of scrapie</b>	<b>Contact: Dr J K Fazakerley, Edinburgh University</b>
<b>Abstract:</b> The contribution of interferons, cytokines, chemokines and inflammatory cells in the neuropathogenesis of TSEs is unknown. The aim is to carry out a detailed investigation into the temporal and spatial changes in transcript levels in defined brain regions and to relate these to other aspects of the neuropathology in a murine model system. We will use the ME7 strain of scrapie in CV mice since several aspects of the neuropathology in this system are already well-characterised or are under investigation. An RNase protection assay will be used to simultaneously measure levels of a number of transcripts including interleukins 1 alpha/beta, 2, 4, 5, 6, 7, 10, 12 and 13, interferons-(alpha, beta, gamma), GMCSF, TNFalpha/beta, TGF/beta, CD3, CD4, CD8, chemokines and metalloproteinases.	

<b>Project Title: The role of calcium and voltage- and calcium- gated potassium channels in TSE-related apoptotic cell death</b>	<b>Contact: Dr N K Macleod, Edinburgh University</b>
<b>Abstract: <i>Abstract is currently unavailable</i></b>	

<b>Project Title: Investigation of scrapie pathogenesis in the lymphoreticular system using in vivo germinal centre cultivation as a model system</b>	<b>Contact: Dr M E Bruce, IAH, Edinburgh</b>
<b>Abstract:</b> Recent data from our laboratory and elsewhere suggests that different mouse passaged strains of scrapie may use different cells within the LRS to process and/or replicate infectivity. In the programme outlined below, we intend to use the in vitro cultivation of germinal centre cells as a model in which to study (I) the expression of PrP by various cell populations within the germinal centre, (II) which cell populations are important for replication of a variety of scrapie strains, (III) the effect of immune activation on susceptibility to infection with scrapie, (IV) the induction of either scrapie-specific immune responses to scrapie-induced alterations to normal function and (V) the role of PrP in normal immune function.	

<b>Project Title: Role of lymph-borne cells in the early stages of scrapie agent replication</b>	<b>Contact: Professor J Hopkins, Edinburgh University</b>
<b>Abstract:</b> The transmissible spongiform encephalopathies are associated with alterations in the structure of prion-related protein, PrP. The conversion of PrPC to PrPSc may be the actual infectious process, or be a manifestation of that process. Natural infection usually occurs via the gut or skin and it is clear that lymphoid tissues are important in agent replication and dissemination. This project will utilise the cannulated lymphatic model to access cell populations trafficking from the skin and the draining lymph node. Using this model we will focus on the role played by the different lymphoid cell populations in PrPSc dissemination, with particular emphasis on cells of the Langerhans cell/dendritic cell (DC) lineage. The consequences of PrPSc carriage on DC function will also be assessed by assays of T cell proliferation and semi quantitative PCR for DC and T cell, cytokine production.	

<b>Project Title: Oral TSE pathogenesis: identification of neuroanatomical pathways and interaction with lymphoid tissue</b>	<b>Contact: Mrs P McBride, IAH Edinburgh</b>
<b>Abstract:</b> The oral route of infection is the most relevant pathway for natural transmission of TSEs within different species. However, the mechanisms involved are poorly understood and a better understanding is vital to intervention and prevention. The lymphoreticular and peripheral nervous systems are involved in the uptake of infection. The spleen harbours infectivity soon after infection and this is subsequently targeted to sites in the CNS via the PNS but the interaction between the two systems is unknown. This project aims to use a mouse model of scrapie to identify the neuroanatomical pathways and timing of events involved in spread of infection from the gut through the LRS and peripheral NS to the CNS. We also plan to orally-infect mouse models in which either the immune or sympathetic nervous systems are functionally deficient in order to separate the individual roles in spread of infection.	

<b>Project Title: The immunobiology of prions during peripheral scrapie pathogenesis</b>	<b>Contact: Dr R Bujdoso, Cambridge University</b>
<b>Abstract: <i>Abstract is currently unavailable</i></b>	

<b>Project Title: CNS gene expression in a mouse model of scrapie</b>	<b>Contact: Dr J K Fazakerley, Edinburgh University</b>
<b>Abstract: <i>Abstract is currently unavailable</i></b>	

<b>Project Title: Scrapie strain influences on the cellular targeting of infection in the peripheral lymphoid system</b>	<b>Contact: Dr M E Bruce, IAH Edinburgh</b>
<b>Abstract:</b> Our recent studies of the ME7 mouse scrapie strain indicate that follicular dendritic cells (FDCs) of the spleen produce PrP, support replication and are critical for neuroinvasion. In contrast, studies elsewhere have implicated B cells in replication and neuroinvasion after challenge of mice with the RML scrapie isolate. We will test the hypothesis that different scrapie strains target different cell types of the immune system. Knockout mice lacking specific cells of the immune system will be challenged with different scrapie strains, to determine their susceptibility and whether infectivity can replicate in their spleens. We will also determine the cellular and subcellular location of PrP within the spleen for a range of scrapie strains.	

<b>Project Title: Copper and prion protein function</b>	<b>Contact: Dr D R Brown, Cambridge University</b>
<p><b>Abstract:</b> The aim of the study is to investigate the function of prion protein (PrPc). More specifically the study will aim to identify the relevance of copper binding by PrPc to the metabolism of cells that express PrPc. The project will also seek to determine how loss of PrPc function might contribute to prion disease. Targets to be met are: In year 1-2: Determine relevance of PrPc expression to copper uptake, release and distribution. In year 2-3. Studies of recombinant PrPc to determine functional domains and any enzymatic activity. In year 2-4 Study with cells expressing PrPc and mutants give details of relevance to cell phenotype. In years 3-4 studies of copper starvation of PrP-knockout mice and will information on PrPc protection from changes in copper availability. End of Project: PrPc function defined; change in function of proteinase resistant PrP defined, functional domains defined; function changes with possible relevance to prion disease identified.</p>	

### 2.3 Characterisation of the different strains, compare scrapie strains with BSE

<p><b>Project Title: SE1909</b>  <b>To study the cellular and humoral responses of distal ileum mucosa and mesenteric lymph nodes in the pathogenesis of BSE</b></p>	<p><b>Contact: Mrs Y I Spencer VLA, Weybridge</b></p>
<p><b>Abstract:</b>  This is an investigation of the local immune response in the intestine during the early pathogenetic events of BSE-challenged cattle and mice. Methods for investigating the biochemical and cellular immune responses by ELISA and immunocytochemistry, respectively, will be developed in conjunction with improving methods for detecting early PrP accumulation in distal ileum and associated lymphoid tissue. These investigations will provide information regarding the immunological changes which occur either in response to direct challenge or as a result of PrP accumulation at specific sites in the gut during the initial stages of infection. An understanding of such events could allow for the development of better methods of early detection of disease and for disease control and prevention.</p>	

<p><b>Project Title: SE1413</b>  <b>Strain typing of scrapie agent in meat and bone meal.</b></p>	<p><b>Contact: Dr Robert A Somerville, IAH, Edinburgh</b></p>
<p><b>Abstract:</b> A pool of TSE-infected sheep brains, previously used in rendering studies, will be subjected to further analysis to strain-type the TSE infection present.</p>	

<p><b>Project Title: SE1427</b>  <b>Association of PrP gene non-coding region polymorphisms with incidence of natural scrapie in sheep and with differences in expression of PrP</b></p>	<p><b>Contact: Dr Nora Hunter</b>  <b>IAH, Edinburgh</b></p>
<p><b>Abstract:</b></p> <ul style="list-style-type: none"> <li>• Variation in sheep PrP amino acid codons 136, 154 and 171 is clearly associated with susceptibility to scrapie in most outbreaks. However scrapie has been described in animals of genotypes expected to be resistant and also in sheep with susceptible genotypes have remained healthy (anomalous animals).</li> <li>• It may be that in the anomalous animals, there are differences in levels of PrP gene expression which could affect the outcome of scrapie infection.</li> <li>• The main objective of this proposal is therefore to assess sheep PrP gene 5' and 3' untranslated regions for linkage of polymorphisms to susceptibility and to discover any associated differences in gene expression and pathology.</li> <li>• MAFF policy objectives addressed include, in Scrapie ROAME A: A2.5 (the relationship between genotype and clinical disease); A3.5 (genotype and epidemiology and pathogenesis).</li> <li>• The results will clarify our understanding of the progression of the disease through a flock as PrP coding region genotype alone does not explain all cases (and absence of cases) of scrapie.</li> </ul>	

<p><b>Project Title: SE1942</b>  <b>The attack rate and phenotype of scrapie-like disease on transmission to cattle of fresh and rendered pools of scrapie</b></p>	<p><b>Contact: Christine Berthelin-Baker,</b>  <b>VLA, Weybridge</b></p>
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**Abstract:**

The main aim of this research is to establish attack rate and dose response data of any scrapie-like agent which proved pathogenic for cattle after oral exposure to pools of sheep scrapie inoculum (of known titre by mouse assay), before and after rendering to meat and bone meal. The study would furthermore determine the phenotypes of any infections established in cattle, both by comparison with data on the BSE phenotype in cattle and on subsequent strain typing in mice. The attack rate data achieved would also be compared to that obtained in cattle for BSE (SE1918, SE 1930). The potential occurrence of BSE presenting in sheep (as scrapie) as a result of foodborne exposure of domestic sheep populations to BSE via contaminated meat and bone meal has been considered and this study has the potential to identify this possibility in sheep which were likely to have been exposed to the agent via feed. This study addresses policy concerns regarding deficits in our knowledge of the relationship between BSE and scrapie, the occurrence of BSE in sheep (presenting as scrapie) after oral exposure during the BSE epidemic and potential food safety hazards should this have occurred. In the absence of identifying the BSE agent, a further outcome of this study may identify the pathogenicity of sheep scrapie for cattle after oral exposure, before and after rendering of the inoculum. Not only could there be differences in pathogenicity, but possible changes in phenotype of disease after rendering might also become evident. The results will contribute to and underpin policy decisions on food safety.

**Project Title: SE1929**  
**Studies of experimental BSE in sheep**  
**of known PrP genotypes**

**Contact: Sue Bellworthy**  
**VLA, Weybridge**

**Abstract:**

The project addresses concerns relating to the possible presence of BSE in British sheep flocks introduced via food-borne exposure. It is designed to provide data on the distribution of BSE infectivity in specified tissues of sheep of known PrP genotype, as determined by bioassay using RIII mice, at known time intervals following experimental challenge with a standard oral dose of BSE-infected bovine brain material. Replicates of harvested tissues will also be archived pending the development of alternative bioassay models. The genotypes selected have been based on knowledge from experimental studies which have shown that the AA<sub>136</sub>RR<sub>154</sub>QQ<sub>171</sub> PrP genotype seems to be the most susceptible following BSE challenge in Cheviot sheep. In addition to AA<sub>136</sub>RR<sub>154</sub>QQ<sub>171</sub> sheep, it is proposed that studies will also be undertaken in AA<sub>136</sub>RR<sub>154</sub>RQ<sub>171</sub> and AA<sub>136</sub>RR<sub>154</sub>RR<sub>171</sub> sheep, but with less frequent sampling. Though these latter two groups are anticipated to be at lower risk of developing disease, especially the RR<sub>171</sub> group, it will be important to determine whether infection can be established in sheep which may not develop clinical signs. Two sources of sheep have been identified for this project, i.e. two identical studies will be undertaken, one in Romney sheep of British origin and the other in Suffolks of New Zealand origin. The latter represent sheep with equivalent PrP genotypes but bred in an infection-free environment. It may be that the projects will run simultaneously, but this will depend on the timing of the importation and the successful breeding of the NZ sheep in the UK. (Pilot studies will need to be undertaken to demonstrate the experimental susceptibility of the Romneys and the NZ sheep: these will be the subject of a separate proposal).

<p><b>Project Title: SE0230</b>  <b>Development and maintenance of a flock of UK sheep naturally infected with scrapie</b></p>	<p><b>Contact: Sue Bellworthy</b>  <b>VLA, Weybridge</b></p>
<p><b>Abstract:</b></p> <ul style="list-style-type: none"> <li>• ROAME Project SE0213 (An epidemiological study of sheep scrapie to determine means of natural transmission) monitored scrapie in farms around the UK. Selected sheep that would otherwise be lost to follow-up were brought from these farms to VLA and were kept together as a single flock at grass.</li> <li>• The result has been an established, diverse flock of known origin, endemically infected with scrapie and presumably with a number of scrapie strains. Such a flock is a valuable source of material for current and future TSE research studies defined in the MAFF TSE research strategy. This research is totally dependent on the scrapie flock and cannot take place in its absence. Continued management of the flock outwith SE0213 is now considered to be the most efficient and effective approach.</li> <li>• The purpose of this project is to maintain and develop this flock through planned introduction of new breeds, PrP genotypes and through breeding so that it continues to support and anticipate MAFF and VLA TSE research requirements.</li> </ul>	

<p><b>Project Title: SE1429</b></p> <p><b>Characterisation (by transmission to mice) of BSE from experimentally infected sheep of different PrP genotypes</b></p>	<p><b>Contact: Dr Jim Foster</b> <b>IAH, Edinburgh</b></p>
<p><b>Abstract:</b></p> <ul style="list-style-type: none"> <li>• The proposal addresses aspects of whether BSE has transmitted to UK sheep and, if so, whether it could be identified as BSE in the presence of a concurrent scrapie infection. This is highly relevant to the formulation of MAFF policy on BSE in sheep.</li> <li>• The main objective is to establish whether natural scrapie and BSE can be identified within the body of a single sheep.</li> <li>• Previous studies have established that, following experimental BSE challenge, BSE can be reisolated from brain and spleen of affected sheep of AA<sub>136</sub>QQ<sub>171</sub> PrP genotype.</li> <li>• Mixtures of BSE and a NPU Cheviot natural scrapie isolate will be transmitted to mice to establish whether BSE can be identified in a mixed inoculum. Spleens from BSE challenged sheep of VV<sub>136</sub>QQ<sub>171</sub> and VA<sub>136</sub>QQ<sub>171</sub> genotypes, both of which are susceptible to natural scrapie, will be transmitted to mice to establish whether injected BSE can be detected in the spleens of sheep which died from natural scrapie. Brain and spleen from BSE challenged sheep of VA<sub>136</sub>QR<sub>171</sub> and AA<sub>136</sub>QR<sub>171</sub> genotypes will be transmitted to mice to assess the possible effect of PrP genotype on BSE transmission characteristics.</li> <li>• Comparisons with natural scrapie isolates (separate project SE1423), including from the NPU Cheviot flock and from BSE primary transmissions, will provide valuable epidemiological information concerning the possibility of BSE causing infection in the national sheep flock.</li> </ul>	

<p><b>Project Title: SE1435</b>  <b>Is the BSE strain phenotype stable on sub-passage in sheep?</b></p>	<p><b>Contact: Dr Nora Hunter</b>  <b>IAH, Edinburgh</b></p>
<p><b>Abstract:</b> BSE is experimentally transmissible to sheep with shortest incubation periods (so far) in animals of ARQ/ARQ PrP genotype. At IAH we have already shown that infectivity re-isolated from brain and spleen of such animals retains the mouse transmission characteristics of primary BSE and have established the glycoform patterns of PrPSc in the sheep and mice. Several studies are underway at IAH and elsewhere to use this information to search for evidence of BSE in the UK national sheep flock, however it is unlikely that many animals are alive at the moment that have been infected with primary BSE. The aim of this proposal, therefore, is to put infectivity recovered from sheep clinically affected following experimental infection with primary BSE through a second round of sheep infection and to establish whether, as a result of this second passage in sheep, there is any adaptation or difference in the subsequent mouse transmission properties and glycoform profiles as compared with the infectivity and PrPSc from the first round of infection. The proposed study is relevant to MAFF policy on scrapie (development of alternative tests for scrapie; specificity and sensitivity of alternative diagnostic tests) and on BSE (incidence of TSEs in other species; possibility of subclinical infections; Diagnosis and Epidemiology). The results will indicate whether, if BSE has become established in UK sheep, its phenotype is stable and still recognisable by any of the currently used criteria.</p>	

<p><b>Project Title: SE1938</b>  <b>Strain typing of isolates of natural scrapie: correlations with host PrP genotype, clinical and pathological phenotype</b></p>	<p><b>Contact: Dr M Simmons</b>  <b>VLA, Weybridge</b></p>
<p><b>Abstract:</b>  The TSE agent(s) exists as a number of different strains, characterised in laboratory mouse models, in which they produce distinctive patterns of lesions and incubation periods. While a single stable strain has been shown to be the cause of BSE in cattle, at least 20 strains of scrapie are defined in rodents. However, little is known about the occurrence of strains in naturally occurring scrapie in the UK.</p> <p>Strain typing currently entails protracted studies in mice. Recent studies have suggested that the pattern of lesions in the affected host animal, or the analysis of PrP glycoforms may offer alternative means of informing on strain. Full assessment of the interrelationships between clinical phenotype, pathological phenotype, PrP distribution patterns, PrP glycoforms patterns, host PrP genotype and strain type (by mouse inoculations) in natural scrapie from a number of different sources, will enable the predictive value of each of these parameters for agent strain definition to be evaluated.</p> <p>This study will improve understanding and detection of different naturally occurring scrapie strains in the UK. Comparison of these parameters in natural scrapie and those in experimental BSE in sheep may assist in surveillance of the national flock for the presence of BSE.</p>	

<p><b>Project Title: SE1945</b>  <b>Investigation of sheep to sheep passage on the BSE strain phenotype.</b></p>	<p><b>Contact: Sue Bellworthy</b>  <b>VLA, Weybridge</b></p>
<p><b>Abstract:</b>  The contaminated feed that initiated the BSE epidemic in cattle was also used on a smaller scale in sheep and it is possible BSE is now present in the British sheep flock where it is likely to be misdiagnosed as scrapie which is endemic in the UK</p> <p>Strain typing studies have so far failed to identify BSE in UK sheep but the numbers examined are small and the effects of sheep to sheep passage on the strain characteristics are unknown.</p> <p>The MAFF TSE strategy document, 1999, emphasises the need to investigate the links between TSEs in other species and BSE in cattle, and risks of transmission back to cattle or to man. The overall objective of this project is to document the clinical and pathological presentation of BSE in sheep and to investigate the stability of the BSE strain during sheep to sheep passage both within and between different breeds with the aims of improving diagnosis - particularly by identifying differential characteristics between BSE and scrapie. identifying changes in the strain characteristics of the BSE agent after sheep passage and providing a better understanding of the human health risks associated with the potential presence of BSE in the sheep population.</p>	

<p><b>Project Title: The relationship between neuron damage and clinical disease: relating murine and ovine scrapie to BSE and CJD</b></p>	<p><b>Contact: Dr J R Fraser,</b>  <b>IAH Edinburgh</b></p>
<p><b>Abstract:</b> The pathology of the TSEs is immensely varied and yet shows considerable congruity between species; however, the pathophysiological basis for clinical disease is not known. This study will combine three areas of pathological research: experimental studies on murine scrapie, scrapie in sheep and BSE in cattle, and CJD in man. Evidence from murine scrapie suggests that neuronal dysfunction initiated at the synapse underlines clinical disease. We propose to test this observation in sheep scrapie, BSE, and CJD by quantifying neuron morphology using confocal imaging and morphometric techniques and correlating with synaptic degeneration. A reliable method of estimating synapse number and integrity will be developed and applied to murine, ovine, bovine and human CNS.</p>	

<b>Project Title: PrPsc glycoform analysis and the origins of natural TSE strains in sheep</b>	<b>Contact: Dr N Hunter, IAH Edinburgh</b>
<p><b>Abstract:</b> PrPSc, the TSE associated isoform of PrPC a neuronal glycoprotein, shows molecular heterogeneity due to differential glycosylation and partial proteolysis which have been said to be TSE strain dependent. The particular patterns and apparent molecular weights of bands produced by PrPSc on Western blots following treatment with proteinase K are called glycoforms and have shown some consistency in association with different TSE sources, for example BSE and nvCJD. This proposal seeks to take advantage of the large scale project already funded by BBSRC to study epidemiology of natural scrapie in 100 flocks throughout the UK by carrying out PrPSc glycoform analysis of scrapie affected animals from these flocks. This study will test the hypothesis that PrPSc glycoforms are useful in revealing the origins of different strains of natural TSEs.</p>	

#### 2.4 The structure of both PrPc and PrPsc, the normal function of PrPc and the mechanisms of conversion of PrPc into PrPsc in vitro

<b>Project Title: Defining the role of PrP in normal development &amp; neurodegeneration in novel transgenic mice in which PrP gene expression is temporally &amp; spatially controlled</b>	<b>Contact: Dr J C Manson, Institute for Animal Health, Edinburgh</b>
<p><b>Abstract:</b> We propose to generate two new lines of PrP mutant mice, in which normal PrP expression will be controlled temporally using the bacteriophage P1 cre/loxP site-specific recombination system. Single rounds of gene targeting will be used, followed by in vitro selection, to produce two embryonic stem cell lines. One of these will carry two lox sites flanking PrP exonic sequences and one will carry a lox flanked stop cassette prior to oxon 3. Subsequent intercrossing with animals transgenic for an inducible form of the cre recombinase will produce strains in which it will be possible to functionally activate or inactivate PrP by expressing the cre recombinase. The major advantages of these lines over the currently available PrP null strains are: first, the ability to ablate or upregulate PrP gene expression at any time point during development and within any tissue. This will allow phenotypic abnormalities to be precisely attributed to loss of PrP and the normal function of PrP to be more accurately defined. Second, the ability to ablate PrP gene expression at specific time points and in specific tissues during scrapie infection. This will permit analysis of the role of PrP in control of disease progression in terms of agent replication, transport of infectivity from the periphery to the CNS and the development of CNS neurodegeneration.</p>	

<b>Project Title: Copper binding by prion protein: conformation and consequence</b>	<b>Contact: Professor I M Jones, Reading University</b>
<p><b>Abstract:</b> Prion diseases such as BSE of the cow and Creutzfeldt-Jakob disease of the human remain an enigma. They are proposed to occur because of the generation of an aberrant form of a protein, the prion protein (PrP), that is a normal constituent of the brain and other tissues. Generation of the aberrant form of PrP is more likely if particular forms of the PrP gene are present in the host or if the host is inoculated with pre-existing disease tissue, for example by ingestion. Yet the disease form of PrP is identical in sequence to the normal cellular form and differences between the two therefore must occur when the PrP protein assumes its final three dimensional shape, a process of protein folding. Thus, PrP folding and the overall conformation of the molecule is crucially important for an understanding of how PrP can give rise to disease. In addition, if the key elements of the change can be understood it may be possible to develop sensitive tests which can distinguish one form of PrP from another and so offer rapid detection of the presence of infected material. This proposal concentrates on understanding the conformation of normal PrP protein and to what extent the shape of the protein can be influenced by copper and other metal ions, a recently discovered normal constituent of the protein. The work will contribute to the basic understanding of PrP and its associated diseases and may give rise to information or reagents that could benefit diagnosis or treatment.</p>	

<b>Project Title: Carbohydrate-mediated interactions of PrP<sup>C</sup></b>	<b>Contact: Professor T Feizi, ICSM at Northwick Park Hospital, Middlesex</b>
<p><b>Abstract:</b> There is considerable evidence that the normal cellular prion protein PrP<sup>C</sup> binds to exogenous acidic polysaccharides and that such interactions inhibit the accumulation of the abnormal isoform, PrP<sup>SC</sup>. There is no information however on endogenous saccharide sequences with which PrP<sup>C</sup> interacts. The aim of this project is to investigate in detail the carbohydrate binding specificities of the rodent, ruminant and human PrP<sup>C</sup>, and identify oligosaccharide ligands which could provide clues not only to the mechanism of PrP<sup>C</sup> to PrP<sup>SC</sup> conversion, but also the molecular basis of barriers to transmissibility between different animal species. In these investigations it is proposed to use (a) the soluble bacterially expressed forms of the murine, hamster, bovine and ovine protein, and later in the programme the human protein and (b) 'libraries' of lipid-linked saccharide probes. First, a library of saccharide probes representative of mammalian N- and O-glycans and glycosaminoglycans will be generated from readily available glycoconjugates, with special emphasis on polyanionic sequences. Second, saccharide libraries will be generated from N- and O-glycosylated glycoproteins, proteoglycans and glycolipids to be isolated from murine and hamster brains. State-of-the-art ligand discovery techniques will be exploited that enable the sensitive detection of oligosaccharide ligands in conjunction with their structural elucidation.</p>	

<b>Project Title: Heparan sulphate antigen 10E4: Complete characterization and investigation of its role in prion biology and disease</b>	<b>Contact: Professor T Feizi, ICSM at Northwick Park Hospital, London</b>
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**Abstract:** A major challenge in the structural and functional characterisation of the infectious lesions in transmissible encephalopathies has been to determine whether the molecular players include host components in addition to the prion protein, PrP. Sulphated carbohydrates of the type that occur on heparan sulphate proteoglycans have been among strong candidates for such a role. This project is focused on a carbohydrate antigen, 10E4, which is expressed on heparan sulphate chains, and has the hallmarks of a prion disease-associated host component even in the earliest detectable lesions in the brain of scrapie-infected mice. Working with fragments of heparan sulphate, we have partially characterised this antigen, and found that it contains a unique tetrasaccharide sequence. We will make two complementary approaches aimed at the complete characterisation of 10E4 antigen. In the first, we will isolate hexasaccharide fragments from natural heparan sulphate glycosaminoglycan, convert them to fluorescent lipid-linked oligosaccharide probes by the neoglycolipid technology, for monitoring and isolating antigenic components, in conjunction with electrospray mass spectrometry (MS), and collision-induced dissociation (CID) MS/MS, to determine composition, size and sequence. In the second approach, we will take advantage of the advances in combinational chemical synthesis to generate an array of thirty-two hexasaccharides related to the 10E4 tetrasaccharide, in order to assign the specificity of the 10E4 antibody, and to select a 10E4 antigen-positive hexasaccharide to produce tens of milligrams. We will prepare formulations of 10E4 hexasaccharide, including fluorescent neoglycolipids with saturated and unsaturated lipids, which will allow evaluation of its role in prion trafficking and infectivity.

**Project Title: The mechanism of TSE infection of neurons**

**Contact: Dr R J Morris , Kings College London**

**Abstract:** Only within living cells does the conversion of cellular prion protein (PrPC) to its pathogenic isoform (PrPSc) produce detectable infectious TSE agent. How cells contribute to the interaction of exogenous PrPSc with cellular PrPC is unknown, although the trafficking of PrPC through endosomes appears to be involved. We have been studying the molecular environment of PrPC on neurons as it leaves the cell surface and is endocytosed, providing a baseline to assess how exogenous PrPSc enters cells. We also find, using polyclonal antibodies we have made to peptide epitopes in the C-terminal domain of PrP, that we can identify protease-resistant PrP accumulating in cultured sensory neurons within days after exposure to TSE agent. We propose to bring these two lines of research together to identify the initial events of infection of neurons leading to the conversion of PrPC to the pathogenic isoform, including: 1.) determination of whether exogenous PrPSc interacts with a specific receptor complex on the neuronal surface or endosomal membrane, or whether its interaction with PrPC is the result of less specific processes; 2.) if there is a specific receptor for PrPSc, identification of its components by microsequencing using mass spectrometric methods; 3.) determination of whether exogenous PrPSc is internalised in neurons; 4.) if so, identification of the vesicular compartments involved; 5.) determination of the relationship of exogenous PrPSc to PrPC within these compartments; and 6.) identification of the subcellular compartment in which the accumulation of newly converted protease-resistant PrP is first detectable.

Overall, this work will discover the molecular and cellular context in which exogenous PrPSc interacts with, and converts, PrPC in neurons. Analysis of the factors controlling the initial stages of this interaction in neurons will assist in the rational design of drugs to inhibit early events in the propagation of TSE.

<b>Project Title: Structural studies on prion proteins and related molecules</b>	<b>Contact: Professor G Dodson, National Institute for Medical Research</b>
<p><b>Abstract:</b> This proposal aims to obtain structural information to elucidate the role of the prion protein PrP in the molecular pathology of spongiform encephalopathies, using spectroscopic methods and X-ray crystallography. Particular emphasis is given to understanding the physical properties of the protein, and to developing new approaches involving protein engineering and novel crystallographic techniques. Intact murine PrPc and selected mutants will be expressed in an optimised CHO system and in E. coli systems. The recombinant full-length aglycosyl protein is currently secreted from the CHO cells at ca 5mg/L, and can be purified in non-denaturing conditions. A variety of constructs coding for intact PrPc and fragments will be expressed from E. coli in experiments in which selected and random mutations will be screened for high and soluble expression. The three-dimensional structure of PrPc 121-231 will be used to design point mutations, and to identify suitable sites for the deletion of loops and making other modifications. Biophysical studies on the recombinant proteins will be carried out to characterise and to improve their solubility, monodispersity and stability. Spectroscopic experiments will be used to characterise the folded states of the intact PrPc and its mutant derivatives, in relation to the PrPc&gt;PrPsc switch. The effects of organic solvents, metal ions, antibodies and of perin (and other anti-scrapie prophylactic molecules) will be studied as possible stabilisers of the conformation of PrPc. These results will provide the basis for the systematic programme to crystallise purified and soluble PrPc and fragments for X-ray analysis.</p>	

<b>Project Title: Membrane trafficking and expression of prion protein: their role in TSE</b>	<b>Contact: Dr R J Morris, Kings College London</b>
<p><b>Abstract:</b> The conversion of prion protein from its cellular (PrPc) to scrapie (PrPsc) form is both diagnostic of TSE infection and central to pathogenesis. We will identify the cellular basis of this conversion and its propagation from sites of infection to target neurons in the CNS. The endosomal trafficking of PrPc appears to be central to its pathogenic conversion. We will investigate, in cultures of polarised neurons, the cycling of PrPc between the cell surface and endosomes, and study the acute and chronic effects of scrapie infection upon this. Definitive proof of the role of endocytic trafficking upon the ability of PrPc to support TSE infection will be sought in cultured cells and in transgenic mice expressing hybrid proteins in which the membrane microenvironment and endocytic behaviour of PrP have been altered. To understand how expression of PrPc in vivo influences routes of infection and selective neurotoxicity, more effective immunohistochemistry is needed. We demonstrate an improved method that displays the cellular and subcellular distribution of PrPc, and its degradative processing; this will be used to study PrPc expression along routes of transmission to CNS target neurons. The increased sensitivity of this method may also enable the <i>in vivo</i> cellular response to infection to be studied at much earlier stages than has been possible. These approaches will substantially define the involvement of PrPc in TSE infection, and provide a rational basis for pharmacological intervention.</p>	

<b>Project Title: Chaperone interactions with PrP</b>	<b>Contact: Dr M E Cheetham, Institute of Ophthalmology, UCL</b>
<p><b>Abstract:</b> The prion protein (PrP) plays a central role in Transmissible Spongiform Encephalopathy (TSE) disease progression and transmission. It is now clear that conformational changes in the PrP molecule are pivotal in disease pathogenesis. Molecular chaperones have been shown to be important facilitators of protein conformational change in many cellular systems, but their effects upon PrP conformation are little studied. This proposal aims to test the hypothesis that chaperones can modulate PrP conformation and may be involved in disease related conformational changes by studying the biochemical basis of their interaction in vitro. We will analyse chaperone/PrP binding by a variety of methods and delineate the chaperone binding sites in PrP. The effects of hsp70 and co-chaperones on the re-folding of recombinant PrP after denaturation will also be investigated, including analysis of PrPSc and peptide mediated alterations in PrPc conformation. Studies of 'prion-like' phenomena in yeast have suggested that manipulation of heat shock proteins and chaperones can inhibit prion activity. Therefore, demonstration that chaperones can mediate changes in PrP conformation may highlight new strategies to combat TSE.</p>	

<b>Project Title: Development and application of an antisense based system to study the biological function of PrP in the hippocampus</b>	<b>Contact: Dr N K MacLeod, University of Edinburgh</b>
<p><b>Abstract:</b> Mice lacking a functional prn-p gene do not develop spongiform encephalopathy when challenged with scrapie-infected material, indicating an important role for the gene product. Yet, PrP knockout mice have failed to yield a consistent phenotype. Possible reasons for this surprising result may be incomplete functional knockout of the gene product or the compensatory up- or down-regulation of other genes. A strategy that may be more revealing than germ line knockout might be the acute knockdown of gene expression through the use of antisense sequences. Organotypic hippocampal cultures will be exposed to PrP antisense sequences and the consequences of rapid PrP depletion on normal cell functions, such as copper binding, cell metabolism, membrane properties, pre- and post-synaptic mechanisms, synaptic plasticity and protein phosphorylation will be assayed by electrophysiological and biochemical means.</p>	

<b>Project Title: The role of the prion protein in copper metabolism</b>	<b>Contact: Dr N M Hooper, Leeds University</b>
<p><b>Abstract:</b> Prion diseases are characterised by the conversion of the normal form of prion protein (PrPC) into an insoluble abnormal form (PrPSc). The prion protein is the causative agent of the transmissible spongiform encephalopathies. The role of PrPC in normal cellular function is unclear, although recent studies have implicated PrPC in cellular copper metabolism. The aim of this project is to investigate the biological role of PrPC, particularly its potential role in copper metabolism. To this end we will investigate how PrPC binds copper and whether disrupting this binding affects cellular functions or alters the conversion of PrPC to PrPSc. We will also examine whether PrPC through interaction of its GPI anchor with caveolae is involved in the cellular uptake of copper, and initiate the characterisation of prion-like proteins in <i>C. elegans</i>.</p>	

<b>Project Title: Prion protein function and neurodegeneration in a prion disease model</b>	<b>Contact: Professor A Compston, Cambridge University</b>
<p><b>Abstract:</b> The aim of this project is to investigate the mechanism of neurotoxicity of a prion protein peptide in cell culture. Four aspects will be investigated: (i) direct toxicity of the prion protein peptide (ii) cellular uptake of prion protein peptide (iii) changes in copper metabolism induced by the peptide (iv) the role and function of prion protein expression in different types, and if loss of this function is induced by the peptide. The function of the prion protein has been suggested to be related to some aspect of copper metabolism and this shall be further investigated. This project should indicate loss of prion protein function is important to neuronal death in prion disease models and in which cell types this loss of function is important. A completed model of the mechanism of action of PrP106-126 involving route of uptake and binding partners will be developed.</p>	

<b>Project Title: Characterisation of the N-linked glycans of the prion protein by HPLC/mass spectrometry</b>	<b>Contact: Dr J Hope, IAH, Compton</b>
<p><b>Abstract: <i>Abstract is currently unavailable</i></b></p>	

<b>Project Title: Spectroscopic, genetic and structural studies on bacterial rPrPc</b>	<b>Contact: Professor G Dodson, National Institute for Medical Research.</b>
<p><b>Abstract:</b> Intact PrPc and selected constructs will be produced in large amounts using bacterial expression. The recombinant protein will be used for spectroscopic and biophysical experiments to probe the molecule's folding, stability and structure in solution and where relevant, its conversion to the PrPsc form. Mutations will be introduced on PrPc and constructs, such as the 121-231, to modify the stability of the tertiary structure. The mutated material, and the information it provides, will be used in folding and crystallisation experiments, in the PrPc to PrPsc conversion studies at IAH and to support the transgenic programme. The crystallisation programme will include experiments with the 121-231 construct and similar constructs trimmed of mobile segments, investigations on other constructs and mutated constructs and the N-terminal octapeptide repeat. There will be co-crystallisation studies with a range of ligands, concentrating on monoclonal Fab's and metal ions. The research will be carried out jointly with the IAH and will be co-ordinated with the recently initiated programme to study the behaviour and structure of the mammalian produced intact PrPc.</p>	

<b>Project Title: Investigating the properties of prion proteins using modelling linked to protein characterisation</b>	<b>Contact: Dr J Warwicker, UMIST</b>
<p><b>Abstract:</b> Solution structures of the PrP-sen folding core and characterisations of recombinant PrP folding and unfolding are available, but definitive links to the mechanism of PrP-sen/PrP-res conversion have not been established. Insolubility (PrP-res) and flexibility (PrP-sen) complicate further structure determination. The proposal links PrP modelling expertise with biophysical studies and in vitro conversions, to probe the folding and interactions of wild-type and mutant PrPs. For example, unfolding of mutants PrPs will test the predicted basis for beta-rich intermediate formation, whilst the conversion reaction will be used with mutants, pH-variation, and under varying conditions, to develop models for PrP-PrP interaction.</p>	

<b>Project Title: Elucidating the regulated expression of the prion protein mRNA in the rat brain: a novel circadian rhythm</b>	<b>Contact: Dr C W Coen, Kings College London</b>
<p><b>Abstract: <i>Abstract is currently unavailable</i></b></p>	

<b>Project Title: The characterisation and development of peptides designed to switch structural state</b>	<b>Contact: Dr D N Woolfson, Sussex University</b>
<p><b>Abstract: <i>Abstract is currently unavailable</i></b></p>	

<b>Project Title: Design, folding and co-operative interactions in beta-sheet peptides</b>	<b>Contact: Dr M S Searle, Nottingham University</b>
<p><b>Abstract:</b> The importance of a beta-sheet structure in the formation of amyloid fibrils, and their role in a variety of pathological disease states (Alzheimer's and BSE) suggests that model beta-sheet peptides may provide insight into the molecular basis and sequence dependence of sheet formation, the origin of their stability and the importance of co-operative interactions in their propagation. We investigate these phenomena using rationally designed two and three-stranded beta-sheet peptides using a variety of spectroscopic probes and theoretical methods to simulate folding. We focus on a quantitative description of stability and co-operative interactions both parallel and perpendicular to the strand direction using model systems already established in our group, and expand the design strategy to incorporate arrays of ionic interactions in addition to conventional hydrophobic motifs.</p>	

<b>Project Title: In vitro study of yeast prion structure, stability and folding</b>	<b>Contact: Professor A R Fersht, Cambridge University.</b>
<p><b>Abstract:</b> The genetic properties of two non-Mendelian genetic elements from the yeast <i>Saccharomyces cerevisiae</i> suggests that they are infectious protein, or prion, forms of chromosomal proteins. These prion-like factors are termed (URE3) and (PSI), and they are associated with the chromosomal genes <i>Ure2</i> and <i>Sup35</i>, respectively. Both proteins possess an N-terminal region which is required for induction of the prion state. The molecular chaperone <i>Hsp104</i> is involved in both formation of (PSI) and in 'cure' of this prion phenotype. The prion conformation is associated with increased protease activity and formation of aggregates in the cell. It is proposed to overproduce these proteins in <i>Escherichia coli</i> in order to study their stability, folding and structure. In particular, the effect of the N-terminal prion-inducing regions on folding and stability will be examined. The effect of chaperones and other agents on protein conformation will also be studied.</p>	

## 2.5. The basis of species barrier limiting inter- and intra- species transmission

<b>Project Title: Investigation into transmissibility of TSEs via blood (DH)</b>	<b>Contact: Dr C J Bostock, IAH, Compton</b>
<p><b>Abstract: <i>Abstract is currently unavailable</i></b></p>	

## 2.6. The susceptibility factors for the development of animal and human prion diseases

<p><b>Project Title: SE1751</b>  <b>Paternal Genotype and recessive inheritance effects on susceptibility to Bovine Spongiform Encephalopathy (BSE)</b></p>	<p><b>Contact: Dr W Vernon Wijeratne</b>  <b>Reading, Berkshire</b></p>
<p><b>Abstract:</b> The main objective of this research is to investigate the possible importance of genetic factors influencing liability to BSE by studying the differences between incidence of disease in the daughters of BSE affected and unaffected sires in the Holstein Friesian breed and determining the role of recessive inheritance.</p> <p>This information is important for policy decisions relating to the epidemiological control of BSE in cattle.</p> <p>These results can be useful in cattle breeding programmes to produce BSE-resistant cattle where environmental control of the BSE agent is difficult. Genetic improvement of resistance to BSE in cattle offers a permanent solution to the control of BSE.</p>	

<p><b>Project Title: SE1759</b>  <b>A comprehensive analysis of transcripts and proteins in blood of uninfected and BSE-infected cattle to identify markers of TSE infection</b></p>	<p><b>Contact: Dr Michael Clinton, Roslin Institute, Midlothian, Scotland</b></p>
<p><b>Abstract:</b> : The aim is to look for diagnostic markers for TSE infection in accessible tissue prior to slaughter:</p> <ol style="list-style-type: none"> <li>1. Blood fractions will be analysed for potential diagnostic markers; blood proteins from cattle orally challenged with BSE will be studied at various stages of the disease progression. This will be compared with blood from uninfected control cattle. The nature of any protein differences will be established and antibodies specific to these proteins produced.</li> </ol> <p>Any gene expression differences between lymphocytes present in the blood from uninfected and BSE infected cattle will be identified</p>	

<p><b>Project Title: SE1761</b>  <b>Production, characterisation and utilisation of antibodies to ovine abnormal PrP for scrapie diagnosis and research.</b></p>	<p><b>Contact: Roy Jackman</b>  <b>VLA, Weybridge</b></p>
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**Abstract:**

Immunodetection methods for abnormal forms of PrP (PrP<sup>Sc</sup>), play an important role in both diagnosis and research of the TSEs. Although in many areas these are the methods of choice, serious deficiencies in the availability of antibodies and the need to optimise and validate the new immunoreagents in existing protocols, continue to restrict their potential and use for the diagnosis and study of scrapie. This proposal presents the following course of study:

1. Production of antisera specific for ovine PrP<sup>Sc</sup> and to selected PrP-peptides.
2. Assessment of commercially and collaboratively available antibodies
3. Assessment of available antibodies by immunohistochemistry (IHC); improvements to and optimisation of the IHC detection of ovine PrP in CNS and non-neural tissue.
4. Development of Immuno-based methods for tissues using PrP<sup>Sc</sup> and PrP-peptide antisera.

MAFF are committed to setting up a scrapie surveillance programme. As with BSE, there are not even sufficient antibodies available to resource the programme as far as immunohistochemical and Western blot detections of PrP are concerned let alone undertake a development process for improved assays for ovine PrP for use in future scrapie surveillance and control.

**Project Title: SE1762**

**Assessment and validation of emerging methods and reagents for BSE diagnosis**

**Contact: Roy Jackman  
VLA, Weybridge**

**Abstract:**

New techniques, methods and (immuno)reagents for the diagnosis of BSE and the detection of the BSE agent (PrP<sup>Sc</sup>) are being produced at VLA, other laboratories worldwide and by commercial companies. It is essential that there is a facility for the assessment of these methodologies and reagents, on a selective basis, so that claims can be examined comparison made with Gold Standard protocols in order to provide advice and consultancy to aid the formulation of MAFF policy on BSE diagnostics. As the major centre for BSE diagnosis, VLA must also maintain its ability to incorporate the latest technology (when appropriate) and expertise in the diagnostic process to ensure that MAFF is provided with up to date advice and data on BSE. At the same time, there is a major gap in our appreciation of the performance of the current detection methods for bovine PrP, particularly with regard to the relative sensitivities and comparative quantification aspects of the different techniques. It is proposed that Western blot, SAF electron microscopy, PrP immunohistochemistry, ELISA / dot blot and infectivity be compared directly for relative performance on CNS material, against which new techniques can be then be related.

<b>Project Title: SE1757</b> <b>Detection, quantification and conformational determination of abnormal PrP by Immuno Capillary Electrophoresis (ICE)</b>	<b>Contact: Roy Jackman</b> <b>VLA, Weybridge</b>
<p><b>Abstract:</b></p> <ol style="list-style-type: none"> <li>1. To set up a collaboration between NCDC Ames and VLA Weybridge to expand, validate and “popularise” ICE as the major technology for the determination of the prion protein and its isoforms.</li> <li>2. To import and develop ICE technology for the determination of PrP<sup>Sc</sup> and PrP<sup>C</sup> from bovine and ovine tissues, into the UK</li> <li>3. To assess and validate ICE as a means of detecting PrP<sup>Sc</sup> in the blood and other body fluids of TSE affected animals.</li> <li>4. To develop, conjointly, the means of describing the conformational properties of abnormal PrP in different species and in different strains of TSE agents by ICE.</li> <li>5. To study, conjointly, the distribution of PrP, its isoforms and glycoforms in neural tissue and then in non-neural tissues and fluids.</li> </ol>	

<b>Project Title: Identification and mapping of BSE susceptibility genes in the mouse and human</b>	<b>Contact: Dr I J Jackson,</b> <b>Edinburgh University</b>
<p><b>Abstract:</b> The mouse strains RIII and C57BL6 differ in their incubation period following infection by primary BSE isolates. The strains have the same Sinc type, suggesting that susceptibility to disease is mediated by genes other than Sinc. Preliminary data indicates that there are up to three loci of major influence. We propose to carry out a backcross between two strains and infect each offspring with BSE. We will determine the genotype and the incubation period of the disease in each individual and using interval mapping we will localise the quantitative trait loci, or genes that affect susceptibility to BSE. Using a second series of crosses we will further characterise the QTL's and will refine their map location. Comparison of the gene maps of mouse and human will indicate where the homologous QTL's are located in the human genome, and will indicate possible candidate genes corresponding to the QTL's.</p>	

<b>Project Title: Host genetic factors in transmission and pathology of spongiform encephalopathies</b>	<b>Contact: Dr S A Whatley, Institute of Psychiatry London</b>
<p><b>Abstract:</b> We propose that in mice of the same sinc genotype disease progression and pathology in the transmissible encephalopathies (TSEs) will act as a quantitative trait in which codominant QTLs will be able to be identified independently from sinc. We will utilise the strengths of the recombinant inbred (RI) mouse strain approach to investigate genetic factors involved in transmission and pathology TSEs. The 26 BXD RI strains will be used to nominate quantitative trait loci (QTLs) distinct from sinc that affect transmission to mice of two TSE agents; BSE and cloned scrapie 'strain' ME7, using intraperitoneal and intracerebral routes of transmission. The integrative ability of the largest and most general QTLs will be conducted using F2 individuals derived from the BXD progenitor inbred strains ( C57BL/6 and DBA/2). In addition, a pilot study within this proposal will investigate host genetic influences on quantitative aspects of pathology. This systematic genetic approach will lead to identification of new genetic factors controlling disease progression in the mouse, and, prospectively, homologous human risk factors.</p>	

<b>Project Title: Pathogenesis of scrapie in sheep</b>	<b>Contact: Dr N Hunter, IAH Edinburgh.</b>
<p><b>Abstract:</b> Attempts to control and eradicate scrapie are hampered by a lack of understanding of the aetiology, pathogenesis and epidemiology of the disease, particularly in relation to strains of agent and variation in PrP genotype. With the knowledge of the influence of the ovine PrP gene on the incidence of natural and experimental scrapie and the power of current biomathematical approaches to the study of infectious diseases, there is tremendous scientific opportunity to undertake detailed and integrated epidemiological and pathogenetic studies to gain a full insight into this increasingly important disease of sheep. The specific objectives of this research programme are:1) to describe in detail the pathogenesis of natural scrapie in NPU Cheviot sheep of defined genotypes to establish the role of genotype in determining pathogenesis and the potential for establishing a carrier state2) to study the pathogenesis of experimental SSBP/1 and other scrapie strains in scrapie-free New Zealand sheep carrying scrapie susceptible and non-susceptible genotypes3) to establish whether there is any age related differential susceptibility to infection and/or differences in pathogenesis4) to investigate parameters influencing the probability of maternal transmission, including time of birth in relation to stage of incubation, placental exposure and transmission via colostrum and/or milk5) to investigate mechanisms of horizontal transmission, including animal to animal contact or environmental contamination and exposure.(This programme interacts closely with 201/TSE09857, Epidemiology of scrapie in sheep).</p>	

<b>Project Title: Scrapie control by cull and selective breeding: a randomised controlled trial</b>	<b>Contact: Dr A McLean, IAH, Compton/ Oxford University</b>
<p><b>Abstract: <i>Abstract is currently unavailable</i></b></p>	

<b>Project Title: Alterations in TSE susceptibility defined by specific mutations in PrP</b>	<b>Contact: Dr J C Manson, IAH Edinburgh</b>
<p><b>Abstract:</b> We have produced unique transgenic models for the TSEs by introducing point mutations into the endogenous PrP gene by gene targeting. Mice with four different PrP alleles have been produced (1) 101L (2) 108F189V (3) 108L189V and (4) 108F189T. These mutations are associated with disease susceptibility in human and murine TSEs. We now aim to use these lines of mice to define the mechanism by which the specific mutations in the PrP gene alters the susceptibility of the host to different strains of TSE agent. The control of incubation period of disease by the mutant PrP alleles will be assessed. The extent to which the mutations define the targeting and the severity of the pathological lesions will also be established.</p>	

### Other support

<b>Project Title: The pathogenesis of transmissible spongiform encephalopathies</b>	<b>Contact: Dr C J Bostock, IAH, Edinburgh</b>
<p><b>Abstract:</b> The research will study various aspects of the pathogenesis of TSEs, the knowledge gained from which should enable the development of rational approaches to the prevention and spread of human, and potentially zoonotic, TSEs. The scientific aims of the programme are to (i) dissect in vivo the cellular basis of lymphoreticular system involvement in peripheral pathogenesis; (ii) understand the molecular (PrP) and cellular (LRS) determinants that block or delay transmission between species; (iii) define the diversity of human TSE strains and the consequences of these for differences in pathogenesis, and (iv) unravel the mechanisms by which TSE agents gain access to and induce degeneration in the CNS.</p> <p>The programme brings together IAH scientists and support staff who have expertise in a wide range of murine models of TSEs, the structural and functional analysis of PrP, mutational analysis of the PrP gene and the production of transgenic mice expressing modified PrP genes, the in vivo and in vitro manipulation of cells of the murine lymphoreticular system and the pathological characterisation of TSE infections.</p>	

<b>Project Title: A molecular dissection of the prion-forming domain of the yeast Sup35p prion protein</b>	<b>Contact: Professor M F Tuite M. F., Kent University</b>
<p><b>Abstract: <i>Abstract is currently unavailable</i></b></p>	

### 3. Diagnosis of Ses

#### 3.1. Further development of cell cultures and banks for tissues and cells

<b>Project Title: SE1432</b> <b>The susceptibility of New Zealand sheep to TSE infection and linkage with PrP genotypes</b>	<b>Contact: Dr Nora Hunter</b> <b>IAH, Edinburgh</b>
<b>Abstract:</b> The main objective is to establish whether sheep from New Zealand (NZ), which is free of scrapie, respond to experimental infection with scrapie and BSE in the same manner as sheep from the UK which show distinct PrP genotype linkage with susceptibility. Once validated in this way, other scrapie-free NZ sheep derived from the main breeding flock, which is to be established in the UK by MAFF, can be used experimentally to study aspects of scrapie and BSE in sheep without the potentially confusing factor of sheep incubating a natural scrapie infection.  There are two main groups of sheep defined by PrP genetic association with scrapie: those which encode the PrP allele with valine at codon 136 (valine sheep) and those which do not (non-valine sheep). Representatives of both these sheep groups from both UK and New Zealand, with a suitable range of PrP genotypes will be challenged with SSBP1 scrapie and with BSE, which target different sheep genotypes, and incubation period of any resulting disease established and compared.	

<b>Project Title: SE1736</b> <b>Experimental production of bovine tissues for validation of BSE diagnostic tests</b>	<b>Contact: Steve Hawkins,</b> <b>VLA, Weybridge</b>
<b>Abstract:</b> To provide a bank of sequentially harvested tissues and body fluids from cattle of known BSE incubation or clinical status, inoculated with a known infectious titre of BSE, and from age-matched controls, for present and future research studies.  Currently, confirmation of a diagnosis of BSE can only be made post mortem. There is an urgent need for tests which can detect evidence of BSE in the live animal and novel methods are being explored and developed. A supply of tissues and fluids from animals of known BSE status is required for test validation. This project will generate such tissues experimentally.	

<p><b>Project Title: SE1737</b></p> <p><b>Provision of "pre-clinical BSE" body fluid samples from bovines experimentally challenged with infected brain material</b></p>	<p><b>Contact: Roy Jackman</b> <b>VLA, Weybridge</b></p>
<p><b>Abstract:</b></p> <p>The emphasis for the development and validation of diagnostic procedures in BSE has now moved from tests to differentiate disease in the clinically affected animal to procedures for assessment of exposure to the infectious agent -so called "pre-clinical" assays. This is central to MAFF policy and not only affects the control and elimination of the disease in the UK but has implications for trade in ruminants, their meat and meat products.</p> <ul style="list-style-type: none"> <li>• Although no such tests are currently available, much resource is actively engaged in this area, particularly with MAFF funding. In this laboratory, a procedure for indicating BSE by the electrochemical quantification of urine metabolites is undergoing validation on samples from clinically affected animals and it is expected that an equivalent blood assay can be produced in the near future. In addition, ELISAs for the highly sensitive detection of abnormal PrP are under development.</li> <li>• However, when (or if) these or other assays for pre-clinical BSE are developed, no materials are currently available for validation or even initial assessment of these procedures. Samples of body fluids such as blood, urine or cerebrospinal fluid (CSF) have not been collected on a regular basis nor in sufficient quantities from previous transmission studies or from high-risk cattle prior to the clinical phase of the disease. Although a major pathogenesis-type project is currently under discussion, this cannot start to produce appropriate materials for test validation for nearly three years. Body fluid samples from pre-clinical, BSE-exposed bovines are urgently required for test assessment and validation.</li> <li>• The major objective of this project is therefore to provide such samples of blood (serum, plasma, RBCs and WBCs), CSF, urine and saliva at and following exposure to BSE, throughout the course of the disease, albeit on a limited scale, but at the earliest opportunity. The experimentally challenged animals must be followed to termination of the disease. This should occur by the fourth year but provision is made for extension into year 5.</li> </ul>	

<p><b>Project Title: SE1931</b></p> <p><b>Maintenance of a TSE-free sheep flock after importation from New Zealand</b></p>	<p><b>Contact: Dr H A Simmons</b> <b>VLA, Weybridge</b></p>
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**Abstract:**

There is a current and forecast objective for MAFF to commission research into TSE in sheep. The presence of, or possibility of, scrapie in the sheep used for these experiments would undermine the scientific validity of any experiment done. The vast majority, if not all, of the sheep in the UK cannot be guaranteed free from scrapie due to the lack of flock monitoring and history, persistence of the infectious agent and lack of definitive test in the live animal. New Zealand is recognised as being scrapie-free. This project intends to maintain an imported breeding flock of sheep from New Zealand by establishing them in purpose-built accommodation on a guaranteed scrapie-free farm (ADAS Arthur Rickwood, history of not having ruminants). This flock would then supply guaranteed scrapie-free sheep so MAFF can achieve its objective in this area.

<b>Project Title: TSE Resource Centre</b>	<b>Contact: Dr P Minor, NIBSC, Hertfordshire</b>
<b>Abstract: <i>Abstract is currently unavailable</i></b>	

<b>Project Title: The development of panels of CSF for the evaluation of tests for CJD</b>	<b>Contact: Dr E Miller PHLS CDSC, London</b>
<b>Abstract: <i>Abstract is currently unavailable</i></b>	

<b>Project Title: Reagent Resource Centre</b>	<b>Contact: Dr C J Bostock, IAH Compton</b>
<b>Abstract:</b> The TSE Resource Centre will provide research groups in Europe with reagents and materials to facilitate projects. The use of common reagents will help standardise work and therefore help in comparative work.	

### 3.2 Development of rapid and sensitive early diagnostic tests including surrogate markers especially in living animals and humans

<p><b>Project Title: SE1728</b></p> <p><b>Relationship between conformation of PrP, infectivity and pathogenicity of BSE as a basis for diagnosis</b></p>	<p><b>Contact: Roy Jackman</b>  <b>VLA, Weybridge</b></p>
<p><b>Abstract:</b></p> <p>This application is for covering 50% funding for an agreed EU FAIR 5 project No. CT97 .3314 involving VLA, the universities of Munich and Gottingen, the CNRS Laboratories in Paris and Connex GMBH of Munich. The project seeks to determine the molecular properties of PrP<sup>C</sup>, PrP<sup>BSE</sup> and PrP-peptides in order to study the relationship of conformation with strain characteristics, pathogenesis, behaviour and ligand interaction as a basis for the development of diagnostic aids in the disease itself and exposed animals. The major role for VLA is the preparation, purification and characterisation of PrP<sup>BSE</sup> from clinically affected animals and the supply of such materials to collaborating scientists. In addition, antibodies to the various forms of PrP and to required PrP peptides will be produced and supplied. The development of diagnostic procedures for the disease itself, for the presence of characterised forms of PrP<sup>Sc</sup> and for the detection of exposed animals is a central requirement for MAFF for the control, management and risk assessment aspects of the epidemic.</p>	
<p><b>Project Title: SE1760</b></p> <p><b>Differential diagnosis of TSEs: characterisation and identification of prion protein strains by mass-spectrometry</b></p>	<p><b>Contact: Dr M J Sauer,</b>  <b>VLA, Weybridge</b></p>

**Abstract:**

**Objective:** To develop and validate sensitive and specific mass spectrometric (MS) techniques for discrimination of BSE and scrapie prion strains in routine ante- or post-mortem tests.

**Policy relevance:** Successful completion of the proposed project, will enable the explicit requirement of MAFF TSE strategy to be met, by providing practical methods for differential analysis of PrP<sup>Sc</sup>. As a consequence of this approach, fundamental strategic questions regarding the molecular basis for strain variation will be addressed, including investigation of the routes by which these proteins take on their final conformation.

**Approach:** Several complementary MS techniques will be applied to study PrP<sup>Sc</sup> structure to determine the most practical and effective means of achieving differential analysis. This will involve comparison of electrospray ionisation using tandem, ion-trap instruments and Time of Flight MS. Studies will include use of collision induced dissociation, deuterium exchange, selective derivatisation and surface topology probing, ESI charge state distribution and differential proteolysis.

**Advantages:** Prion protein analysis by MS offers many advantages compared with other techniques, these include:

- the ability to determine definitive structural characteristics of PrP and PrP<sup>Sc</sup> especially post-translational modifications.
- potential for differential diagnosis through quantitative, semi-automatable and specific analysis of PrP<sup>Sc</sup>
- evaluation of protein structure in relation to known gene sequences and the disease mechanism.
- identification of specific protein 'footprint' fragmentation patterns associated with various prion isoforms.
- analysis of PrP<sup>Sc</sup> structural modifications and conformation in relation to the molecular basis of strain variation.
- potential to identify diagnostic protein surface structures to expedite immunogen and ELISA development.

**Project Title: Cellular Immune responses to prion proteins in null mice**

**Contact: Dr P D Minor, NIBSC, Hertfordshire**

**Abstract:** We propose to undertake a program of immunisation to generate and study T cell mediated immune responses directed against the human and murine prion (PrP) proteins in mice. We will use vaccination protocols that have been shown to generate a wide range and/or schedules that have elicited responses of novel specificity. The studies will be carried out using inbred PrP(-/-) deficient (null) mice immunised with plasmids containing an appropriate prion gene or with cells transfected with such plasmids, or cells from the inbred parental wild type PrP(+/+) mice from which the null mice were derived. The cellular responses generated will be used as tools to investigate PrP structure, their prophylactic or therapeutic properties will be explored and they will be assessed as diagnostics to differentiate the normal and protease resistant isoforms of the PrP protein. The use of null mice and DNA immunisation technology makes it likely that hitherto unrecognised immune responses may be generated, with potentially novel characteristics.

<b>Project Title: The Identification &amp; Characterisation of Early Diagnostic Clinical and Neuroimaging Features of Creutzfeldt-jakob Disease</b>	<b>Contact: Professor M N Rossor, National Hospital for Neurology and Neurosurgery London</b>
<p><b>Abstract:</b> Sixty patients referred to a specialist dementia and prion disease clinic with suspected Creutzfeldt-Jakob disease (CJD) will be studied to identify early diagnostic markers. Clinical, neuropsychological and behavioural assessments will be combined with quantitation of cerebral atrophy by the registration of serially acquired magnetic resonance image (MRI) data sets. Subsequent follow-up will identify three groups: cases with CJD, cases with other progressive cerebral degenerations, and cases with other conditions such as functional psychiatric disorders in the absence of progressive cerebral degeneration. The diagnosis of CJD will be confirmed at autopsy. Clinical and MR data will be compared across the groups. The novel method of registration of serially acquired MR data sets permits accurate quantitation of rates and patterns of regional brain atrophy. Preliminary data suggest that significant cerebral and cerebellar tissue loss in CJD will be identified with scan intervals of 3 months or less. It is proposed that this method will be valuable not only in diagnosis but in monitoring potential treatments.</p>	

<b>Project Title: The Development of Immuno Detection Methods for a Prion-Specific Blood Test</b>	<b>Contact: Professor J Collinge, MRC Prion Unit</b>
<p><b>Abstract:</b> A research programme jointly funded by the Department of Health and the Medical Research Council aimed at improving immunodetection methods for human prions for the development of a prion specific blood test. A complete immunisation and fusion programme of transgenic mice will produce an array of monoclonal antibodies that will be screened for their specificity and sensitivity to native and recombinant prion proteins.</p>	

<b>Project Title: Development of monoclonal antibodies against normal and disease-related isoforms of human prion proteins</b>	<b>Contact: Professor J Collinge, MRC Prion Unit</b>
<p><b>Abstract: <i>Abstract is currently unavailable</i></b></p>	

<b>Project Title: The development of a sensitive and specific in vitro diagnostic test for the detection of TSEs in blood &amp; urine samples</b>	<b>Contact: Dr. R. Eglin, NBS Colindale, London</b>
<p><b>Abstract: <i>Abstract is currently unavailable</i></b></p>	

<b>Project Title: Bacteriophage display antibodies recognising PrP (BBSRC)</b>	<b>Contact: Dr J R Young IAH, Compton</b>
<b>Abstract: <i>Abstract is currently unavailable</i></b>	

<b>Project Title: RNA aptamers against disease isoform bovine prion protein</b>	<b>Contact: Dr W James, Oxford University</b>
<b>Abstract: <i>Abstract is currently unavailable</i></b>	

<b>Project Title: Molecular characterisation of the pathogenesis of TSE infection</b>	<b>Contact: Dr M Clinton, Roslin Institute, Midlothian</b>
<b>Abstract:</b> In TSEs, the molecular events leading to the resulting neurodegeneration are poorly understood and, at present, there is no effective pre-mortem diagnostic test or therapy for TSE infection. In order to identify early molecular markers of disease and the molecular pathways involved in neurodegeneration following TSE infection, we will characterise the differences in gene expression between infected and non-infected mouse brain with particular emphasis on the early stages of disease. The transcripts identified will be cloned, differential expression confirmed and the expression profiles established by in situ hybridisation. Genes coding for these mRNAs will be isolated, sequenced and characterised and their expression patterns analysed in the blood and peripheral tissues of infected and control mice.	

### 3.3. Development of sensitive assay in transgenic mice

<b>Project Title: SE1426 Generation and validation of transgenic mice expressing multiple copies of sheep and bovine PrP gene alleles</b>	<b>Contact: Dr Nora Hunter IAH, Edinburgh</b>
<b>Abstract:</b> The main objective is to generate mice which will become faster bioassays for the detection of scrapie and BSE infectivity than those mouse lines which are currently available. This will be achieved by means of making transgenic mouse lines with multiple copies of various sheep and cattle PrP gene alleles and validating these lines as models for sheep and cattle by carrying out challenges of the transgenic mice with SSBP/1 scrapie and BSE.  Once validated, the mouse lines will subsequently be used in infectivity bioassays in, for example, natural and experimental scrapie and BSE pathogenesis studies and will be made available to MAFF for their own purposes.  This proposal is relevant to MAFF policy as one of the bottlenecks which delay the objectives is the length of time involved in, and the lack of sensitivity of, the infectivity bioassays.	

<p><b>Project Title: SE1416</b></p> <p><b>Development of mouse models for the study of bovine transmissible spongiform encephalopathy</b></p>	<p><b>Contact: Dr Jean C Manson</b> <b>IAH, Edinburgh</b></p>
<p><b>Abstract:</b></p> <ul style="list-style-type: none"> <li>• The work addresses policy issues related to the development of sensitive, rapid and validated models for the detection and characterisation of BSE.</li> <li>• Transgenic mice in which the mouse PrP gene has been replaced with the bovine PrP gene will be produced to study the susceptibility of cows to scrapie and BSE isolates.</li> <li>• Gene targeting techniques will be used to replace the coding region of the endogenous murine PrP gene for that of the bovine PrP gene.</li> <li>• Two different bovine genes constructs will be used for the targeting experiment coding for 5 or 6 octapeptide repeats. <ul style="list-style-type: none"> <li>• Targeting constructs will be electroporated into the HM1 murine embryonic stem cell line.</li> </ul> </li> <li>• Targeted clones will be identified by selection in thioguanine and confirmed by PCR and genomic Southern analysis.</li> <li>• Clones in which one copy of the murine gene has been replaced by the bovine gene will be injected into blastocysts and reimplanted into foster mothers for the production of chimaeric mice.</li> <li>• The chimaeras will be bred to produce mice homozygous for the introduced bovine PrP genes.</li> <li>• The two different lines of mice will be crossed to produce mice with all three genotypes of the bovine PrP genes.</li> <li>• The transgenic mice will be validated as a model for bovine TSEs by infection with scrapie and BSE isolates.</li> </ul>	

<p><b>Project Title: SE1753</b></p> <p><b>Preparation of more sensitive bioassay models for the improved detection, differentiation and diagnosis of the BSE agent</b></p>	<p><b>Contact: Peter Griffiths</b>  <b>VLA, Weybridge</b></p>
<p><b>Abstract:</b></p> <p>At present, the conventional mouse bioassay model represents the only feasible method for the detection and characterisation of prion disease agents from naturally and experimentally infected cases of BSE and scrapie. However, conventional mice are 1000 times less sensitive in detecting the BSE agent than in an homologous bovine infection model. There is clearly a need for the development of more sensitive bioassay models for the evaluation of prion disease agents in a whole range of tissues and body fluids from cattle and sheep affected by BSE and scrapie. In addition to the high susceptibility of cattle to BSE, kudu may represent an alternative model for susceptibility and sensitivity to prion disease. Preparation of transgenic mice expressing prion proteins or chimaeric prion proteins derived from cattle, or kudu, could provide more susceptible and sensitive animal models, leading to the improved detection, diagnosis and differentiation of prion diseases in a range of mammals. In transgenic lines that show particular sensitivity to prion disease, it might be possible to detect the presence of prion agents in subclinically infected, or carrier, hosts, not showing signs of disease. Information may be gained to further assess food safety issues as well as the dietary exposure of humans to the BSE agent. The results could provide benefits to MAFF, the beef industry, and to the general public, in terms of food safety and consumer confidence.</p>	

<p><b>Project Title: SE1756</b></p> <p><b><i>Detection of Bovine Prions from Cattle With BSE Using Transgenic Mice and Conformation-Dependent Immunoassay (CDI)</i></b></p>	<p><b>Contact: Stanley B Prusiner</b>  <b>University of California</b></p>
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**Abstract:**

Until the advent of transgenic mice expressing Bovine PrP, there was no practical and sufficiently sensitive bioassay for BSE prions. The back-titration of BSE prions in cattle is impractical due to the long incubation period (over 4 years for undiluted inoculum) and high cost of maintaining BSE-infected cows. As a result, previous efforts to bioassay potentially contaminated sources for BSE prions have been restricted to the use of inbred mice (C57Bl/C6 or RIII). These studies suffer due to a relative lack of sensitivity of these mice to native BSE prions derived from cattle. Transgenic mice which express Bovine PrP solve this problem by abrogating the species barrier to infection with Bovine prions. Although transgenic mice are the most efficient animal bioassay system available; a rapid and economical in vitro assay would be desirable as a practical alternative to bioassays, providing that a similar level of sensitivity can be achieved. Consequently, we will evaluate two complementary methods for detection of BSE and sheep scrapie prions. Our first objective is to calibrate our Bovine PrP transgenic mice for use in detection of BSE prions. The sensitivity of these transgenic mice to BSE and sheep scrapie prions will be established by endpoint titration of standardized BSE stock inocula and the data will be used to develop an incubation time period bioassay. The same inocula will be tested in parallel in RIII mice, to effect a direct comparison of the relative sensitivities of transgenic mice and inbred mice to BSE and sheep scrapie prions. Our second objective is to evaluate the conformation dependent immunoassay (CDI) for application in the detection of BSE prions. The CDI assay will be calibrated using standard inocula of known titer, determined by bioassay in transgenic mice. Once the CDI assay has been calibrated to standards of known titer, it will provide a rapid and sensitive method for detection of BSE prions.

<p><b>Project Title: Development of monoclonal antibodies against normal and disease-related isoforms of human prion protein</b></p>	<p><b>Contact: Professor J Collinge, MRC Prion Unit, London</b></p>
<p><b>Abstract:</b></p> <p>The occurrence of new variant CJD has dramatically highlighted the need for ante-mortem diagnostic tests for prion disease. It remains possible that a substantial epidemic of this disease will occur over the coming years. We are therefore faced with the possibility that significant numbers in the population may be infected and that they might pass it on to others via blood transfusion, blood products, tissue and organ transplantation and other iatrogenic routes. The prion diseases are all associated with the accumulation of a disease specific isoform of the cellular prion protein, designated PrP<sup>Sc</sup>, the detection of which in the brain is diagnostic. We propose to develop an extensive panel of high affinity monoclonal antibodies against both normal and disease related isoforms of human PrP by immunisation of PrP null mice with a range of recombinant derived human prion proteins and to use them for the development of immunodetection methods of sufficient sensitivity to detect PrP<sup>Sc</sup> in peripheral tissues, in particular in peripheral blood samples. The development of such a blood based diagnostic test will be extremely challenging but is essential for the management of this serious public health concern. Furthermore, should rational therapeutics become available for this group of diseases, early diagnosis, before extensive neurodegeneration has ensued, will be crucial.</p>	

## 4. Risk assessment of SEs

### 4.1. An evaluation of SEs transmission modalities from cattle to man and other food animals, environment vectors

<b>Project Title: SE1941</b> <b>Studies to examine the pathogenicity, phenotype and pathogenesis of endemic scrapie in cattle</b>	<b>Contact: Steve Ryder,</b> <b>VLA, Weybridge</b>
<b>Abstract:</b> There is a clear need to examine transmissions of Scrapie to cattle in the UK in the context of understanding the relationship of the BSE agent to that of TSEs. Such studies address principally two concerns for future control strategies: <ul style="list-style-type: none"><li>• the dynamics of the origin of the BSE agent.</li><li>• the potential occurrence of an endemic form of scrapie, resulting from feed borne exposure of domestic sheep populations to BSE via contaminated meat and bone meal, which could remain at least as pathogenic for other species as BSE</li></ul> We propose studies: <ul style="list-style-type: none"><li>• to establish by parenteral inoculation the pathogenicity for cattle of pools of sheep scrapie isolates sourced before and during the BSE epidemic and to determine the pathological phenotype of any disease established in cattle;</li><li>• to establish the pathogenicity for cattle, by oral exposure, of two different pools of sheep scrapie inoculi (segregated by PrP genotype into donor populations theoretically susceptible or resistant to BSE infection);</li><li>• to obtain indications of the tissue distribution and spread of infectivity, over the incubation period, in cattle infected by oral exposure, to the two different pools of sheep scrapie inoculum;</li><li>• to determine the strain typing characteristics in a standard panel of mice of isolates from those cattle which develop scrapie-like disease.</li></ul>	
<b>Project Title: SE1942</b> <b>The attack rate and phenotype of scrapie-like disease on transmission to cattle of fresh and rendered pools of scrapie</b>	<b>Contact: Christine Berthelin-Baker,</b> <b>VLA, Weybridge</b>

**Abstract:**

The main aim of this research is to establish attack rate and dose response data of any scrapie-like agent which proved pathogenic for cattle after oral exposure to pools of sheep scrapie inoculum (of known titre by mouse assay), before and after rendering to meat and bone meal. The study would furthermore determine the phenotypes of any infections established in cattle, both by comparison with data on the BSE phenotype in cattle and on subsequent strain typing in mice. The attack rate data achieved would also be compared to that obtained in cattle for BSE (SE1918, SE 1930). The potential occurrence of BSE presenting in sheep (as scrapie) as a result of foodborne exposure of domestic sheep populations to BSE via contaminated meat and bone meal has been considered and this study has the potential to identify this possibility in sheep which were likely to have been exposed to the agent via feed. This study addresses policy concerns regarding deficits in our knowledge of the relationship between BSE and scrapie, the occurrence of BSE in sheep (presenting as scrapie) after oral exposure during the BSE epidemic and potential food safety hazards should this have occurred. In the absence of identifying the BSE agent, a further outcome of this study may identify the pathogenicity of sheep scrapie for cattle after oral exposure, before and after rendering of the inoculum. Not only could there be differences in pathogenicity, but possible changes in phenotype of disease after rendering might also become evident. The results will contribute to and underpin policy decisions on food safety.

**Project Title: SE0213****An epidemiological study of sheep scrapie to determine means of natural transmission and role of PrP genotype****Contact: Linda Hoinville,  
VLA, Weybridge****Abstract:** The main objectives are:

- To understand the epidemiology of scrapie.
- To quantify the effect of and investigate the relationship between possible risk factors, including PrP genotype and parental scrapie status.
- To determine the effect of introducing resistant rams in a scrapie-affected flock.
- To investigate the dynamics of infection within flocks using a mathematical model which will be validated using data collected during the study and used to predict the impact of various control measures on scrapie incidence in order to provide advice to farmers.

The results of this research project will be an improved understanding of the aetiology and dynamics of scrapie within affected flocks. This will improve our ability to advise farmers with affected flocks on control policies, as well as to give general advice about prevention of scrapie. Control of scrapie is important because of the economic impact of TSEs on the livestock industry, animal welfare considerations and the potential human health risk if BSE has been transmitted and maintained in the sheep population.

<p><b>Project Title: SE1423</b>  <b>Transmission studies for the detection of BSE in sheep</b></p>	<p><b>Contact: Dr Nora Hunter</b>  <b>IAH, Edinburgh</b></p>
<p><b>Abstract:</b></p> <ul style="list-style-type: none"> <li>• The main objective is to look for evidence of BSE infection of sheep by carrying out transmissions to mice from sheep of PrP genotypes susceptible to BSE but resistant to scrapie.</li> <li>• The work will address MAFF policy requirements in relation to the risk of BSE to other species of domestic animals and epidemiological changes requiring further action.</li> <li>• Samples of brain tissue from scrapie affected animals will be collected and stored under conditions suitable for subsequent transmission.</li> <li>• PrP genotyping will be carried out on DNA made from the samples.</li> <li>• Transmissions to a panel of mouse lines will be initiated from up to ten individual brain samples from animals found to be of genotypes known to be susceptible to BSE.</li> <li>• Incubation periods and lesion profiles will be recorded from the mouse panel and compared with those obtained from similar previous studies of BSE and scrapie.</li> </ul>	

<p><b>Project Title: SE1821 Comparative efficiencies of the bioassay of BSE infectivity in cattle and mice.</b></p>	<p><b>Contact: Steve Hawkins, VLA, Weybridge</b></p>
<p><b>Abstract:</b> The study has three main aims</p> <ol style="list-style-type: none"> <li>1) To obtain a measure of the underestimation of the infectivity titre of BSE tissues when titrated across a species barrier in mice.</li> <li>2) To produce an approximate dose-incubation curve for infectivity of brain from clinically BSE affected cattle by simultaneous titration of a primary inoculum in cattle and mice.</li> <li>3) To provide order of magnitude estimates of concentrations of infectivity in selected tissues other than brain from clinically BSE affected cattle by testing in cattle at a single low dilution.</li> </ol> <p>A report is being prepared.</p>	

<p><b>Project Title: SE1828</b></p> <p><b>The exposure of British sheep and cattle to mites</b></p>	<p><b>Contact: Dr John Chambers</b>  <b>Central Science Laboratory,</b>  <b>Sand Hutton, York</b></p>
<p><b>Abstract:</b></p> <p>It has been suggested that mites found on sheep farms in Iceland could represent a self-sustaining reservoir for scrapie-like agents and could be involved in the continuing occurrence of BSE in the UK. The main objective of the current proposal is to establish whether there is a connection between TSEs in sheep and cattle and their exposure to mites and, if so, what can be done to reduce it. This will be done by assessing the exposure to mites, identifying the mite species and testing their ability to cause TSE-like symptoms. The proposed work lies within the policy objective to identify and eradicate causes of scrapie and BSE. The results will be used to ensure that studies to investigate whether TSE prions can be replicated in mites are conducted on mite species relevant to the UK situation. If there is a connection between mite presence and the incidence of TSEs a strategy to minimise the occurrence of mites on farms will be drawn up and evaluated. The results will be used to help MAFF formulate policy, guide industry to avoid the problem and alleviate the concerns of consumers.</p>	

<p><b>Project Title: SE1829</b></p> <p><b>Replication of scrapie and BSE prions in mites</b></p>	<p><b>Contact: Dr Alan D MacNicoll</b>  <b>Central Science laboratory , York</b></p>
<p><b>Abstract:</b></p> <p>A report recently published in the Lancet (Wisniewski <i>et al</i>, 1996, Lancet, 347, 1114) provides evidence of a role for mites in the transmission of scrapie. The report is of a very preliminary study and leaves many questions unanswered. This proposal relates to the question of whether or not prions can replicate in mites, such that they could become a significant source of infection, and whether the mites themselves may be the original source of prions leading in turn to scrapie, BSE and CJD. CSL and CVL expertise in, and facilities for, studying mites and spongiform encephalopathies will be used to evaluate the potential for replication of the scrapie prion in mites. The results will provide evidence on the role of mites in the transmission of spongiform encephalopathies, and is relevant to MAFF policies towards eradication of those diseases.</p>	

<b>Project Title: SE1433</b> <b>Studies on the Environmental Persistence of TSE infectivity</b>	<b>Contact: Dr Robert Somerville</b> <b>IAH, Edinburgh</b>
<p><b>Abstract:</b> To assess whether TSE infectivity can persist and migrate after disposal into the ground, a co-ordinated series of experiments will be conducted. Laboratory based experiments will examine the persistence and migration of infectivity through different soil types under various conditions. Secondly, a lysimeter based experiment will simulate, as far as is reasonably practical and safe to do so, the deposition of a bolus of infectivity in the ground. Survival and migration of infectivity from the source will be measured. Thirdly to assess the survival of infectivity within buried carcasses, a series of TSE spiked bovine heads will be buried. They will be serially exhumed and their residual TSE infectivity measured.</p>	

<b>Project Title: SE1840/ MO3010</b> <b>Further studies on the transmissibility of BSE to pigs</b>	<b>Contact: Dr S J Ryder</b> <b>VLA, Weybridge</b>
<p><b>Abstract:</b></p> <p>Further investigation of the possible transmission of BSE to pigs is proposed, involving investigation of the pathology by immunocytochemistry, investigation of the, presumably incidental, vacuolation of the rostral colliculi by examination of pigs from outside of the UK and of pigs alive prior to the appearance of BSE in 1986 by passage of the rostral colliculi of unchallenged control pigs into pigs parenterally challenged. In addition further examination will be carried out of the apparently normal brains from pigs orally challenged with BSE to confirm lack of transmission by this route.</p> <p>The results of this study will enable a more informed approach to the diagnosis of TSE in the pig to be taken, and will answer in greater depth the question of possible infection of the national pig herd by feeding contaminated MBM.</p>	

<p><b>Project Title: SE0219/MO3001</b>  <b>The epidemiology of TSEs in ruminants and assessment of possible associated risk to human health</b></p>	<p><b>Contact: Roy M Anderson, Department of Infectious Disease Epidemiology, Imperial College School of Medicine, London.</b></p>
<p><b>Abstract:</b> The primary objective of the research is the continued analysis of the UK BSE databases (maintained by Central Veterinary Laboratory (CVL) and the Department of Agriculture for Northern Ireland (DANI). Of particular interest is the fine-scale analysis of spatial heterogeneity in incidence and its relation to the underlying heterogeneity of infectivity in feed, the further characterisation of the age-dependent susceptibility and the distribution of incubation periods, and any direct evidence of maternal transmission or genetic predisposition to disease/infection in the database (i.e. possible overabundance of dam-offspring pairs of BSE cases). Herd-based models (requiring considerable additional effort) will also be constructed which would facilitate these analyses as well as the further analysis of possible culling strategies. The analyses would also facilitate the estimation of the pattern of human consumption of BSE-infected meat products (by stage of incubation and abnormal prion density).</p> <p>Parallel analysis will be carried out on the available data on scrapie in the UK. Long term data on scrapie cases in individual sheep flocks in Edinburgh will be analysed to determine the importance of both maternal transmission and genetic predisposition in the disease transmission process. In addition, the potential effect of BSE introduction into sheep flocks will be explored, with particular emphasis on the fact that the genetically determined susceptibility to scrapie of different breeds does not appear to correlate with susceptibility to the BSE agent.</p>	

<p><b>Project Title: MO3004 (formerly SE1817) Transmissibility of BSE to pigs by oral exposure to brain homogenate.</b></p>	<p><b>Contact: Steve Hawkins, VLA, Weybridge</b></p>
<p><b>Abstract:</b> To determine the transmissibility of BSE to pigs by oral exposure to brain homogenate from BSE affected cattle.</p> <p>No clinical signs have been observed in pigs, and no evidence of infectivity from any interim or final kills.</p>	

<p><b>Project Title: MO3003 (formerly SE1806) Transmissibility of BSE to domestic fowl by oral exposure to brain homogenate</b></p>	<p><b>Contact: Steve Hawkins, VLA, Weybridge</b></p>
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**Abstract:** The objective of this project is to determine the transmissibility of BSE to domestic fowl by oral inoculation of brain homogenate from BSE affected cattle. Selected tissues are assayed for infectivity by mouse inoculation and for histopathological examination.

**Project Title: MO3002 (formerly SE1805) Transmissibility of BSE to domestic fowl by injection with brain homogenate**

**Contact: Steve Hawkins, VLA,  
Weybridge**

**Abstract:**

The objective of this project is to determine the transmissibility of BSE to domestic fowl by injection with brain homogenate from BSE affected cattle. Selected tissues are assayed for infectivity by mouse inoculation, subpassage in domestic fowl and for histopathological examination.

**Project Title: MO3005 (formerly SE1822) Transmissibility of scrapie to pigs by oral exposure to brain homogenate**

**Contact: Steve Hawkins, VLA,  
Weybridge**

**Abstract:** This study has two main aims:

- 01) To determine the transmissibility of scrapie to pigs by oral exposure to brain homogenate from scrapie affected sheep.
- 02) To determine the principal tissue distribution and titre of agent during the incubation period.

#### 4.2. Extended surveillance programme on BSE and related diseases

<p><b>Project Title: SE0209</b> <b>Epidemiological studies of BSE and related TSEs</b></p>	<p><b>Contact: John Wilesmith, VLA, Weybridge</b></p>
<p><b>Abstract:</b> This project represents a continuation of previous research on the epidemiology of BSE and related TSEs. The main objectives are to provide a fuller understanding of the epidemiology of BSE, and related TSEs, notably to determine whether a means of transmission other than the feedborne source occurs, to understand the important factors in the propagation of the epidemic and to continue to investigate the origin of BSE. The results of the project will assist in the assessment of current and potential future control policies, assist in the identification of more targeted surveillance for BSE, and provide relevant information for scientific discussions on international trade of cattle and bovine products, notably within the European Union. In addition the improved understanding of the propagation of the epidemic could assist the understanding of the vCJD epidemic. Finally, the results of modeling will assist in preparing budgets for compensation for cases and destruction of carcasses.</p>	
<p><b>Project Title: SE0227</b> <b>An investigation of risk factors for scrapie at flock level</b></p>	<p><b>Contact: Alies Hoek, VLA, Weybridge</b></p>
<p><b>Abstract:</b> The main objective of this project is to identify risk factors for introduction and maintenance of scrapie in sheep flocks in the UK.</p> <p>The results of this research project will be an improved understanding of the dynamics of scrapie between flocks. This will improve our ability to advise all sheep farmers on possible measures to prevent introduction of scrapie into their flock and sheep farmers with a scrapie affected flock on possible measures to prevent the maintenance of the disease in their flock.</p> <p>Control of scrapie is important because of the economic impact of TSEs on the livestock industry, animal welfare considerations and the potential human health risk if BSE has been transmitted to and maintained in the sheep population.</p>	

<b>Project Title: SE0228</b> <b>Investigations of the transmission of Scrapie within and between flocks</b>	<b>Contact: Dr Cerian Webb, VLA, Weybridge</b>
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<p><b>Abstract:</b> The main aim of this project is to evaluate strategies for the control of scrapie in the British sheep population. This will be achieved by the development of a mathematical model to represent the transmission and maintenance of scrapie infection within and between flocks. The model will be used to address three key questions:</p> <ul style="list-style-type: none"> <li>• How is scrapie transmitted within and between flocks in Great Britain?</li> <li>• What impact will different control policies have on the level of scrapie in the GB sheep population?</li> <li>• How much will each policy cost the farmer, the industry and the government?</li> </ul>	
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<b>Project Title: Epidemiology of scrapie in sheep</b>	<b>Contact: Dr A McLean, IAH, Compton / Oxford University.</b>
<b>Abstract: <i>Abstract is currently unavailable</i></b>	

<b>Project Title: Analysis of natural scrapie outbreaks in Suffolk sheep</b>	<b>Contact: Professor M E J Woolhouse, Edinburgh University</b>
<p><b>Abstract:</b> This project concerns a model-based analysis of two databases describing outbreaks of scrapie in Suffolk sheep. The aims are: to apply recently developed mathematical models to field data; to determine the range of parameter values consistent with the data; to relate these findings to current understanding of scrapie pathogenesis; to compare the dynamics of scrapie outbreaks in Suffolks and Cheviots; and to explore the potential impact of scrapie control measures. Different hypotheses concerning possible differences in the incubation period between genotypes, different stages at which sheep are infectious, and whether clinically 'resistant' sheep can act as carriers will be tested for consistency with the epidemiological data.</p>	

### 4.3. Identification of covert disease in cattle

<b>Project Title: SE0225</b> <b>The diagnosis and neuropathological monitoring of suspect BSE cases</b>	<b>Contact: Dr Marion Simmons, VLA, Weybridge</b>
<p><b>Abstract:</b> This proposal forms a logical continuation of previous similar studies (SE0203, SE0216 and SE0220 (ongoing)), and suspect cases born on or after 01/01/94 will be assessed using the same methods as these, together with well-established PrP detection assays. Broadly, the lesion distribution and severity (the lesion profile) in 100 positive cases will be determined by a previously validated semi-quantitative method. A sample of clinical suspects in which BSE was not confirmed (up to a maximum of 140 cases) will also be examined for evidence of an alternative neurohistological diagnosis or an atypical presentation of BSE. All cases will also be sampled for SAF and Western blotting analysis and the obex section stained immunocytochemically (ICC) for protease-resistant PrP.</p> <p>In addition, the examination of field cases born in 1993, which are currently under study within SE0220, will be extended to include ICC and Western blotting using archive material, providing a further 240 cases for the comparison of diagnostic sensitivity and specificity. These results will be compared with the results from the earlier studies, and also with the results from a similar comparative diagnostic study in sheep scrapie currently underway at CVL (TS4002).</p> <p>Apart from confirming, or refuting, the theory that the BSE epidemic continues to be sustained by a single stable strain of BSE agent, this also provides a background against which the profiles of other cases of BSE (e.g. cases arising outwith the UK) can be compared, to see if the same strain of agent is responsible, without the need for rodent strain-typing.</p>	

#### 4.4. Determination of the infectivity titres in cattle tissues and cattle derived products entering the human food chain or used in pharmaceutical and cosmetics products

<p><b>Project Title: SE0229</b></p> <p><b>The effects of tallow separation and solvent extraction on PrPSc in the rendering process: a laboratory-scale study</b></p>	<p><b>Contact: Roy Jackman, VLA, Weybridge</b></p>
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**Abstract:** A laboratory-scale apparatus will be designed and constructed to allow the fate of PrPSc in animal carcass remains to be studied in a simulation of the commercial rendering process employing tallow separation and solvent extraction. The primary objective is to determine whether infectivity or the PrPSc molecule itself is inactivated or partitioned during the process.

Changes to methods of tallow extraction after rendering has been held as a major contributory factor in the exposure of the cattle population to TSE infection through contaminated meat and bone meal. It is not clear which part of the process offered protection from the spread of the agent, nor whether this was due to inactivation or partition into the tallow and related extracts.

It is essential to determine whether any of the side products of rendering, such as tallow, contain infectivity and to quantify any partitioning or inactivation that occurs during the process.

<p><b>Project Title: SE1755</b></p> <p><b>Detection and identification of species-specific nucleic acids in rendered animal material</b></p>	<p><b>Contact: Dr T C Martin VLA, Weybridge</b></p>
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**Abstract:** MAFF has introduced a complete exclusion of mammalian meat and bonemeal from all farm animals' feed (The Bovine Spongiform Encephalopathy (Amendment) Order 1996 SI 1996 No. 962). In order to ensure that feed companies comply with this ban, specific methods that can detect and differentiate between mammalian proteins are required. This proposal offers a possible solution to this problem.

The objective of this project is to develop a flexible, specific, sensitive and cost-effective assay to detect and differentiate between different species protein (sheep, cattle, pig and chicken) in animal feedstuffs.

The proposed assay is based on the detection of nucleic acids and, when developed, would complement the existing immunoassay by acting as a backup (confirmatory) test - or it could be used as a stand-alone assay.

<p><b>Project Title: MO3011 (formerly SE1901) Pathogenesis of experimental BSE in cattle</b></p>	<p><b>Contact: Steve Hawkins, VLA, Weybridge</b></p>
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**Abstract:**

The objective of this project is to determine the temporal and spatial development of infectivity and pathology following oral exposure of calves to a single 100g dose of affected cattle brain homogenate.

**Project Title: MO3006 (formerly SE1824)**  
**Bioassay of BSE infectivity in non-neural tissues by intracerebral inoculation of cattle**

**Contact: Steve Hawkins, VLA,  
Weybridge**

**Abstract:**

To determine, qualitatively, infectivity in certain tissues, including SBM, of cattle infected orally with 100g BSE-affected brain and killed at specified times after exposure, using a within species bioassay. This provides the most sensitive means of detection of infectivity in the tissues in order to identify public health risks from exposure to bovine tissues.

The study provides the most sensitive available assay of BSE infectivity in bovine tissues which form a potential human health risk or have been identified as SBM. The results will indicate qualitative differences in the use of cattle versus mice for bioassay of BSE infectivity in experimentally, orally infected cattle. They will also provide information for use in quantitative analysis of risks of human exposure to infected bovine tissues and necessity to retain restrictions on SBM entering the human food chain.

**Project Title: MO3007 (formerly SE1825)**  
**Bioassay of BSE infectivity in further tissues by intracerebral inoculation of cattle (extension to SE 1824)**

**Contact: Steve Hawkins, VLA,  
Weybridge**

**Abstract:**

To determine, qualitatively, infectivity in certain tissues, including SBM, of cattle infected orally with 100g BSE-affected brain and killed at specified times after exposure, using a within species bioassay. This provides the most sensitive means of detection of infectivity in the tissues in order to identify public health risks from exposure to bovine tissues.

The study provides the most sensitive available assay of BSE infectivity in bovine tissues which form a potential human health risk or have been identified as SBM. The results will indicate qualitative differences in the use of cattle versus mice for bioassay of BSE infectivity in experimentally, orally infected cattle. They will also provide information for use in quantitative analysis of risks of human exposure to infected bovine tissues and necessity to retain restrictions on SBM entering the human food chain.

#### 4.5. The potential exposure of the human population

<b>Project Title: Prevalence studies in haemophiliacs</b>	<b>Contact: Professor C Lee, Royal Free Hospital, London</b>
<b>Abstract: <i>Abstract is currently unavailable</i></b>	

#### 4.6. Determination of the level of meat contamination by brain / spinal cord after standard butchering procedures

<b>Project Title: SE1826/ MO3008</b> <b>Measures to reduce contamination of meat and environment with CNS tissue during slaughter and processing of cattle and sheep (REMCORD)</b>	<b>Contact: Andy Knight, Silsoe Research Institute, Bedfordshire</b>
<b>Abstract:</b> MAFF provides funding to the Silsoe Research Institute to undertake work which feeds into EU funded research, with a number of collaborators (REMCORD).  Silsoe Research Institute's work will include: assessing indirect measurement of cross-contamination by the use of tracer dyes injected into the CNS and developing aerosol sampling techniques;  - providing engineering advice, monitoring equipment and expertise for other partners to use in determining routes of cross-contamination and evaluating proposed improvements.  - concurrently, innovative equipment will be developed and tested for their ability to reduce or eliminate cross-contamination. Practices or equipment that decrease the risks under experimental will be under commercial conditions for economic and practical viability.  New practices or equipment will be assessed and demonstrated to industry. A set of guidelines for avoidance of CNS cross-contamination in the meat industry will be drafted for presentation to the EU.	

#### 4.7. Determination of oral feeding and intracerebral dose responses to BSE agent and whether multiple dose is cumulative

<b>Project Title: SE1842 Comparative titration of Infectivity of BSE in Sheep and Mice</b>	<b>Contact: Dr Hugh Reid, Moredun Research Institute, Scotland</b>
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**Abstract:** This experiment aims to obtain a measure of the underestimation of the infectivity of sheep BSE tissues when titrated across a species barrier in mice. An approximate dose-incubation curve for infectivity of brain from clinically BSE-affected sheep will be produced by simultaneous titration of a primary inoculum in sheep and mice. The relative infectivity of BSE-affected sheep CNS and spleen will also be determined by bioassay in R111 mice.

**Project Title: SE1918**  
**Effect of oral inoculum dose on attack rate and incubation period of BSE in cattle**

**Contact: Steve Hawkins, VLA, Weybridge**

**Abstract:** This study has three objectives (continuing the work done in SE1902)

1) to determine the attack rate and incubation period of BSE in cattle exposed orally to four different dose levels of brain homogenate from affected cattle.

2) to determine also if there is a dose-response effect on the incubation period.

3) To establish the effect of multiple exposures on attack rate and incubation period.

Note that this project is nearing completion.

**Project Title: SE1930**  
**Further studies of the effect of oral inoculum dose on attack rate and incubation period of BSE in cattle.**

**Contact: Steve Ryder VLA, Weybridge**

**Abstract:**

The transmissible spongiform encephalopathies (TSE) are unusual, long-incubation-period diseases for which the infectious agent, and the dynamics of its replication relative to dose, have not yet been fully characterised. The infective dose of the ingested scrapie-like pathogen necessary to produce the perceived attack rate in the BSE epidemic is unclear. Although addressed only in part by this study, the experiment provides a baseline for further studies toward an understanding of what constitutes effective exposure in the field.

The study continues work carried out in ROAME SE1918, where preliminary results indicate that the limiting dose for infectivity has not been reached using dose levels of 1g to 3x100g

The study aims to determine the attack rate and incubation period of BSE in cattle exposed orally to lower dose levels of the same pooled brainstem homogenate from affected cattle. The dose range will be 1mg to 1g

**Project Title: SE1841**  
**Stochastic dynamics of TSE agents**

**Contact: Dr Chris Bostock, IAH, Compton**

**Abstract:** The objective of this project is to design an experiment (or experiments) which will address the question of the accumulation of risk of infection with accumulated small doses of BSE. This will be achieved using mathematical models of the within host-dynamics of TSE agents.

Using NPU archive data on 76 titration curves, meta analysis describing 76 titration curves aligned around their individual LD50s will be compiled to describe the relationship between dose and incubation. Stochastic models will use this set of observations in order to describe the observed relationship between dose and incubation period. The distribution of incubation periods will be of particular interest for each dose. Using this information, BSE titration curves will be compared with scrapie titration curves using Diringer's data which compares the outcome of giving 10 serial doses at one tenth dilution with one large dose

#### 4.8. Investigation of possible biological mechanisms of maternal transmission of BSE

**Project Title: SE1424**

**The study of BSE in sheep and the possibility of its vertical transmission**

**Contact: Dr J Foster  
IAH, Edinburgh**

**Abstract:** This study will compare the responses to BSE challenge of sheep which differ in PrP genotype at codon 171 and which are resistant to natural scrapie. The effect of genotype of dam and offspring on the potential for vertical transmission of BSE will be examined and the study will also provide further information about the susceptibility of RR<sub>171</sub>, RQ<sub>171</sub> and QQ<sub>171</sub> sheep to BSE challenge.

Genotyped ewes will be challenged with BSE just after mating (natural or AI) with genotyped rams. Resultant progeny and their dams will be monitored for clinical signs of disease and selected tissues will be recovered from clinical and non-clinical cases for agent detection/typing using mouse bioassay.

The results will help to elucidate the pathogenesis of BSE in sheep by establishing whether BSE could be maintained naturally within a flock and, therefore, could identify possible public health hazards.

<p><b>Project Title: SE1801</b></p> <p><b>Bovine Spongiform Encephalopathy: embryo transfer studies (in cattle)</b></p>	<p><b>Contact: Dr A E Wrathall</b> <b>VLA, Weybridge</b></p>
<p><b>Abstract:</b></p> <p>Transmission studies with bovine embryos were recommended in 1989 by the MAFF Consultative Committee on Research on Spongiform Encephalopathies, and the project started in October that year.</p> <p>The main objective is to show that embryos from BSE-affected cattle can be transferred into healthy, uninfected recipients without transmitting the disease.</p> <p>Successful demonstration that the risk of BSE transmission via embryos is negligible will reassure potential customers abroad that genetic material from British cattle is safe. It will also provide an effective way of salvaging genetic material from valuable, BSE-infected cattle.</p> <p>The project is scheduled to finish in June 2001, but so far there has been no evidence of BSE transmission. The plan is to monitor the cattle for BSE for seven years, then to pathologically examine them.</p>	

<p><b>Project Title: SE1834</b></p> <p><b>The role of the pre-implantation embryo in the vertical transmission of natural scrapie infection</b></p>	<p><b>Contact: Dr C Low</b> <b>Scottish Agricultural College</b></p>
<p><b>Abstract:</b></p> <p>The objective of this project is to determine the role of the pre-implantation stage embryo in the vertical transmission of natural Scrapie.</p> <p>Scrapie is one of a group of diseases called transmissible spongiform encephalopathies (TSEs). The presence of TSEs in the national flock costs the agricultural industry millions of pounds in lost revenue. The UK also loses further money in agricultural compensation. The possibility of the TSE contamination of human foodstuffs has also given rise to widespread public health concerns.</p> <p>Once the mode of vertical transmission of natural Scrapie infection is determined the results can be used as follows:</p> <ol style="list-style-type: none"> <li>a) To provide advice and a model to allow the possible reduction in levels of TSE infection in the national flock. This information will supplement earlier MAFF funded BSE transmission studies at High Mowthorpe.</li> <li>b) To allow informed decisions to be made by import/export regulatory authorities on the relative risks of importing disease in embryos from infected flocks/countries.</li> <li>c) Possibly increase UK agricultural exports.</li> </ol>	

<b>Project Title: SE1819 BSE Embryo transfer studies</b>	<b>Contact: Dr L Heasman, ADAS, High Mowthorpe.</b>
<b>Abstract:</b> This project involves the management of cattle which are used in another experiment, SE1801	

<b>Project Title: SE1823 Investigation of the aetiology of scrapie transmission in sheep</b>	<b>Contact: Nora Hunter IAH, Edinburgh</b>
<p><b>Abstract</b></p> <p>The MAFF Open Contract on <i>Investigation of the role of the embryo in maternal transmission of scrapie in sheep</i>, has now formally come to an end although the work initiated has to go on for another six years to be completed.</p> <p>There are at present 121 surviving sheep of various ages and Sip/PrP genotypes which, as part of the experiment, are being maintained by IAH.</p> <p>Important observations from these sheep which relate to the aetiology of scrapie:</p> <p>There is the probability that scrapie is an infectious disease and does not have a purely genetic origin. This is being assessed by the longevity of highly susceptible sheep (Sip sAsA, currently &gt;1350 days old), which had they been born and reared normally in the NPU Cheviot flock could be expected to develop natural scrapie at about 800 days of age.</p> <p>A better understanding of the timing of scrapie transmission from an infected ewe to her offspring, either as a result of <u>in utero</u> or peri-/ post-natal infection may emerge. It is essential that the work which has already been funded should be maintained until completion over the next six years by additional funding.</p>	

<b>Project Title: SE1919 Studies to identify possible homologies between scrapie agents in the British sheep population and the agent of BSE</b>	<b>Contact: Steve Ryder VLA, Weybridge</b>
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**Abstract:**

The proposed study has one aim: to determine whether there is any evidence that the BSE agent has become endemic in the British sheep population.

This proposal uses mouse transmission and strain typing of scrapie-like agents to detect isolates from individual cases and defined pools of scrapie-affected sheep brains and to selectively identify an agent with typing characteristics of the BSE agent, if present.

Two temporally separated samples of suspected natural scrapie cases will be acquired. The first sample of approximately 400 brains from scrapie-affected sheep will be harvested from suspects born after 01.01.1991 in order to reduce the risk of food-borne exposure, i.e. infection would be assumed to be endemic. Samples will be subject to histopathological examination and PrP genotypes will be determined for histopathologically confirmed scrapie cases. Two sheep brain homogenate pools will be prepared based on genotype (A<sub>136</sub>Q<sub>171</sub> or V<sub>136</sub>Q<sub>171</sub>). It is assumed that the former homogenate pool will be from sheep genetically susceptible to BSE but resistant to scrapie and the latter from sheep genetically susceptible to scrapie. Standard methods of strain typing for each homogenate will be carried out at primary passage in a panel of mice (R/III, C57BL and VM) (20 mice/group) injected i.c. and i.p. Secondary passage of material from successful transmissions in VM mice will be undertaken in further VM mice for additional characterisation. Serial passage of each resultant mouse transmission in more mice may also be performed.

The second sample for suspect scrapie cases will be taken from sheep born after March 1996. Brain, spleen and lymph node samples from this later cohort will be collected also and subject to the same protocol. This sample of cases is to allow acquisition of BSE cases in sheep with an incubation period greater than that proposed in the initial sample.

In addition, single brain assays will be undertaken on the basis of one brain per genotype per farm using PrP homozygotes only.

## 5. Treatment and prevention of SEs

### 5.1. Assessment and development of inactivation procedures currently used in industry

<b>Project Title: SE1425</b> <b>Inactivation of TSE agents by a novel biorefinement system</b>	<b>Contact: Dr Robert Somerville</b> <b>IAH, Edinburgh</b>
<b>Abstract:</b> Rendering processes fail to inactivate TSE infectivity and this failure is thought to have contributed to the spread of BSE. A novel system of biorefinement, involving high pressure steam which processes the tissue at 190° C has been developed. It produces mineral and protein products which are still useful as fertilisers or animal feed products. We propose to test the survival of TSE infectivity in the system. If validated, there is the potential for using this system for processing large animal carcass waste and the UK meat and bone meal mountains for use or for safe disposal.	

<b>Project Title: SE1434</b> <b>Pressurised steam with alkaline hydrolysis as a means of inactivating TSE agents</b>	<b>Contact: Dr Robert Somerville</b> <b>IAH, Edinburgh</b>
<b>Abstract:</b> Neither autoclaving nor exposure to alkali reliably and totally inactivate TSE infectivity but their combination is very effective. An American company, WR <sup>2</sup> , has developed a system that combines these procedures in a system that is capable of quickly and completely digesting large animal carcasses. We will validate whether this system completely destroys TSE infectivity. If so, it will provide a commercial alternative to the incineration of large animal carcasses, SRM and MBM	

<p><b>Project Title: SE 0224</b></p> <p><b>Inactivation of the causative agents of Transmissible Spongiform Encephalopathies by Thermophilic and Hyperthermophilic Proteases</b></p>	<p><b>Contact: Dr Neil Raven, Centre for Applied Microbiology and Research, Porton Down, Wiltshire</b></p>
<p><b>Abstract:</b> The main objective of the proposal is to demonstrate the inactivation of the infective agents implicated in the causation of transmissible spongiform encephalopathies (TSEs) by the action of highly thermostable proteolytic enzymes. This will be measured using the most stringent assay available, namely the intracerebral inoculation of VM mice with protease- treated high titre infectivity stocks from a mouse-passaged strain of the most thermostable TSE variant reported BSE (301V). The proposal contributes to an EU supported collaboration involving, the group of Prof. Garo Antranikian in Hamburg, who have considerable expertise in the purification and characterisation of thermostable proteases from hyperthermophiles and the group of Prof. Rossi in Naples who have expertise in the cloning and expression of genes from hyperthermophiles to permit large scale production.</p> <p>The proposal involves the growth of a diverse range of thermophilic, thermoacidophilic, hyperthermophilic, and thermoalkalophilic microorganisms from both the archaeal and bacterial domains. CAMR have already developed novel strategies for the cultivation of thermophiles and hyperthermophiles to high cell densities which will allow production of sufficient material for the initial characterisation of extracellular proteases. This will enable the effect of high temperature proteolysis on the inactivation of TSE agents to be studied over a wide range of pH. A breeding colony of mice will be established which will be used to provide a supply of high titre infectious mouse brain for inactivation tests. Purified proteases will be screened for pH and temperature optima, sensitivity to protease inhibitors and substrate specificity. Optimised conditions will then be used to study the proteolysis of high-titre mouse passaged BSE agent present in the infected mouse brain homogenate. This will be monitored by 2D-gel electrophoresis of total protein looking for elimination of the spot associated with the misfolded form of PrP. Mouse brain homogenates showing significant depletion of the abnormal protein will be tested for complete inactivation of the TSE agent by intracerebral injection into mice. Those enzymes indicating inactivation of TSE agents will be cloned by the group of Mose Rossi to enable larger quantities of enzyme production for use in subsequent studies using infected bovine brains.</p> <p>Currently, as recognised by MAFF, inactivation of TSE agents is an intractable problem, whether in medical or veterinary research, food handling and processing, or the pharmaceutical and cosmetic industries. Reliable inactivation of TSE agents using highly thermostable proteases will be a major advance in several areas. For processes involving potentially infected animal materials (food, pharmaceutical and cosmetics industries) it will allow the safe recovery of non-proteinaceous components rather than their destruction. It will also permit the introduction of effective non-destructive disinfection cycles for most standard equipment used in handling and processing. In medical and veterinary applications, highly thermostable proteases will provide a research tool for the characterisation of causative agents of TSEs and allow a greater range of techniques to be used in their study, since dedicated equipment will not need to be destroyed after use.</p>	

Project Title: Validation of TSE decontamination	Contact: Dr N Raven, Centre for Applied Microbiology and Research, Porton Down, Wiltshire
<b>Abstract: <i>Abstract is currently unavailable</i></b>	

Project Title: Removal or destruction of TSE agents by absorption onto zeolite	Contact: Dr R Somerville, IAH, Edinburgh
<b>Abstract: <i>Abstract is currently unavailable</i></b>	

Project Title: Surgical instrument damage during decontamination procedures	Contact: Karen Fernie, IAH Edinburgh
<b>Abstract: <i>Abstract is currently unavailable</i></b>	

Project Title: The relative resistance to inactivation of different TSE agent strains	Contact: Dr R Somerville, IAH Edinburgh
<b>Abstract: <i>Abstract is currently unavailable</i></b>	

Project Title: The development, validation and application of a model to study and improve sterilisation of prion-contaminated surgical instruments	Contact: Professor J Collinge, MRC Prion Unit
<b>Abstract: <i>Abstract is currently unavailable</i></b>	

<p><b>Project Title: Adsorption of prion isoforms to stainless steel surfaces: implication for surgical decontamination procedures</b></p>	<p><b>Contact: Professor C. Lowe, University of Cambridge</b></p>
<p><b>Abstract:</b></p> <p>A magnetic acoustic resonator sensor (MARS) will be used to investigate the adsorption of normal and abnormal prion isoforms onto stainless steel and also their desorption under a variety of washing conditions. Infectivity of immobilised prion protein will be tested. Chemical modifications of the steel in order to reduce or eliminate adsorption will be investigated.</p> <p>The detailed protocol is as set out in the application form submitted to the Department of Health. Funding will be provided initially for the first 3 years of work but a review after 12 months will determine whether to extend funding for a further 2 years.</p> <p>Overall, the 5 year project would cover:</p> <ul style="list-style-type: none"> <li>* preliminary assessment of stainless steel samples</li> <li>* prion adsorption onto stainless steel</li> <li>* surface modification of stainless steel</li> <li>* dynamic changes in the adsorbed prion proteins</li> <li>* infectivity studies on stainless steel surfaces</li> <li>* development of high throughput prp immunosensors</li> </ul> <p>Detailed milestones for the first 3 years are attached as an annex.</p>	

<p><b>Project Title: Proposal to investigate the efficiency of decontamination of surgical instruments and recommended improved procedures.</b></p>	<p><b>Contact: Professor D Perrett, St Bartholemews and the Royal London School of Medicine and Dentistry</b></p>
<p><b>Abstract:</b></p> <p>The normal use of surgical instruments is accompanied by adsorption of tissue materials on those surfaces of the device that come into contact with the tissue and/or blood. The compounds deposited could be proteins, nucleic acids, oligosaccharides and lipids. In light of the emergence of vCJD, a qualitative and quantitative study to determining the removal of such macromolecules could be used to monitor the efficiency of the washing and decontaminating procedures. Proteins are the major macromolecule component of tissues. It is well known that proteins adsorb with varying tenacity to surfaces.</p> <p>This proposal aims to study, in detail, methods of removing biological materials, particularly protein, from surgical instruments and other instruments. Following an evaluation of high sensitive protein assays, the most sensitive will, in the first instance, be used to monitor the removal of model proteins from various stainless steel and other surfaces. The removal efficiency of various cleaning procedures in biological systems will be evaluated using test pieces. The second objective is to develop easily applied screening tests for assessing efficiency of cleaning in a CSSD setting. Qualitative investigations using MALDI-ToF MS will be undertaken to understand the nature of the absorbed proteins.</p>	

## 5.2. Development of therapeutic approaches

<p><b>Project Title: SE1758</b></p> <p><b>To maintain a nucleus flock of scrapie resistant Swaledale sheep, to use this resource to further refine a PrP genotype test, and to quantify the relationship between PrP genotype and other important production traits.</b></p>	<p><b>Contact: Brian Merrell</b> <b>ADAS Consulting Ltd.</b> <b>Newcastle-on-Tyne</b></p>
<p><b>Abstract:</b> Government aims to safeguard human and sheep health and to improve the efficiency and economic performance of the livestock industry. Scrapie is a progressive, fatal, degenerative disorder of the central nervous system, and it is estimated that up to one third of the UK flocks may be affected by the disease causing economic losses through the death of breeding stock, higher culling and hence higher replacements rates, and through the disruption of international trade, particularly in breeding animals. It has been established that not all animals are equally susceptible to scrapie and that high susceptibility appears to be genetically linked. A flock of resistant (low susceptibility) Swaledale sheep has been maintained at ADAS Redesdale (Project SE1713) since 1975 to provide a source of sheep which give a predictable response to induced scrapie and which can be used to study the disease. It also produces a source of scrapie resistant rams, which are returned to the industry as a practical means of reducing the incidence of scrapie in affected flocks thereby helping to safeguard sheep health. The nucleus flock maintained to (1) act as an important source of genetically resistant breeding stock for use by the industry and to allow further improvement in breed characteristics to be made such that pedigree breeders used rams bred from the nucleus thereby increasing considerably the dissemination of scrapie resistance, (2) act as a research base to further refine the current gene-marker test, specifically to quantify the role of the AHQ allele in the development of the disease, (3) to quantify the effects of selecting for scrapie resistance on other important production traits, particularly lamb survival and growth rates, (4) to act as a research base for evaluating new potential tests under controlled conditions, and (5) to act as a resource which can be used to address future issues as they relate to scrapie and its control mechanisms in sheep. The project provides information, which addresses the concerns of commercial producers with respect to genotyping and selective breeding for scrapie resistance</p>	

<b>Project Title: New inhibitors of PrP-res formation</b>	<b>Contact: Dr I H Gilbert, Cardiff University</b>
<p><b>Abstract:</b> There is an urgent need for the development of drugs for the treatment and prophylaxis of transmissible spongiform encephalopathies (TSE) such as CJD and variant CJD. There are no current drugs available to treat these diseases; however there are a number of lead compounds, such as azo dyes, sulphated polysaccharides, polyamines, porphyrins etc. None of these compounds are suitable as drug candidates due to toxicity, poor uptake across cell membranes (and in particular the blood brain barrier) and lack of in vivo activity in rodent models of infection.</p> <p>Our starting point has been the azo dye Congo red. Whilst this compound is not a suitable drug candidate, it is a good starting point for drug design. It is a low molecular weight compound, which is amenable to structure activity relationship studies. Particular problems with Congo red are its poor permeability across the blood brain barrier and inherent carcinogenicity due to metabolism to the known carcinogen benzidine. We have carried out structure activity studies on the compound and shown a number of points of possible variation in the structure. In particular, it may be possible to replace the benzidine moiety with other less toxic groups and to replace the highly charge sulphonic acid groups with less charged carboxylate groups, hence increasing cell permeability.</p> <p>In this project we plan to exploit this information and design compounds which should have lower toxicity and better blood brain barrier permeation. Compounds will be tested against a persistently scrapie-infected cell line and in a cell-free assay system. Computer modelling will be used to rationalise the data and to aid in the drug design process.</p>	

<b>Project Title: A system for cloning factors involved in PrPRes accumulation</b>	<b>Contact: Dr J G Connolly, University of Strathclyde</b>
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**Abstract:** The aim of this proposal is to develop a cell based system whereby novel protein factors involved in the disease processes of Transmissible Spongiform Encephalopathies (TSE's) can be functionally cloned. This study will attempt to identify factors involved in protease resistant prion protein (PrP<sup>Res</sup>) formation and disassembly, and also create a clinically relevant model in which the mode of action of therapeutic drugs can be evaluated.

To achieve this, we will overexpress epitope tagged PrP<sup>C</sup> along with brain poly A+ RNA in non-mammalian cells. The cells will then be additionally injected with infectious scrapie (PrP<sup>Sc</sup>). Western blotting will then be used to detect the formation of new, epitope tagged, PrP<sup>Res</sup> material. If control experiments confirm that the new PrP<sup>Res</sup> formation is dependent upon the co-injection of Poly A+ RNA, then it will be possible to functionally clone those protein factors (e.g. protein X) which catalyse the conversion of PrP<sup>C</sup> to PrP<sup>Res</sup>/Sc. We may also be able to functionally clone factors which are involved in PrP<sup>Res</sup> disassembly. Eventually, if it proves possible to produce PrP<sup>Res</sup> in the model system, the newly formed material will be tested for infectivity.

Another use of the model will be to help understand the mechanism of action of therapeutic drugs. If PrP<sup>Res</sup> can be produced, then drugs will be tested for their ability to inhibit this production. However we will also transplant brain scrapie material into the cells by injection of PrP<sup>Sc</sup> inocula. Drugs can then be tested for their ability to destabilise the PrP<sup>Sc</sup> complex in the absence of new PrP<sup>Sc</sup> formation. Thus drugs which destabilise PrP<sup>Sc</sup> can be distinguished from those which prevent its accumulation.

If successful, this model may contribute not just to the understanding and treatment of TSE's, but also to other amyloid diseases such as Alzheimer's Disease and Huntington's Chorea.

**Project Title: Defining a role for therapeutic immunisation in Prion disease**

**Contact: Dr S Hawke, ICSM at St Mary's Hospital, London**

**Abstract:** Transmissible spongiform encephalopathy (TSE) is a serious potential health risk in the UK without any effective treatment. The aim of this work is to demonstrate that disruption of the interaction between PrP<sup>C</sup> and PrP<sup>Sc</sup> by immunological means will influence the course of TSE in mice, preliminary to developing therapeutic immunisation in variant CJD.

**Project Title: Will clusterin modify the progression of TSEs**

**Contact: Professor N Edington, Royal Veterinary College, London**

**Abstract:** Mouse adapted BSE will be given to C57bl mice as a model of prion induced spongiform encephalopathy. The objective is to then increase levels of clusterin both peripherally and in the CNS at different, or several, time points in the incubation period in order to investigate whether increased clusterin levels will modify the time of onset or the progression of prion disorder in the CNS.

The work is based on our observations that in vivo bovine clusterin has a similar localisation to abnormal PrP in BSE, and that in vitro clusterin will prevent aggregation of the 106-126 neuropeptide which is common to both abnormal PrP and to amyloid beta of Alzheimer's Disease.

TGF beta 1 will be used to increase levels of clusterin since this has been established as an activator of clusterin mRNA in astrocytes by several different groups. The specificity of any ameliorating action of TGF beta 1 operating via clusterin will be investigated by the use of clusterin knock out C57 bl mice.

**Project Title: Investigation of pentosan polysulphate as a potential prophylactic agent against the transmission of nvCJD by blood products**

**Contact: Dr. C. Farquhar,  
IAH Edinburgh**

**Abstract:**

This project aims to evaluate whether pentosan polysulphate (PPS) has general efficacy in reducing susceptibility to TSE agents at doses, and by routes, that are clinically relevant and without deleterious effects. PPS is known to reduce susceptibility to scrapie in animal studies when administered at, or close to, the time of infection and may therefore have practical application where the timing of potential exposure to vCJD in a laboratory accident or by blood transfusion, blood products or surgery is known.

The efficacy of a number of doses and routes of PPS administration will be analysed with regard to their effect on the susceptibility of rodents to a range of TSEs. The tissue and cellular location of PPS and its metabolites will be determined. The *in vivo* effects of PPS on PrP accumulation will be determined by western blotting analysis.

This joint project combines experienced TSE researchers with those from transfusion medicine. Both groups have published studies using PPS.

<b>Project Title: Genetic and biochemical analysis of prion elimination in yeast.</b>	<b>Contact: Professor M F Tuite, Kent University</b>
<b>Abstract:</b> The realisation that PSI, a non-Mendelian genetic determinant of yeast, actually represents a prion-like state of the Sup35p protein has opened up an entirely new genetic approach to the study of prions. The [PSI] prion will be used to determine the mechanism by which certain non-mutagenic compounds (e.g. guanidine HC1) can eliminate yeast prions. The cellular response of yeast to such chemicals will be characterised paying particular attention to the stress response. The importance of heat shock proteins in the maintenance and curing of [PSI] will be defined using both in vitro and in vivo assays. Finally, genetic screens will be used to isolate non-curable forms of [PSI]. A full understanding of the mechanism of prion curing in yeast will facilitate the development of a chemical strategy for curing mammalian prions.	

### 5.3 Generation of cattle and sheep devoid of PrP

<b>Project Title: The effect of leucocyte depletion on the generation and removal of microvesicles and prion-related protein in blood components</b>	<b>Contact: Dr T Wallington, NBS, Bristol</b>
<b>Abstract: <i>Abstract is currently unavailable</i></b>	

<b>Project Title: A cost consequence study of leucocyte-depleted blood in prevention of post operative infection following elective surgical procedures</b>	<b>Contact: Dr L Williamson, University of Cambridge</b>
<b>Abstract: <i>Abstract is currently unavailable</i></b>	

<b>Project Title: TSE spiking study in blood fractionation</b>	<b>Contact: Dr C Prowse, Scottish National Blood Transfusion Service, Edinburgh.</b>
<b>Abstract: <i>Abstract is currently unavailable</i></b>	

**Other Support:**

<b>Project Title: The Medical Research Council Prion Unit (MRC PU)</b>	<b>Contact: Professor J Collinge, MRC Prion Unit</b>
<p><b>Abstract:</b> The MRC PU was formally established in 1998, following on from interim funding awarded in 1996 in response to the need to develop a stable research environment and long-term support in an area of national importance. The Unit is directly supported by the MRC, but also receives support from the Wellcome Trust, BBSRC, the Department of Health and the European Commission. The Unit plays a key role in linking basic science to clinical research and focuses principally on human prion diseases. Its aim is to understand the molecular basis of prion propagation and human prion strain diversity and to fully characterise human genetic susceptibility to prion infection. The main areas of research are:</p> <ul style="list-style-type: none"> <li>• Molecular genetic studies of human prion disease susceptibility</li> <li>• Mapping novel prion disease incubation loci in mice and identifying human homologues</li> <li>• Transgenic modelling of prion diseases and interspecies transmission barriers</li> <li>• Normal cellular function of PrP</li> <li>• Molecular and phenotypic analysis of human prion strains</li> <li>• Structure of normal and mutant prion proteins</li> <li>• Clinical research: early diagnostic markers and longitudinal studies</li> <li>• Development of human diagnostics and therapeutics</li> </ul>	

<b>Project Title: Transgenic Transmission Facility for human prion disease (MRC/DH funded) (part of the MRC Prion Unit)</b>	<b>Contact: Professor J Collinge, MRC Prion Unit</b>
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**Abstract:**

Aims of the programme:

- 1) To develop and fully characterise a transgenic transmission model of human prion disease
- 2) To study the “species barriers” limiting transmission of prions between mammalian species, in particular those between cattle, sheep and humans
- 3) To determine whether human prion diseases are caused by one or many different prion strains, and to isolate and characterise such putative strains
- 4) Determination of transmission parameters for BSE passaged in several species and “new variant” CJD in transgenic mice overexpressing wild type human prion protein.
- 5) To perform a structure/function analysis of PrP by attempted phenotypic rescue of a PrP null phenotype using human PrP with known and experimental mutations. These transgenic lines will also be inoculated with prions to determine the structural components of PrP necessary for prion propagation.
- 6) To further delineate the normal cellular function of PrP using neurophysiological and behavioural assessment of mice expressing mutant PrP and mice in which conditional knockout of PrP at defined developmental stages can be performed.
- 7) To directly test the “protein only” hypothesis of prion propagation by attempting production of prions in vitro from recombinant derived material, using suitably designed transgenic mice as bioassays.

**Project Title: Additional animal facilities for work involving transgenic mice**

**Contact: Professor J. Collinge, MRC Prion Unit**

**Abstract: *Abstract is currently unavailable***

**Project Title: Category 3 containment facilities for the MRC Prion Unit**

**Contact: Professor J. Collinge, MRC Prion Unit**

**Abstract: *Abstract is currently unavailable***

**Project Title: Neuropathogenesis Unit (BBSRC) Core Support**

**Contact: N/A**

**Abstract: *Abstract is currently unavailable***

### 3. Principal research teams and their areas of expertise with contact details.

The principal research teams funded by the UK Government and their areas of expertise are described below. Information on tissue resources is also given where appropriate. Some teams receive funding from more than one funder. Smaller groups are listed at the end of the section.

#### **Institute for Animal Health**

Institute for Animal Health  
Compton  
Berkshire  
RG20 7NN

Contact: Dr Geoff Oldham  
Tel: 01635 578411  
[geoff.oldham@bbsrc.ac.uk](mailto:geoff.oldham@bbsrc.ac.uk)

- **Mathematical Biology**

To consider ways epidemiology can help in the control and eventual eradication of scrapie, using the techniques of mathematical biology. The Group has expertise in modelling and field studies.

- **PrP Structure and Function**

PrP is central to TSE infections, with its conversion from the normal (PrP<sup>c</sup>) to abnormal (PrP<sup>Sc</sup>) isoform the key to pathogenesis. PrP<sup>Sc</sup>, either alone or in association with other molecules, is regarded by many as the infectious agent. This series of diseases is characterised by long incubation periods, neurodegeneration and lack of host immune responses. It is crucial that this simple protein be thoroughly understood.

The Group has expertise in the biophysical analysis of proteins and their interactions with other molecules.

- **TSE Inactivation**

It has long been recognised that the infectious agent is extremely resistant to standard inactivation and sterilisation procedures. Why this should be so, and how inactivation can be achieved has become particularly urgent since BSE agent was shown to infect humans causing variant CJD.

The Group has expertise in biochemistry, measuring TSE infectivity and setting up pilot scale or laboratory models of industrial rendering and other treatment processes.

- **TSE Mouse Genetics**

PrP is central to TSE infections, with its conversion from the normal (PrP<sup>c</sup>) to abnormal (PrP<sup>Sc</sup>) isoform being the key to pathogenesis. It exists in a number of variant forms which are genetically determined, some forms giving the host greater susceptibility or resistance to the TSE agents.

Through the development of appropriate models the group aims to define: the role of PrP in pathology; the role of allelic variation in susceptibility to TSEs; the normal function of PrP and the regulation of PrP expression.

The Group has expertise in molecular biology, transgenic mouse technology and measurement of infectivity.

- **TSE Pathogenesis**

The Group is engaged in fundamental studies on the pathogenesis of TSEs in peripheral tissues using experimental models. An understanding of the disease mechanisms involved, particularly in the early stages of infection, will suggest possible approaches to prevention and therapy. The Group is also concerned with the basis of variation in the pathogenesis of the TSEs, associated with strain variation in the causative agent, genetic variation in the infected host and transmission between species.

The Group has particular expertise in studying routes of transmission and spread within the host, characterisation of different strains of TSE agent, immunocytochemical detection of PrP in tissues and investigating the role of cells of the immune system.

- **TSE Sheep Scrapie Genetics**

PrP is central to TSE infections, with its conversion from the normal (PrP<sup>c</sup>) to abnormal (PrP<sup>Sc</sup>) isoform the key to pathogenesis. This Group aims to understand the basis of the genetic control of scrapie in sheep, to understand the aetiology of the disease and from that knowledge seek ways of controlling this disease that is now controlled by statutory means.

The Group has expertise in scrapie sheep genetics, molecular biology, transgenics, reporter gene constructs and cell lines

- **Neuropathogenesis**

The main observable effects of infection with TSE agents are in the CNS. This Group is carrying out studies to understand the sequence of events and the mechanisms involved in neurodegeneration in TSEs in order to be able to target strategies aimed at treating the clinical manifestations of disease.

The Group has expertise in neurobiology, neurohistochemistry, immunocytochemistry, confocal microscopy, image analysis and morphometric techniques.

- **The TSE Resource Centre, Institute for Animal Health**

Important TSEs in the UK are scrapie, BSE and CJD, with current emphasis on vCJD linked to BSE. World wide there are other recognised TSEs including kuru, Gerstmann-Strausler-Scheinker disease(GSS), transmissible mink encephalopathy(TME) and chronic wasting disease (CWD).

### **Background to Resource Centre**

The TSE Resource Centre, directly supported by the BBSRC and MRC, was established in 1999 to collect, store, characterise, produce and distribute a range of reagents, from monoclonal antibodies to teaching aids. The primary role of the Centre is to supply a range of specialised research reagents needed for TSE research. By providing an infrastructure to produce sufficient amounts of quality reagents to meet the needs of users, much of the unfunded workload and cost currently placed on individual laboratories have been removed and the reagents are available for prompt distribution. The Resource Centre is providing reagents to TSE researchers worldwide.

### **Reagents**

The reagents available fall under 3 main categories:

- Anti-recPrP(prion protein) Monoclonal Antibodies
- Infective Material
- Miscellaneous Reagents

Full details can be found at the Resource Centre's web site: <http://www.iah.bbsrc.ac.uk/tse-rc>

### **Donations**

Donating reagents or teaching aids to the Resource Centre is no different in principle from making reagents available to other researchers in the "usual way". However the benefits to a donor are considerable, with the Resource Centre undertaking all the duties involved in

distribution. If the reagent cannot be propagated at the Resource Centre a batch of aliquots could be donated. Donor's interests are protected by the Conditions of Use, which must be accepted when a reagent is requested.

The Resource Centre will welcome all TSE reagent donations.

If you are willing to donate a reagent contact the Resource Centre (see below).

At any time most, but not all, reagents are likely to be listed on the web pages below. An up-to-date listing will be emailed on receipt of a Latest List Request form.

**For Further Information contact the TSE Resource Centre:**

**Tel:** +44(0)1635-577294

**Fax:** +44 (0)1635-577295

**e-mail:** [tse.rc@bbsrc.ac.uk](mailto:tse.rc@bbsrc.ac.uk)

By Post The TSE Resource Centre, Institute for Animal Health,  
Compton, Newbury, Berkshire. RG20 7NN U.K

**Website** <http://www.iah.bbsrc.ac.uk/tse-rc>

**Veterinary Laboratories Agency**

The following research groups are based at three VLA sites. Unless otherwise indicated, the group is based at VLA Weybridge. Their addresses are as follows:

VLA Weybridge	VLA Luddington	VLA – Lasswade
Woodham Lane	Stratford upon Avon	Pentlands Science Park
New Haw	Warwickshire	Bush Loan
Addlestone	CV37 9SJ	Penicuik
Surrey KT15 3NB		Midlothian E26 OPZ

• **Epidemiology Department**

[John.JW.Wilesmith@vla.maff.gsi.gov.uk](mailto:John.JW.Wilesmith@vla.maff.gsi.gov.uk)

Scrapie and BSE epidemiological studies

Field investigation of risk factors for BSE, and scrapie and other animal TSEs

Mathematical modelling of disease

Design of surveillance studies

Statistical analyses of surveillance data

• **Neuropathology Unit**

[Marion.MM.Simmons@vla.maff.gsi.gov.uk](mailto:Marion.MM.Simmons@vla.maff.gsi.gov.uk)

Statutory diagnosis of scrapie

Ovine TSE lesion profiling

Murine TSE lesion profiling

Analysis of IHC/ICC tests

Scrapie and BSE pathogenesis

Transmission studies

Clinical TSE studies

Specialised *post-mortem* techniques for tissue collection

- **Molecular biology/Diagnostics**

[D.Matthews@vla.maff.gsi.gov.uk](mailto:D.Matthews@vla.maff.gsi.gov.uk)

Molecular genetics- PrP genotype testing

PrP and Antibody production

Immunological testing- ELISA

Western blot

ICE

FRET analysis

Biochemical analysis/detection of molecular markers

- **Animal Services Unit**

[Paul.P.Townsend@vla.maff.gsi.gov.uk](mailto:Paul.P.Townsend@vla.maff.gsi.gov.uk)

Maintenance and care of experimental animals for TSE research.

- **Regional Laboratories**

c/o VLA Weybridge

[D.Matthews@vla.maff.gsi.gov.uk](mailto:D.Matthews@vla.maff.gsi.gov.uk)

Large scale surveillance projects

Receipt, sampling and distribution for testing

- **VLA - Luddington**

[M.Ansfield@vla.maff.gsi.gov.uk](mailto:M.Ansfield@vla.maff.gsi.gov.uk)

ELISA testing of feedstuffs for mammalian protein

Introduction & validation of tests for detecting animal proteins/structures in cooked & uncooked feedstuffs

- **VLA – Lasswade**

[R.Munro@vla.maff.gsi.gov.uk](mailto:R.Munro@vla.maff.gsi.gov.uk)

[M.Jeffrey@vla.maff.gsi.gov.uk](mailto:M.Jeffrey@vla.maff.gsi.gov.uk)

Electron microscopy

ICC: Development of immunogold techniques for use at ultrastructural level

Neuropathological examination of laboratory and domestic animals

Statutory diagnosis of BSE

- **Histology Unit**

[Yvonne.YI.Spencer@vla.maff.gsi.gov.uk](mailto:Yvonne.YI.Spencer@vla.maff.gsi.gov.uk)

Histological examination

Preparation of materials for IHC testing and neuropathological examination.

- **VLA TSE Archive**

The VLA and the TSE Research & Surveillance Unit in MAFF co-ordinate a large programme to supply scientists with tissues from animals infected with TSEs. Researchers from over 25 countries have made over 160 requests for material from the archive.

The VLA was asked to establish the archive under the remit of the Chief Veterinary Officer's TSE Emergency Account (April 1996-March 1999). In 1996, the demand for further research grew at a time when the epidemic was declining. A bank of materials for future research was therefore considered essential.

Tissues for the archive come from animals that are either naturally or experimentally infected with BSE or scrapie. MAFF funds one project at the VLA, specifically supplying tissues and fluids from healthy and infected cattle to help with the evaluation of diagnostic tests.

The archive currently stores 140 different tissues and fluids from cattle and 104 from sheep. It contains over 46 litres of cerebrospinal fluid, over 16 litres of blood and over 21 kg of brainstem from cattle infected with BSE. Much of the material has been collected opportunistically when processing suspects for statutory diagnosis. It has to be recognised therefore that not all stored material is suitable for transmission studies in laboratory animals. Such samples are still suitable for in vitro work. Samples suitable for transmission work are collected at designated autopsies on naturally or experimentally infected animals, and are therefore likely to be available in smaller volumes.

The TSE Office at the VLA co-ordinates all requests. Requests from non-MAFF scientists need to be authorised by the TSE Research & Surveillance Unit before the final release of the material. MAFF scientists receive equal treatment at the time of project evaluation.

All requests must be made in writing to the TSE Office:

Mrs Dianne Bunce  
TSE Office  
Veterinary Laboratories Agency  
New Haw  
Addlestone  
Surrey KT15 3NB  
UK  
Phone 01932 357 875  
Fax 01932 357 230  
Email: [d.e.bunce@vla.maff.gsi.gov.uk](mailto:d.e.bunce@vla.maff.gsi.gov.uk)

You will need to supply the TSE Office with the following:

- a detailed description (if an EU project, the technical annexe) of the studies involved;
- details of the EU contract number, title and co-ordinator (please state if not applicable)
- the minimum quantities of material required, i.e. number and size of samples;
- details of any specific sampling protocol e.g. if you require aseptic collection, or freezing in liquid nitrogen;
- details of when the material is required by;
- details of the receiving airport (if applicable); and
- confirmation that your laboratory has suitable containment facilities for handling TSE agents.

If approval is given to supply you material, the following conditions must be adhered to:

- the material to be provided will only be used for TSE research;
- the material to be provided will be used solely for this purpose and will not be made available to other workers or organisations without prior permission from MAFF through the TSE Office at VLA; and
- if it is proposed to publish any results from this project, the manuscript will be made available in confidence, and for information only, to MAFF who fund the TSE archive. The manuscript should be made available to MAFF through the TSE Office, prior to submission for publication. In effect this archive is seen as a means of extending the research base on

TSEs, and samples are usually made available free of charge (other than handling costs if excessive) in exchange for scientific information that derives from the ensuing project.

Therefore, please send your request directly to the TSE Office at the VLA confirming that you have a laboratory that meets the appropriate containment requirements, and agreeing to the above release conditions. Failure to provide sufficient information may prejudice the supply and/or the outcome of your studies. Past experience indicates that misinterpretation is likely if the request does not include sufficient detail to ensure that the material supplied is exactly what is being requested.

### **MRC Prion Unit**

Imperial College School of Science Technology and Medicine

St Mary's Hospital

Norfolk Place

London W2 1 PG

Tel: 020 7594 3792

Fax: 020 7706 7094

A dedicated MRC Prion Unit, directed by Professor John Collinge, is the focus of MRC funded TSE research. The Unit was set up in 1998 to create an international centre of excellence by bringing together existing expertise and integrating MRC funded TSE research programmes. The Unit is directly supported by the MRC, but also receives support from the Wellcome Trust, BBSRC, the Department of Health and the European Commission. The Unit plays a key role in linking basic science to clinical research and focuses principally on human prion diseases. Its aim is to understand the molecular basis of prion propagation and human prion strain diversity and to fully characterise human genetic susceptibility to prion infection. The main areas of research are:

- Molecular genetic studies of human prion disease susceptibility
- Mapping novel prion disease incubation loci in mice and identifying human homologues
- Transgenic modelling of prion diseases and interspecies transmission barriers
- Normal cellular function of PrP
- Molecular and phenotypic analysis of human prion strains
- Structure of normal and mutant prion proteins
- Clinical research: early diagnostic markers and longitudinal studies
- Development of human diagnostics and therapeutics

## Cambridge University

- Department of Biochemistry  
Old Addenbrookes Site  
80 Tennis Court Road  
Cambridge  
CB2 1QW

The function of normal PrP, relevance of copper binding by PrP<sup>c</sup> to the metabolism of cells expressing PrP<sup>c</sup> and function changes of possible relevance to prion disease

- Department of Clinical Veterinary Medicine  
Madingly Road  
Cambridge  
CB3 0ES

CNS and peripheral pathogenesis and the interaction of prions with cells of the immune system during peripheral pathogenesis

The involvement of cells of the lymphoreticular system in the immune response in scrapie pathogenesis

- Department of Chemistry  
Lensfield Road  
Cambridge  
CB2 1EW

Prion protein structure (yeast), stability and folding

- Cambridge Group for Brain Repair.  
Edgar Adrian Building  
Forvie Site  
Robinson Way  
Cambridge

Mechanism of neurotoxicity of prion protein peptides in culture, prion protein function and neurodegeneration in prion disease models

- **Institute for Biotechnology**

University of Cambridge

Tennis Court Road

Cambridge CB2 1QT

Mechanisms for detecting prions on surfaces and assessing the efficacy of novel cleaning and decontamination protocols.

University of Edinburgh

- Royal (Dick) School of Veterinary Studies

Faculty of Veterinary Medicine

Summerhall

Edinburgh

EH9 1QH

Scotland

The molecular immunology and neuropathology of TSE pathogenesis (mice and *in vitro*)

The role of lymphoid tissue in pathogenesis and the immune response, the molecular pathogenesis of TSEs

The analysis of natural scrapie outbreaks in Suffolk sheep using the application of recently developed mathematical models to field data. To relate findings to current understanding of scrapie pathogenesis

- Faculty of Medicine

University of Edinburgh

Teviot Place

Edinburgh EH8 9AG

Scotland

TSE neuropathology and immunocytochemistry (CJD Surveillance Unit, see below)

The neurophysiological mechanisms involved in TSE pathogenesis, neurophysiology on acute gene knockout mice through anti-sense sequences

- **National CJD Surveillance Unit**

The Creutzfeldt-Jakob Disease Surveillance Unit aims to monitor the characteristics of all forms of CJD, to identify trends in incidence rates and to study risk factors for the development of disease.

Key objectives:

- To investigate the incidence of, and risk factors for, variant vCJD
- To determine whether human BSE infection is occurring additionally in individuals with a heterozygote or valine homozygote genotype.
- To determine whether cases of vCJD are occurring outside the age range of cases already identified.
- To investigate the aetiology of sporadic CJD through epidemiological and pathological studies.
- To investigate determinants that influence the variation in the clinico-pathological phenotype of CJD
- To assess potential diagnostic markers for all forms of CJD including those that might allow an estimate of the prevalence of BSE infection in the UK population
- To collaborate with other research projects in the UK and elsewhere
- To provide timely information to the Department of Health, the Scottish Office Department of Health, the Welsh Office, the Northern Ireland Office, the British Parliament, the British public, the European Commission and the World Health Organisation.
- To provide professional advice to relevant scientific committees and, as appropriate, to agencies involved in the investigation and management of CJD.
- To provide information and support to patients with CJD and to their relatives and carers.

In 1999, the National CJD Surveillance Unit became a WHO Collaborative Centre for Reference and Research on the Surveillance and Epidemiology of Human Transmissible Spongiform Encephalopathies (TSEs).

The Unit has research expertise in clinical neurology, neuropathology, molecular biology, image analysis, CSF protein analysis, epidemiology and statistics.

Since 1993, staff at the Unit have co-ordinated the EU funded project on surveillance of CJD in Europe.

For further details, please contact:

The National Creutzfeldt-Jakob Disease Surveillance Unit

Western General Hospital

Crewe Road

Edinburgh

EH4 2XU

UK

Clinical Office Telephone: 0131 537 2128

Pathology Telephone: 0131 537 1980

Fax: 0131 343 1404

[Jan.Mackenzie@ed.ac.uk](mailto:Jan.Mackenzie@ed.ac.uk)

<http://www.cjd.ed.ac.uk/jan.html>

**Roslin Institute**

John Withers

Roslin Institute

Roslin

Midlothian

EH25 9PS

Scotland

Molecular characterisation of TSE pathogenesis and the development of diagnostics tests

**Institute for Ophthalmology**

University College London

11-43 Bath Street

London

EC1V 9EL

Molecular chaperones in relation to conformational changes

**National Institute for Medical Research**

Protein Structure Department

Mill Hill

London

NW7 1AA

Structural studies on prion proteins

**Kings College London**

Division of Clinical Laboratory Sciences

Molecular Neurobiology Group

New Hunts House

Guy's Campus

London

SE1 9RT

The cellular basis of the conversion of normal PrP to abnormal PrP and the propagation of abnormal PrP in pathogenesis

**University of Oxford**

- Sir William Dunn School of Pathology

South Park Road

Oxford

OX1 3RE

Development of a diagnostic test using RNA aptamers against disease isoform bovine prion protein

Mechanisms of pathogenesis following the oral route of infection

- Department of Zoology

South Parks Road

Oxford

OX1 3PS

Scrapie epidemiology, risk factors and routes of transmission in the field, the genetic basis of susceptibility, selective breeding to control and eliminate scrapie

### **National Institute for Biological Standards and Control**

- **WHO Repository of internationally agreed-upon reference materials for use in TSE research**

At the second meeting of the Working Group on International Reference Materials for Diagnosis and Study of Transmissible Spongiform Encephalopathies (TSEs), the importance of establishing a WHO Repository of internationally agreed-upon reference materials was emphasised in order to facilitate development of methods for diagnosis and better understanding of the TSE diseases. The establishment of the reference materials would also be essential for regulatory decisions in the pharmaceutical and biologicals field and for the validation of the ability of pharmaceutical procedures to remove the agents

At the previous meeting, the WHO TSE Working Group recommended that candidate biological reference materials for human TSEs be prepared from brains of cases of sporadic and variant CJDs and from a similarly processed uninfected human brain. Responding to this suggestion, four brains were provided by the National CJD Surveillance Unit, Edinburgh, and homogenised by NIBSC staff with the valuable assistance of the Institute of Animal Health, Compton, UK. The four preparations of human brain homogenate were aliquotted and have now been distributed by NIBSC to participants in the WHO collaborative study. The preparations were from one uninfected brain, one brain from a case of variant CJD (vCJD) and two cases of sporadic CJD (sCJD). All four brains were from individuals homozygous for the allele encoding methionine at codon 129 of the prion-protein gene (PRNP).

These materials are presented as 10% homogenates in 0.25M sucrose in pyrogen-free water. All specimens have been stored at -80°. A collaborative study whereby the materials will be assayed by immunoblot and other *in vitro* and *in vivo* methods is being performed. The aim is to assess the suitability of preparations as references for use in the detection of infectious material in homogenates of brain samples from cases of CJD by assaying for:

1. PrP<sup>Sc</sup> by immunoblot.
2. PrP<sup>Sc</sup> by other *in vitro* methods.
3. PrP<sup>Sc</sup> by *in vivo* methods.

After conclusion of the collaborative study, WHO intends to offer these reference materials as calibrants to laboratories attempting to optimise a variety of *in vitro* and *in vivo* diagnostic procedures for TSEs. They will also be used later to establish WHO proficiency panels of serially diluted, randomised and coded samples.

Details of the collaborative study and the protocol for storage can be found at <http://www.who.int/technology/biologicals/tsereport2.doc>

## **Smaller Groups**

Smaller groups are defined as those receiving funding for a small number of TSE related projects, usually two. There are numerous other groups with only one TSE related project, these are not listed here, but the project can be found described in the abstracts, section 2.

- **University of Kent at Canterbury**

Department of Biosciences

Canterbury

CT2 7NJ

Genetic and biochemical analysis of prion elimination in yeast

- **University of Leeds**

Department of Biochemistry and Molecular Biology

Leeds

LS2 9JT

Function of normal PrP and its potential role in copper metabolism

Structural investigations of yeast prions

#### **4. Collaboration with other countries and openness of the UK programme to collaboration**

UK funders are currently producing a call for research proposals on diagnostics in which it will be explicitly stated that applications from outside the UK will be welcomed.

As indicated previously, the various tissue archives and tissue banks supported by the UK funders have supplied tissues outside the UK for independent and collaborative research. In addition, a number of projects underway in the UK involve non-UK collaborators, some have been provided with matching funding by the EU LINK scheme, and a small number of UK funded research is underway outside the UK.

The UK Government funders recognise the value of effective collaboration between groups of experts and strongly encourage this.

# JOINT RESEARCH CENTRE

(Status January 2001)

## Introduction

Since 1998, the European Commission's Joint Research Centre (JRC) has been playing a vital role in the detection of BSE by evaluating and validating tests and developing methods to both prevent and control the spread of this formidable disease.

This it does on behalf of DG SANCO and in collaboration with leading EU expert institutes. Two JRC Institutes are engaged in this research, namely the Institute for Reference Materials and Measurement (IRMM) in Geel, Belgium, which is mainly concerned with the validation of tests for BSE infection and the Institute for Health and Consumer Protection (IHCP) in Ispra, Italy, which concentrates its efforts on the quality control of animal feed and the detection of risk material in food. A brief resume of past, ongoing and future projects are documented henceforth.

### **Activity: Evaluation of post mortem BSE tests**

*Scope:* An important step forward was made in the fight against BSE. Co-ordinated by DG SANCO, the IRMM performed the practical part of the work for the evaluation of four candidate post-mortem BSE diagnostic tests. IRMM supervised the 4 EU laboratories that conducted the analysis and evaluated a total of 5290 results generated by these tests. The European Commission's scientific steering committee concluded that three of the tests could identify animals clinically infected with BSE. This complete project was executed within months and the preparation of infected samples required the set-up of a special laboratory and the use of specialised glove box facilities to handle the infectious materials. The tests - which can only be performed on brain or spinal cord samples of dead animals but not on meat - are extraordinarily reliable when dealing with animals in the clinical stage of BSE. The outcome of this work has now led to a Europe wide monitoring programme involving several million cattle where from 1<sup>st</sup> January 2001, post mortem testing for BSE became compulsory throughout the EU.

*Deliverables:* As above

*Action plan:* Diagnosis of TSEs

*JRC Institute:* IRMM, Geel

*Programme:* Framework Programmes 4 and 5 (November 1998 - July 1999)

*Co-op.:* DG SANCO and expert EU laboratories

Prionics AG, Zürich, CH

Enfer Technology Ltd., Newbridge IR

Commissariat à l'Energie Atomique, Gif sur Yvette, F

For the sample supply: Veterinary Laboratory Agency, UK/ Ministry of Agriculture, Fisheries and Food, UK, National Centre for disease Investigation, NZ

*Follow-up:* In continuation of its work on tests for BSE, the JRC is evaluating five new tests. The tests were identified following a worldwide call for expression of interest, which closed on 30<sup>th</sup> September 2000. The evaluation of the five new tests will involve a smaller series of samples. Particular emphasis has been placed on the sensitivity of the tests. The field work, which will be done in stages, has already commenced and will continue until late spring 2001.

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**Activity: Evaluation of tests to identify animals affected with BSE at the pre-clinical stage, i.e. before the animal shows symptoms of the disease**

*Scope:* Further work is being done to detect BSE at the pre-clinical stage, i.e. before the animal shows symptoms of the disease. The IRMM established the collaboration network for obtaining the samples and performing the tests. Experiments using the most sensitive test from the first post mortem study have been completed and the publication of the results is under way.

*Deliverables:* Set up the sampling scheme and collected 146 BSE-positive (UK) and -negative (New Zealand) samples as well as 25 homogenates. Results of the experimental work carried out at Commissariat à l'Energie Atomique (France) will be published soon

*Action plan:* Diagnosis of TSEs

*JRC Institute:* IRMM, Geel

*Programme:* Framework Programmes 5

*Co-operation:* DG SANCO, IRMM, Commissariat à l'Energie Atomique, Gif sur Yvette, F  
For the sample supply: Veterinary Laboratory Agency, UK Ministry of Agriculture, Fisheries and Food, UK, National Centre for Disease Investigation, NZ.

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**Activity: Assessment of relationship between infectivity titre and prion concentration**

*Deliverables:* The comparison between the reference test based on bioassay and a new biochemical test required further confirmation. While the most efficient of the first 4 tests (test D, CEA) could detect the positive control diluted 300 fold, agreement was observed between the mouse bioassay and the biochemical test samples carried out on dilutions of the same sample previously used for the bioassay. The findings were also correlated to results from the study on pre-clinical cases and for the diluted homogenates provided in the first BSE test evaluation exercise. The results demonstrated that, if used to detect and exclude BSE positive samples, the sensitive and rapid diagnostic test would provide a high level of confidence that mice used in the reference bioassay would not be exposed to a lethal level of exposure to BSE via the intracranial route. In the absence of other essential risk management methods (such as specified risk material removal) such tests would also provide an important risk reduction to man from the BSE agent. The extend of any additional synergy between testing and existing risk reduction methods cannot be quantified. In addition the results gave a strong hint that there is a direct relationship between infectivity titre and prion concentration expressed by the abundance of the test signal. The results of this work were recently published in Nature (2001, vol. 409, 476).

*Action plan:* Diagnosis of TSEs, the infectious agent and its mechanism of transmission

*JRC Institute:* IRMM, Geel

*Programme:* Framework Programme 5

*Co-op.:* DG SANCO and CEA, for the sample supply: Veterinary Laboratory Agency, UK/ Ministry of Agriculture, Fisheries and Food, UK

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*Activity:* Quality assurance (QA) of post-mortem testing on BSE for EU monitoring programme

*Scope:* The JRC will establish and operate a QA scheme for the monitoring programme in collaboration with the National Reference Laboratories for BSE-testing laboratories. It intends to set up an appropriate network, organise regular meetings (workshops) to discuss the performance of tests under routine conditions and new developments in testing. The activities include the organisation of a proficiency testing programme for BSE laboratories in the EU and the preparation of reference materials.

Certified Reference Materials are indispensable cornerstones to ensure that Member State laboratories conduct measurements that are comparable, traceable and of renowned provenance. These international reference materials will not only support the execution of proper reference measurements within and beyond the EU but may also prove useful for increasing the sensitivity of BSE detection methods - the key to overcoming problems posed by the lengthy incubation period of BSE.

*Action plan:* Support to the diagnosis, risk assessment, and treatment of prevention of SEs

**Deliverables: The supply of material needed for production of BSE positive and negative Certified Reference Materials (CRMs) for BSE test calibration is under discussion.**

*JRC Institute* IRMM, Geel

*Programme:* Framework Programmes 5

*Co-operation:* DG SANCO, National Reference Laboratories

For the sample supply: Veterinary Laboratory Agency, UK/ Ministry of Agriculture, Fisheries and Food, UK, National Centre for Disease Investigation, NZ

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***Activity:* Evaluation of the ability of tests to detect TSE in sheep, including the adaptation of tissue other than brain**

*Deliverables:* In continuation of its work on tests for BSE and other TSE, the JRC plans to analyse the ability of BSE tests to distinguish the various Transmissible Spongiform Encephalopathies (TSE) diseases.

This is a much larger and more complicated evaluation but is potentially of great importance particularly if BSE is identified in the sheep population. The evaluation would involve the collection and preparation of a bank of positive and negative sheep specimens. Phase 1 would involve the creation of a tissue bank. So far, 2 tests are envisaged to be evaluated for their potential to distinguish BSE and Scrapie.

*Action plan:* Diagnosis of TSEs

*JRC Institute:* IRMM, Geel

*Programme:* Framework Programme 5

*Co-op.:* DG SANCO and expert EU laboratories (CEA, UCSF)

For the sample supply: Veterinary Laboratory Agency, UK Ministry of Agriculture, Fisheries and Food, UK, National Centre for disease Investigation, NZ

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***Activity:* Influence of autolysis and repeated freezing/thawing cycles on the performance of BSE tests**

*Scope:* Preparation of a small series of tissues to assess if the performance of tests is affected by autolysis, putrefaction and repeated freezing/thawing cycles. Enough series should be provided to test the three tests already approved the five new currently under examination and some spare sets for contingencies and future use.

*Action plan:* 20 each of the homogenised samples will be prepared: negative control, untreated, repeatedly frozen and thawed, degraded. 3 times 20 samples each will be shipped to all 8 laboratories whose tests have been evaluated. Completion is scheduled for 6/2001.

*Action plan:* Diagnosis of TSEs, treatment of prevention of TSEs and the infectious agent and its mechanism of transmission

*JRC Institute:* IRMM, Geel  
*Programme:* Framework Programme 5  
*Co-operation:* DG SANCO

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**Activity:** **Evaluation of methods for the detection of bone meal in animal feed**

*Scope:* Animal meat and bone meal infected by the BSE agent is conceived to be the primary source of BSE. Since there has long been a ban on the use of animal meal from mammals in feed for ruminants (there will most probably be a ban on animal meal in general in animal feed for all animals), reliable analytical methods are necessary in order to control the implementation of these bans. The JRC is working on the improvement and validation of suitable methods. One method based on the detection of bovine DNA was validated in 1998; another method based on the detection of specific proteins is currently under validation. The JRC is enlarging the work on meat and bone meal detection with a strong focus on fast screening methods. Screening methods are required in order to increase the number of tests carried out to analyse samples for potential positives, taking into account limited laboratory capacity and thus improving the efficiency of the surveillance programmes. The JRC is going to evaluate the applicability of different techniques such as Differential Scanning Calorimetry (DSC), Pyrolysis Mass Spectrometry and an artificial nose as screening methods for meat and bone meal in feed.

The JRC is also an active partner (one of 10 laboratories) in a study that aims at developing new methodologies for the detection and quantification of illegal addition of mammalian tissues in animal feed which will be financed under the Competitive and Sustainable Growth Programme and will start in January 2001.

*Action plan:* Treatment of prevention of TSEs

*JRC Institute:* IHCP, Ispra

*Programme:* Framework Programmes 4 and 5

*Co-operation:* DG SANCO, DG RECH and 9 partners in the shared cost action (co-ordinator Centre de Recherches Agronomiques, Gembloux, B)

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**Activity:** **Evaluation of methods for the assessment of heat treatment of animal meat and bone meal**

*Scope:* Recently the JRC has validated an immunoassay to check that sterilisation of animal meal has been carried according to the European standard (Commission Decision 96/449/EC). Although the results of the collaborative trial confirmed reliability of the method, the general applicability of the immunoassay is hampered due to the fact that the animal meal has to contain at least 10 % porcine proteins. In order to circumvent this drawback of the test kit the JRC optimised an alternative immunoassay developed for the detection of beef. Results of the validation of the method demonstrate a sufficient sensitivity of the test to differentiate between animal meals treated at different conditions.

*Deliverables:* Two validated methods and threshold value for appropriate heat treatment.

*Action plan:* Treatment of prevention of TSEs

*JRC Institute:* IHCP, Ispra

*Programme:* Framework Programmes 4 and 5

*Co-operation:* DG SANCO and IRMM, Geel and participating expert laboratories in the validation studies

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***Activity:* Detection of central nervous tissue (CNT) in food products deriving from animals**

*Scope:* Tissues of the central nervous system (such as brain and spinal cord) account for nearly all of the infective load in a BSE case approaching the end of the incubation period. Rapid and reliable tests are necessary to assess whether CNT material is kept out of the food chain. The IHCP is co-ordinating the validation study of a commercial test that identifies brain and spinal cord even in heat treated meat products. This method will be used for food enforcement laboratories to facilitate the control of food adulteration, and for producers to check for the absence of CNT at every stage of meat manufacturing.

*Deliverables:* Validation of this method has started and first results are expected for summer 2001.

*Action plan:* Treatment of prevention of TSEs

*JRC Institute* IHCP, Ispra

*Programme:* Framework Programme 5

*Co-operation:* DG SANCO, University of Giessen and Leipzig and EU Member States control laboratories

## **EUROPEAN ACTION PLAN ON TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES (TSE) RESEARCH**

Within months of the announcement, in June 1996, of the appearance of a new variant of Creutzfeldt-Jakob-Disaese (CJD), raising concerns about the transmission of Bovine Spongiform Encephalopathy (BSE) via the food chain, the Commission immediately assessed the scientific advice and present needs through the Weissman report and set out its intention at the Research Council of proposing an action plan for research on Transmissible Spongiform Encephalopathies.

In November 1996 the Commission adopted a communication (COM/96/582) entitled «A European initiative on research on transmissible spongiform encephalopathies» which outlined an action plan which identified the most important research priorities and the necessary budgetary requirements.

This action plan took into account the recommendations of the Weissmann report, the Multidisciplinary Scientific Committee as well as ongoing national and Community research activities. Based on the Council recommendations, the action plan was comprised of two levels:

- the co-ordination of activities between Member States, aimed at harmonization of data collection and diagnostic criteria;
- a specific call for RTD actions, intended to stimulate research efforts at Community level and to mobilise new and complementary expertise in order to attain sufficient European critical mass.

The financial envelope for such an initiative was estimated at 50.7 MECU.

Three calls for proposals for RTD activities within Community research programmes were launched between December 1996 and March 1998 to address the issue.

The Action plan has resulted in an excellent mobilisation of expertise from more than 120 laboratories throughout Member States and associated countries and far-reaching combination of scientific disciplines, which could contribute to speed knowledge acquisition in this field.

54 RTD projects are now being supported. The areas addressed by these projects include:

- 1) Clinical, epidemiological and social research
- 2) The infectious agent and its mechanisms of transmission
- 3) Diagnosis
- 4) Risk assessment
- 5) Treatment and prevention
- 6) Co-ordination of activities between Member states

The list of projects is provided in Annex 1. The abstracts of the projects and the list of participants have been published in the booklet « Transmissible Spongiform Encephalopathies : the European initiative ». The booklet is available for free from the QoL infodesk e-mail : [quality-of-life@cec.eu.int](mailto:quality-of-life@cec.eu.int)

## TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES (TSE) RESEARCH IN THE QUALITY OF LIFE PROGRAMME

The Quality of Life Programme , under the 5<sup>th</sup> Framework programme, continues to support research on animal and human TSE.

Three projects have been funded so far for a total EC contribution of 1.06 M€:

- two concerted actions aimed at supporting networks on neuropathology and validation of diagnostic tests;
- one shared-cost project aimed at evaluating the inactivation / removal effect of the gelatine manufacturing process on TSE infectivity;

The list of projects is provided in Annex 2.

Other projects are likely to be funded during the life-time of 5<sup>th</sup> Frame work Programme.

### ANNEX 1

<b>1. CLINICAL, EPIDEMIOLOGICAL AND SOCIAL RESEARCH ON HUMAN SE'S</b>		
<b>CT963698</b>	Coordination of National Surveillance Programmes for CJD in the European Union	<b>R.G. WILL</b> University of Edinburgh CJD Surveillance Unit E-mail : r.g.will@ed.ac.uk
<b>CT972034</b>	Human Transmissible Spongiform Encephalopathies (Prion Diseases): Neuropathology and Phenotypic Variation	<b>Herbert BUDKA</b> Institute of Neurology University of Vienna E-mail: h.budka@akh-wien.ac.at
<b>CT972216</b>	Creutzfeldt-Jakob Disease in the European Union – Incidence and Risk Factors	<b>R.G. WILL</b> University of Edinburgh CJD Surveillance Unit E-mail : r.g.will@ed.ac.uk
<b>CT976003</b>	Transgenic Mice Expressing Human Prion Protein Use for Characterisation of Human Encephalopathies, and Sensitivity for Detection of Infectivity	<b>Jean-Jacques HAUW</b> Association Claude Bernard pour le Développement des Recherches Biologiques et Médicales dans les Hopitaux de l'assistance Publique à Paris E-Mail : jean-jacques.hauw@psl.ap-hop-paris.fr
<b>CT976015</b>	European Centralized Facility for Human Transmissible Spongiform Encephalopathies (Prion Diseases)	<b>Herbert BUDKA</b> Institute of Neurology University of Vienna E-mail: h.budka@akh-wien.ac.at
<b>PL987022</b>	A study of genetic factors in CJD	<b>Cornelia M. VAN DUIJN</b> Erasmus University Rotterdam E-mail : duijn@epib.fgg.eur.nl
<b>2. THE INFECTIOUS AGENT AND ITS TRANSMISSION MECHANISMS</b>		
<b>CT960601</b>	Basic Mechanism of Neurodegeneration in Transmissible Spongiform Encephalopathies: Role of Amyloidogenic Prion Protein PRP in glial Activation	<b>Jan P.M. LANGEVELD</b> Institute for Animal Science and Health (Id-Dlo) E-mail: j.p.m.langeveld@id.wag-ur.nl
<b>CT960856</b>	Clinico-Pathological features and pathogenesis of fatal familial insomnia	<b>Elio LUGARESI</b> Università di Bologna Istituto di Clinica Neurologica E-mail : lugaresi@bo.nettuno.it
<b>CT972679</b>	CJD and BSE: An integrated molecular and experimental neuropathological analysis of prion neurodegeneration, strain variation and transmission risks	<b>John, COLLINGE</b> , Imperial College School of Medicine at St Mary's Neurogenetics Unit E-mail: j.collinge@ic.ac.uk

<b>CT973305</b>	Improving prospects for scrapie control in sheep and goats by study of host genotypes, TSE isolates and their in vivo and in vitro interaction	<b>Jean-Michel ELSÉN</b> Institut National de la Recherche Agronomique Inra-Saga E-Mail: elsen@toulouse.inra.fr
<b>CT973308</b>	Separation, identification and characterisation of the normal and abnormal isoform of prion protein from normal and experimentally infected fish	<b>Carla BOLIS</b> Institute of Pharmacological Sciences University of Milan Faculty of Pharmacy E-mail: bolis@mailserver.unimi.it
<b>CT973311</b>	Analysis of molecular factors affecting variability in BSE and scrapie susceptibility	<b>John WILLIAMS</b> Roslin Institute (Edinburgh) Division of Molecular Biology E-Mail: john.williams@bbsrc.ac.uk
<b>CT973314</b>	Relationship between conformation of PRP, infectivity and pathogenicity of bovine spongiform encephalopathy (BSE) as a basis for diagnosis	<b>Detlev RIESNER</b> Heinrich-Heine-Universitaet Duesseldorf Institut Fuer Physikalische Biologie Email: riesner@biophys.uni-duesseldorf.de
<b>CT976006</b>	Investigation of putative signal transduction processes of normal prion protein and their role in spongiform encephalopathy pathogenesis	<b>Brian H. ANDERTON</b> Institute of Psychiatry E-mail: b.anderton@iop.kcl.ac.uk
<b>CT976022</b>	Role of PRP in prion spread and establishment of central nervous system infection	<b>Dominique DORMONT</b> Commissariat a l'énergie Atomique E-Mail: dormont@dsvifd.cea.fr
<b>CT976040</b>	Prion diseases: Mechanisms of transmission and identification of targets for potential therapeutics	<b>John COLLINGE</b> The Imperial College of Science, E-mail: j.collinge@ic.ac.uk
<b>CT976045</b>	Maintenance and transmission of yeast prions: a model system	<b>Michael Francis TUIE</b> University of Kent at Canterbury, Department of Biosciences E-mail: m.f.tuite@ukc.ac.uk
<b>CT976050</b>	The bovine prion protein: From structure analysis to the molecular mechanism of conformational transitions	<b>Maria Catia SORGATO</b> Universita' Degli Studi di Padova Dipartimento di Chimica Biologica E-Mail: sorgato@civ.bio.unipd.it
<b>CT976051</b>	Structure, function and interactions of prion proteins and prion protein domains	<b>Hans A. KRETZSCHMAR</b> Institut für Neuropathologie E-mail: Hans.Kretzschmar@inp.med.uni-muenchen.de
<b>CT976055</b>	Trafficking pathways of normal and pathologic isoforms of the prion protein	<b>Sylvain LEHMANN</b> Centre National de la Recherche Scientifique E-Mail: sylvain.lehmann@igh.cnrs.fr
<b>CT976060</b>	Cerebellar networks alterations in prion diseases	<b>Herbert AXELRAD</b> Faculte de Medecine Pitie - Salpetriere Universite Paris VI E-Mail: axelrad@chups.jussieu.fr
<b>CT983265</b>	Cellular pathogenesis of prion diseases	<b>Hans A. KRETZSCHMAR</b> Institut für Neuropathologie E-mail: Hans.Kretzschmar@inp.med.uni-muenchen.de

### 3. DIAGNOSIS OF SE'S

<b>CT973304</b>	For the development of improved bioassays for BSE and scrapie agent detection ovine and bovine PRP transgenic	<b>Martin GROSCHUP</b> Federal Research Centre for Virus Diseases of Animals Institute for Vaccines at the Federal Research E-mail: martin.groschup@tue.bfav.de
<b>CT973306</b>	New approaches to the diagnosis; and control of transmissible spongiform encephalopathies	<b>Mark ROGERS</b> University College Dublin Dept. of Zoology & Biotechnology Centre E-mail: mark.rogers@ucd.ie
<b>CT973315</b>	Development of a novel diagnostic to assist quality assurance procedures in European meat production	<b>Heinz C. SCHROEDER</b> Johannes Gutenberg-Universitaet Mainz Institut fuer Physiologische Chemie E-Mail: hschroed@mail.uni-mainz.de

<b>CT976011</b>	In vitro investigation of PRP-induced neurodegeneration: Development of a system for testing potential therapeutic agents	<b>Alun WILLIAMS</b> University of Glasgow E-mail : a.williams@vet.gla.ac.uk
<b>CT976013</b>	PRPSC distribution and kinetics in lymphoid tissues of sheep with natural scrapie: Effects of sheep PRP genotype and scrapie strains	<b>Lucien VAN KEULEN</b> DLO Institute for Animal Science and Health E-mail : l.j.m.vankeulen@id.dlo.nl
<b>CT976016</b>	Analysis and function of 14-3-3 isoforms. Early diagnosis of Creutzfeld-Jakob disease	<b>Alastair AITKEN</b> Medical Research Council E-Mail: a-aitken@nimr.mrc.ac.uk
<b>CT976046</b>	Diagnosis of Transmissible spongiform encephalopathies using PrPSc/PrPc specific antibodies	<b>Hans A. KRETZSCHMAR</b> Institut für Neuropathologie E-mail: Hans.Kretzschmar@inp.med.uni-muenchen.de
<b>CT976048</b>	Quantitative analysis of MR scans in Creutzfeldt-Jakob disease	<b>Alan COLCHESTER</b> University of Kent at Canterbury Electronic Engineering Laboratory E-Mail: a.colchester@ukc.ac.uk
<b>CT976064</b>	Development of cell culture models of infectious forms of TSE	<b>Hubert LAUDE</b> Institut National de la Recherche Agronomique E-Mail: laude@biotec.jouy.inra.fr
<b>CT983727</b>	Laboratory supported diagnosis of Creutzfeldt-Jacob disease	<b>Sigrid POSER</b> Klinik und Poliklinik für Neurologie Georg-August-Universität Göttingen Fax :49.551.39.7020
<b>PL987024</b>	Development and control of PRP SC based test in humans and animals using cerebrospinal fluid and brain tissue	<b>Hans A. KRETZSCHMAR</b> Institut für Neuropathologie E-mail: Hans.Kretzschmar@inp.med.uni-muenchen.de

#### 4. RISK ASSESSMENT OF SE'S

<b>CT973301</b>	Measures to reduce contamination of meat and environment with CNS tissue during slaughter and processing of cattle and sheep remcord	<b>David HARBOUR</b> University of Bristol E-mail: dave.harbour@bristol.ac.uk
<b>CT976029</b>	Risk assessment in primates of TSE transmission to humans through food and blood products	<b>GERHARD HUNSMANN</b> Deutsches Primatenzentrum GMBH E-Mail: ghunsm@gwdg.de
<b>PL987004</b>	Contamination of meat, and exposure of abattoir workers, by CNS material during standard butchering prevalent in the member states of the European Union eucnsrisk	<b>David HARBOUR</b> University of Bristol E-mail: dave.harbour@bristol.ac.uk
<b>PL987008</b>	Establishing the resources for and examination of transcription changes occurring during BSE infection: A route to early diagnosis of infection and a detailed study of early molecular pathology	<b>John WILLIAMS</b> Roslin Institute (Edinburgh) E-mail : <a href="mailto:john.williams@bbsrc.ac.uk">john.williams@bbsrc.ac.uk</a>
<b>PL987017</b>	Genome scan for loci controlling scrapie incubation time in mouse and sheep	<b>Jean-Michel ELSÉN</b> Institut National de la Recherche Agronomique – Auzeville E-mail : elsen@toulouse.inra.fr
<b>PL987020</b>	PRP- Dimerization and oligomerization as a model for the evaluation of tsetransmission modalities and as a target for a therapeutic intervention against tses	<b>Stefan WEISS</b> Ludwig-Maximilians-Universität München E-mail : weisse@lmb.uni-muenchen
<b>PL987023</b>	Role of environmental and host factors on the horizontal and vertical transmission of scrapie in naturally infected sheep floc	<b>Lucas GRUNER</b> Institut National de la Recherche Agronomique – Nouzilly E-mail : gruner@tours.inra.fr

<b>5. TREATMENT AND PREVENTION OF SE'S</b>		
<b>CT976030</b>	TSE spiking experiments for process validations: Evaluation of different sources of infectivity and spiking approaches	<b>Herwig E. REICHL</b> Haemosan Erzeugung E-mail : herwig.reichl@haemosan.com
<b>CT976054</b>	Development of TSE therapies based on prion protein-binding oligosaccharides	<b>Ruth GABIZON</b> Hadassah University Hospital E-Mail: gabizonr@hadassah.org.il
<b>CT976065</b>	Inactivation of the causative agents of transmissible spongiform encephalopathies by thermophilic and hyperthermophilic proteases	<b>Neil RAVEN</b> Microbial Research Authority Acting Through its Centre for Applied Microbiology and Research E-Mail: neil.raven@camr.org.uk
<b>PL987015</b>	Low levels of TSE infectivity in blood: Determination of titre and evaluation of removal	<b>Herwig Ernst REICHL</b> Haemosan, Erzeugung Pharmazeutischer Grundstoffe E-mail : herwig.reichl@haemosan.com
<b>PL987019</b>	TSE-Agent inactivation, product quality evaluation and sterilization process simulation in rendering processes for the production of feed grade animal proteins	<b>Radulf OBERTHÜR</b> Labor Dr. Oberthür GBMH E-mail : fmb.ob@-online.de
<b>PL987026</b>	Infectivity of blood components in experimental NVCJD: Towards a risk assessment for human blood	<b>Gerald EDER</b> Baxter Aktiengesellschaft e-mail : <a href="mailto:ederg@baxter.com">ederg@baxter.com</a>

<b>6. COORDINATION OF ACTIVITIES BETWEEN MEMBER STATES</b>		
<b>CT976056</b>	Concerted action for the settings up of multicentric epidemiological databases and biological samples banks for small ruminant scrapie	<b>Frederic LANTIER</b> Institut National de la Recherche Agronomique E-Mail: lantier@tours.inra.fr
<b>CT976057</b>	Building a common database on scientific research and public decision on TSE in Europe	<b>Pierre-Benoit JOLY</b> Institut National De La Recherche Agronomique E-Mail: joly@grenoble.inra.fr
<b>CT987006</b>	Development of a pre-clinical test to differentiate between scrapie and BSE infection in sheep	<b>Lucien Johannes VAN KEULEN</b> DLO Institute for Animal Science and Health E-mail : l.j.m.vankeulen@id.dlo.nl
<b>CT987021</b>	The establishment of a European network for the surveillance of ruminant TSE and the standardisation and harmonisation of the process and criteria for the identification of suspected cases	<b>Mark ROGERS</b> University College Dublin E-mail : mark.rogers@ucd.ie
<b>CT987028</b>	Public receptions of BSE and CJD risk in Europe, their interplay with media, policy initiatives and surveillance issues. Drawing the lessons for lessons for information policy	<b>Carlos DORA</b> World Health Organisation E-mail : cdo@who.it
<b>CT983651</b>	A network for the supply of BSE tissues and fluids for European collaborative research	<b>Stan DONE</b> Vet. Lab. Agency E-mail : s.done@vla.maff.gsi.gov.uk

## ANNEX 2

<b>CT9930009</b>	Evaluation of the inactivation / removal effect of the gelatine manufacturing process on TSE infectivity	<b>THOMSON</b> Gelatin Manufacturers of Europe Brussels E-mail : jth@cefic.be
<b>CT9930837</b>	'Human Transmissible Spongiform Encephalopathies: The Neuropathology network'	<b>Herbert BUDKA</b> Klinisches Institut für Neurologie der Universität Wien, AT E-mail : h.budka@vm230.akh-wien.ac.at
<b>CT9930837</b>	EUROCJD	<b>Robert . G. WILL</b> CJD Surveillance Unit E-mail : r.g.will@ed.ac.uk

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