### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>BSE</td>
<td>Bovine spongiform encephalopathy</td>
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<tr>
<td>CDI</td>
<td>Conformation dependent immunoassay</td>
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<tr>
<td>CJD</td>
<td>Creutzfeldt Jakob disease</td>
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<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<tr>
<td>ECDC</td>
<td>European Centre for Communicable Disease Prevention and Control</td>
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<tr>
<td>EEG</td>
<td>Electroencephalogram</td>
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<tr>
<td>EU</td>
<td>European Union</td>
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<tr>
<td>EUROCJD</td>
<td>Collaborative project of European surveillance of CJD</td>
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<td>HPA</td>
<td>Health Protection Agency</td>
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<td>MRC</td>
<td>Medical Research Council</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>NCJDSU</td>
<td>National Creutzfeldt Jakob Disease Surveillance Unit or the Unit</td>
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<tr>
<td>PET</td>
<td>Paraffin embedded tissue</td>
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<tr>
<td>PIND</td>
<td>Progressive Intellectual and Neurological Deterioration</td>
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<tr>
<td>PMCA</td>
<td>Protein misfolding cyclic amplification</td>
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<td>PRNP</td>
<td>Human prion protein gene</td>
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<td>sCJD</td>
<td>Sporadic Creutzfeldt Jakob disease or sporadic CJD</td>
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<tr>
<td>SEAC</td>
<td>Spongiform Encephalopathy Advisory Committee</td>
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<tr>
<td>TMER</td>
<td>Transfusion Medicine Epidemiology Review</td>
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<tr>
<td>TSE</td>
<td>Transmissible spongiform encephalopathy</td>
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<tr>
<td>vCJD</td>
<td>Variant Creutzfeldt Jakob disease or variant CJD</td>
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I  PREFACE

Surveillance and scientific research are both integral and interdependent components of the monitoring and investigation of Creutzfeldt Jakob Disease (CJD). Surveillance of variant and sporadic CJD by the National CJD Surveillance Unit (NCJDSU) provides data that are essential for informing a significant number of scientific research projects carried out in a wide range of professional fields at the NCJDSU, elsewhere in the UK, Europe and the world. While surveillance underpins this research, the findings of the research can also in themselves play an important role in surveillance, for example by providing sensitive and specific methods of diagnosis or by indicating specific risk factors to examine.

The aim of this report is to inform interested parties of details of the current, and planned future, scientific research being undertaken by staff at the NCJDSU, in the context of the Unit’s previous research and its on-going background surveillance.

This Scientific Report complements two other reports:
1) The NCJDSU Annual Report, which provides a description of the clinico-pathological epidemiology of CJD in the previous 12 months, reflecting the Unit’s core surveillance work. The Annual Report is available on the Unit’s web-site (www.cjd.ed.ac.uk).
2) The NCJDSU Business Plan, which provides financial, structural and organisational information.

Section III summaries the planned development of surveillance and research of the NCJDSU over the next five years.

Section IV (Introduction) provides the background for the NCJDSU’s surveillance and scientific research in terms of its main areas of activity: UK and European surveillance, epidemiology and public health, clinical features and diagnosis, neuropathology, the prion protein laboratory, molecular genetics, the Cerebro-Spinal Fluid (CSF) laboratory and transmission studies.
Section V (Key Scientific Questions), shows how the NCJDSU’s scientific research (and, in places, core surveillance) is attempting to answer some of the current key questions regarding prion diseases in humans.

The appendices (Section VI) contain details of NCJDSU staff, grant funding held by the NCJDSU, work for higher degrees (MD, PhD) by NCJDSU staff, relevant membership of committees by NCJDSU staff, material sent nationally and internationally in 2007 from the NCJDSU, details of the methodology of the case control study examining risk factors for variant and sporadic CJD, diagnostic criteria and a full list of publications of work from the NCJDSU since 1990.

The NCJDSU receives funding for its core activities from the Department of Health and the Scottish Government. Its additional research activities have separate funding. In practice, for some areas of work, it is difficult to separate the two sources of funding as the work is interlinked. However, where possible the two sorts of activities are differentiated throughout the report.
II EXECUTIVE SUMMARY

On-going clinico-pathological surveillance of CJD in the UK is imperative in order to determine the full extent of the primary and secondary variant CJD epidemics, how these relate to sporadic CJD and the potential for other animal TSEs to transmit to humans. Given the potentially very long incubation periods seen in other human TSEs, such as 40 years in kuru, it is important to continue surveillance of CJD for the foreseeable future.

Surveillance and scientific research are both integral and interdependent components of the monitoring and investigation of CJD. Therefore, the development of research, in addition to surveillance within the NCJDSU, to help aid the understanding of this fatal group of diseases with wide public health implications, is crucial. As exemplified in this report, staff at the NCJDSU hold a large number of grants for research and publish widely in peer-reviewed literature. The NCJDSU has a renowned international reputation for its knowledge and wide range of expertise in many fields. Its staff continue to make important contributions to relevant national, European and international committees and to discourse in the wider academic, clinical, and public arena.

The aim of this report is to inform interested parties of details of the current, and planned future (Section III), scientific research being undertaken by staff at the National Creutzfeldt-Jakob Disease Surveillance Unit (NCJDSU), in the context of the Unit’s previous research and its on-going background surveillance.

Section IV Introduction: The NCJDSU continues to carry out surveillance of variant (vCJD) and sporadic (sCJD) CJD in the UK. This is wide-ranging and involves describing, where possible, the clinical and diagnostic features, epidemiology, neuropathology, prion protein biochemistry, molecular genetics and CSF biochemistry of all cases of prion disease referred to the Unit. Collaboration with European and international colleagues is an essential part this surveillance mechanism. These findings are key to public health policy development.
Section V Key Scientific Questions:

**Surveillance:** The NCJDSU has comprehensive mechanisms in place in order to attempt complete ascertainment of all cases of variant and sporadic CJD in the UK. Cases in the elderly in vCJD and the very elderly in sCJD may be missed; further work is being considered.

**Variant CJD:** Much research has been carried out by the NCJDSU to describe the clinico-pathological, epidemiological, genetic and molecular features of vCJD. The risk factors have been examined extensively. Key features of on-going NCJDSU research include how to ensure that vCJD in non methionine homozygotes (MM) codon 129 genotypes is detected, examining the risk of secondary transmission of vCJD (including blood, surgery, dentistry) and how it can be determined, providing a fuller biochemical definition of prion protein, further elucidating TSE genomics (PRNP and non-PRNP) and how vCJD in other countries aids the understanding of this disease. The NCJDSU is collaborating with HPA in population prevalence studies.

**Sporadic CJD:** An extensive research programme has been in place for many years examining the clinico-pathological, epidemiological, genetic and molecular features of sCJD, often in collaboration with colleagues in Europe and internationally. On-going clinico-pathological-molecular characterisation is crucial to help differentiate unusual cases of sCJD from vCJD and perhaps transmission of atypical animal TSEs to humans. Collaborative mouse transmission studies are also on-going to determine more fully subtypes of CJD, including those from secondary transmission.

**Diagnostic tests:** This is a vital area of research development, which the NCJDSU is actively involved in, again often in collaboration with colleagues in Europe and internationally. Research of the utility of EEG, MRI and CSF proteins, including 14-3-3 and tau, is being undertaken. Also wide-ranging research of the neuropathological and molecular aspects of diagnosis is on-going, including how the prion protein may correlate with CJD phenotypic diversity and the development and evaluation of methods of detection of prion protein, such as the development and use of novel monoclonal antibodies, conformation dependent immunoassays (CDI), and amplification methods such as protein misfolding cyclic amplification (PMCA).
Treatment: NCJDSU is involved in European Union (EU) research to harmonise treatment trials of CJD.

Public Health: Most of the research carried out by the NCJDSU will ultimately have public health consequences, including the evaluation of the risk of secondary transmission of vCJD, determining whether BSE infection will be found in non-MM codon 129 genotypes, determining whether atypical animal TSEs will infect humans, following up those at risk of CJD in collaboration with the HPA and the development of a blood test for CJD.
III SUMMARY OF PLANNED DEVELOPMENT OF SURVEILLANCE AND RESEARCH OVER THE NEXT 5 YEARS BY NCJDSU

1. Surveillance of CJD
   1.1. To ensure that surveillance of suspect cases of CJD, including atypical cases, in the UK continues by developing and maintaining links with the neurological neuropathological, epidemiological, and scientific communities across the country.
   1.2. To ensure that the surveillance system for CJD in the UK uses the best available tools and continuously develops the most appropriate strategies for the investigation of CJD in the UK.
   1.3. To ensure that data relating to cases of CJD are complete and available for use in research relating to the prediction of trends.
   1.4. To continue the UK’s internationally recognised role in CJD surveillance and to maintain and develop links with international groups for CJD research and surveillance, such as the NeuroPrion Network of Excellence and the European Centre for Disease Control and Prevention.
   1.5. To continue to coordinate the surveillance and reporting of vCJD in the European Union.

2. Epidemiology of CJD
   2.1. To study the primary and secondary epidemics of vCJD and to identify factors influencing their development and outcome.
   2.2. To continue to study the risk of transmission of vCJD through blood transfusion by the Transfusion Medicine Epidemiology Review.
   2.3. To continue to search for cases of vCJD in the paediatric population and for evidence of vertical transmission of infection through the PIND study.
   2.4. To investigate medical risk factors for variant and sporadic CJD directly from medical records.
   2.5. To study dentistry as a risk factor for vCJD.
   2.6. To investigate the epidemiology of UK cases of CJD compared with that in other countries, for example, vCJD in France.
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3. Public Health of CJD
   3.1. To inform promptly relevant bodies, for example, government health
departments, ECDC, and committees, for example SEAC, of any relevant
developments, such as evidence of vCJD in non methionine (MM) homozygous
individuals, evidence of transmission of typical animal TSEs to humans, and the
development of a new potential diagnostic test for CJD.
   3.2. To investigate risk of secondary transmission of CJD, for example through
blood products, dentistry and surgery.
   3.3. To develop methodology and facilitate the follow up of those ‘at risk’ of CJD.
   3.4. To continue to support the CJD Incidents Panel and the Advisory Committee
on Dangerous Pathogens TSE Working Group in their activities concerning
public health issues in relation to CJD.
   3.5. To continue to coordinate the National Care Package for patients with CJD of
all types.
   3.6. To provide information and advice to clinicians, health professionals, CJD
Support Groups, families of patients, the media and the general public.
   3.7. To continue to provide information and statistics on CJD through a dedicated
website.

4. Identification of novel phenotypes of CJD
   4.1. To ensure that surveillance systems are in place to detect potentially novel
clinical, neuropathological, biochemical, or genetic phenotypes.
   4.2. To investigate the distribution of the disease-associated form of the prion
protein in tissues outside the nervous system in all forms of CJD.
   4.3. To develop new discriminate assays to help identify novel forms of human prion
disease such as those that might result from BSE infection of non- methionine
homozygous (MM) individuals or human exposure to atypical forms of scrapie
or atypical BSE.

5. Laboratory-based studies of CJD
   5.1. To maintain the NCJDSU Brain and Tissue Banks as a resource for CJD
research within the Unit and to collaborators in the UK and beyond.
   5.2. To develop pathological techniques for the detection of the disease-associated
from of the prion protein in both neural and extraneural tissues.
5.3. To continue to develop tests for CJD using cerebrospinal fluid as an analyte.

5.4. To develop diagnostic tests and blood screening or confirmatory assays, including those based on protein misfolding cyclic amplification (PMCA) and the infection of cultured human cells.

5.5. To use in vitro systems, such as PMCA and the infection of human cell cultures to model the effects of agent strain and host genotype in human prion disease.

5.6. To continue the study of genetic factors in relation to disease susceptibility and phenotype in CJD.
IV  INTRODUCTION

The national surveillance of Creutzfeldt-Jakob disease (CJD) in the UK was initiated in May 1990 in response to a recommendation in the Report of the Working Party on Bovine Spongiform Encephalopathy (the Southwood Committee). The core activities of the National CJD Surveillance Unit (NCJDSU) are funded by the Department of Health and by the Scottish Government Health Department. In 1999, the NCJDSU became a World Health Organisation (WHO) Collaborative Centre for Reference and Research on the surveillance and epidemiology of human transmissible spongiform encephalopathies (TSEs). The NCJDSU acts as the coordinating centre for the European CJD Surveillance System (EUROCJD), is the reporting hub for CJD to the European Centre for Disease Control and Prevention (ECDC), is a member of the Neuroprion Network of Excellence and is a founding member of the Scottish TSE Network (STN).

The initial aim of the NCJDSU was to identify any change in the pattern of CJD that might be attributable to human infection with the agent responsible for the emergence of bovine spongiform encephalopathy (BSE) in cattle. Such a change was recognised formally in 1996 when variant CJD (vCJD) was first reported. Since first describing vCJD, the NCJDSU has had several core functions: to monitor the characteristics (clinical, pathological, biochemical, epidemiological and genetic) of CJD, specifically sporadic CJD and vCJD, to identify trends in incidence rates and to study risk factors for the development of disease. Together with routine clinico-pathological surveillance of cases of sporadic and variant CJD, scientific enquiry into both typical and atypical cases is an essential and integral part of the work of the unit in order to meet its core objectives. These findings vitally contribute to the development of public health policy aiming to prevent onward transmission of the disease at national and international levels.

The aim of this Scientific Report to describe the scientific work of the NCJDSU, to summarise findings and to describe current work and future plans, will be undertaken with reference to key questions concerning prion diseases that relate to scientific, clinical and public health concerns. Because of the interdependent nature of the surveillance and research activities of the NCJDSU, an understanding of the main surveillance activities of the unit is essential and will be described in the remainder of this section.
The NCJDSU will be referred to as the ‘Unit’ throughout the remainder of the report.

**UK Surveillance and Epidemiology**

The core function of the Unit is clinico-pathological surveillance of CJD in the UK. This underpins all the other activities and research in the Unit. The Unit has detailed records of all UK variant and nearly 1000 sporadic CJD cases, based on case notes, family interviews, direct clinical examinations and pathological materials. It also has a wealth of data on various diagnostic tests, such as magnetic resonance images (MRI), electroencephalograms (EEG) and cerebrospinal fluid (CSF) proteins, with the ability to correlate test results with clinical, pathological and molecular features.

Referral of suspect cases to the Unit occurs in three ways:

**Clinical** - passive ascertainment: neurologists are reminded at least annually of the need to refer any individuals in whom CJD or vCJD is considered a possible diagnosis to the Unit.

**Death certificates** - passive ascertainment: the Office for National Statistics for England and Wales and the General Register Offices for Scotland and Northern Ireland supply all death certificates coded under rubrics A81.0 and F02.1 (10th ICD revision).

**Other sources** - passive ascertainment: neuropathologists and neurophysiologists, psychiatrists, paediatricians (also enhanced surveillance - see below), geriatricians, other health professionals and members of the general public may refer cases to the Unit.

Whenever possible all referrals suspected of CJD are visited in life by a neurologist from the Unit in order to obtain a clinical history, to carry out a physical examination, to take specimen samples and to gather systematic clinical information from the suspect case and their relatives. Following the death of a definite or probable case of CJD, the general practice records are requested and details on past medical history recorded. If notification to the Unit is made after death or death occurs soon after notification and before a visit can be performed, hospital and general practitioner records are requested. In addition, an attempt is made to visit the relatives of the case in order to gather further clinical information. Death certificates are requested for all suspect cases of CJD referred to the Unit. Neuropathology, prion protein biochemistry, CSF marker and genetic analysis within the Unit play crucial roles in core surveillance and are described in the relevant sections below.
Notifications classified as probable or definite genetic or iatrogenic CJD are not followed up by Unit, unless the diagnosis is unclear or a specific request is made by the local clinician for a visit by a neurologist from the Unit. Professor Michael Preece, Institute of Child Heath, London, follows up cases of growth hormone related iatrogenic CJD and the investigation of cases of genetic CJD is undertaken by staff at the National Prion Clinic, London.

The core surveillance activities of the unit are strengthened by enhanced surveillance:

**Paediatric surveillance (‘PIND Study’) -** This is carried out through prospective active surveillance in conjunction with the British Paediatric Surveillance Unit. The aim is to identify cases of Progressive Intellectual and Neurological Deterioration (PIND) and to determine whether or not cases of CJD are occurring in children resident in the UK aged under 16 years at onset of symptoms.

**Transfusion Medicine Epidemiology Review -** Reporting of blood donation and transfusion histories of cases of CJD to the UK Blood Services in order to determine whether any recipients or donors develop CJD.

**Europe and International surveillance** – see section below, “European CJD Surveillance (EUROCJD) and Public health”.

Integral to the core surveillance function is the collaboration between colleagues outwith the Unit in the UK, Europe and further afield from a number of specialties, including neurological, psychiatric, geriatric, neuropathological, epidemiological, public health, statistical, biochemical and genetic. This collaboration enhances a wide range of Unit activities, from the detection of clinical suspect cases of sporadic and variant CJD, through to the characterisation of cases of CJD at the epidemiological, neuropathological, genetic and biochemical levels.

The Unit has on-going epidemiological collaboration with Professors Peter Smith and Simon Cousens at the London School of Hygiene and Tropical Medicine, which has resulted in many publications- see Appendix 8. The wealth of epidemiological and statistical expertise provided by this collaboration and the ability to examine a problem from outwith the Unit has proved invaluable.
Clinical Features and Diagnosis
The initial suspicion of prion disease in life depends on recognition of a suggestive clinical picture. The typical forms of prion disease follow a relatively uniform course, but there is clinical heterogeneity with occasional very atypical forms. There is, currently, no simple, non-invasive clinical diagnostic test for prion disease in general and no pre-clinical test. However, genetic prion disease (and being at risk of it) is identifiable via prion protein gene (PRNP) sequencing, which can be performed on a simple blood test.

Early clinical diagnosis has a number of advantages, including allowing prompt avoidance of potential onward transmission. It is also likely that effective treatment will require diagnosis before severe disability has developed. The Unit continues to collect data on clinical features and diagnostic test results, as well as undertaking specific research in these areas. The Unit’s expertise in this area is of relevance to its surveillance function. In the absence of a simple clinical diagnostic test, and with a rare disease, clinicians are keen to obtain advice from others with particular experience. They therefore receive help in the process of notification of suspect cases and this encourages referral to the Unit. The additional role of the CSF protein laboratory is detailed below.

UK Public Health
A key role of the Unit is to inform public health policy through the results of its core surveillance and research collaborations, either in the ‘acute’ situation when findings necessitate swift action, for example, when vCJD was first identified, or through contributing over the longer term to the development of public health policy. The Unit’s involvement in informing public health policy is undertaken largely by involvement in various committees, such as SEAC (Spongiform Encephalopathy Advisory Committee), and the CJD Incidents Panel, (see Appendix 4), and through collaboration with other agencies, such as the Health Protection Agency (HPA), Health Protection Scotland (HPS) and the National Blood Services. In addition, close working relationships with UK Government Health Departments, ensures that significant or urgent public health issues can be discussed directly with Government Health Departments and, if necessary, before relevant committees meet. This is exampled by the description of the first cases of vCJD in 1996 by the NCJDSU and the identification of transmission of vCJD infection through blood component transfusion in 2003, 2004 and 2006.
The Unit undertakes a wide range of activities and provides information critical to public health policy, including:

- The identification and characterisation of all cases of vCJD in the UK.
- The analysis of potential influences on risk such as gender distribution, social class and frequency of urban or rural residence.
- Providing information on temporal and geographic trends in the vCJD outbreak, for example, the number of deaths and clinical onsets per annum, and analysis of the age distribution of cases.
- Analysing data on the regional distribution of vCJD cases (provided quarterly to public health departments nationally) and of geographical clustering of cases.
- Identification of potential primary and secondary risk factors for the development of disease in all cases, including histories of prior blood transfusion, of exposure to plasma derived products and of past surgery.
- Collating data on potential routes of secondary transmission, including a regularly updated database of past surgical exposures with dates and locations of operative procedures and a database of children of vCJD cases.
- Assessment of putative risk factors that are proposed as potential mechanisms of exposure eg contact lenses, organophosphates, vaccines, plasma products etc.
- Neuropathological validation of the diagnosis of vCJD in a high proportion of all cases.
- Collating data on the regional distribution of prion protein staining and infectivity in peripheral tissues in vCJD.
- Prion protein typing to allow appropriate classification of CJD cases.
- Collecting a bank of tissues and biological fluids for further research nationally and internationally.

European CJD Surveillance (“EUROCJD”) & Public Health.
Collaboration to strengthen surveillance and inform public health decisions internationally has evolved over time. The EUROCJD project began in 1993 with the UK as the coordinating centre and included: the UK, France, Germany, Italy, the Netherlands and Slovakia. After 19961 and in 20002, additional countries joined the

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1 Australia, Austria, Canada, Belgium, Norway, Spain, Sweden, Denmark, Finland, Portugal, Eire, Israel, Switzerland and Iceland

2 Czech Republic, Hungary, Poland and China
project. In recent years, countries including the USA, Japan and Mexico have joined the system, but are self-funding. New EU member states will be invited to join in the future. The Unit continues to coordinate this project.

In the past DG Research and DG SANCO have funded this project. Currently, the research component of the project is funded through the Neuroprion Network of Excellence until December 2008. A further application to continue this activity as part of a larger project coordinated by J-P Deslys (CEA, Paris) has passed the initial assessment stage and a full application was made in February 2008. The public health component of EUROJJD was funded on an interim basis through ECDC until 31st January 2008. Following a formal assessment of the hub functions of the Unit in May 2007, a decision was made to outsource the continuing CJD surveillance function in Europe for 3 years. A call for tender was made in December 2007 and the Unit has applied to ECDC to continue to act as the hub for reporting of vCJD in Europe.

The international collaborative systems have been enormously helpful in a number of ways. Comparative country data allow assessment of differing surveillance techniques and can inform epidemiological theories and development of public health policy, the initial rapid identification of vCJD being a classic example. The collection of data from large combined populations greatly aids the study of rare diseases for a number of reasons, including increasing the statistical power of any study, allowing standardisation and evaluation of tests, such as CSF 14-3-3 and MRI, and validating diagnostic criteria. Furthermore, the accumulation of samples and tissues from a large number of well-documented cases of all forms of human TSE is a vital resource for further research.

The Unit has a crucial role in providing data and expert advice to EU and WHO, for example, through the ECDC, the European Medicines Agency (EMEA) and the EU SCENHIR (Scientific Committee on Emerging and Newly Identified Health Risks).

Neuropathology Laboratory

The NCJDSU Neuropathology Laboratory provides vital contributions to surveillance and currently important research activities. The core functions of the Neuropathology Laboratory are neuropathological analysis of all cases of suspected CJD referred to the Unit, and tissue – based surveillance of prion infection. These functions are critically
dependent on engagement with neuropathologists and their staff. A neuropathological infrastructure has been established across the UK that relates to the Unit, allowing referral of any suspected case, collaborating with other experienced centres of excellence in the assessment of unusual cases and ensuring that appropriate specimens and collected and submitted. The Unit provides advice and guidance on autopsies and laboratory investigations that can be performed in local centres as well as liaising with neuropathologists over referred cases and organising transport of specimens. The Unit also provides a range of specialised investigations (including immunohistochemistry and PET blot analysis for disease associated prion protein), undertakes analysis of individual cases in a way that avoids bias in interpretation of results (by performing neuropathological analysis without reference to detailed clinical, biochemical or genetic data in the first instance) and has developed techniques to increase the sensitivity and specificity of neuropathological diagnosis. The Neuropathology Laboratory also acts as an international reference centre for neuropathological diagnosis and investigations in human prion diseases (used by EU, Centres for Disease Control and Prevention, USA, and WHO).

The Neuropathology Laboratory provides detailed neuropathological analysis (diagnosis and sub-classification) on all cases of suspected CJD referred to Unit. The majority of these cases involve examination of the brain following autopsy on a patient, but occasionally brain biopsy specimens and a range of other tissue samples (including tonsil biopsy, lymph node and appendix) are examined. Additionally, the Neuropathology Laboratory supports a number of tissue – based surveillance projects in the UK by undertaking immunohistochemical and biochemical investigations for disease-associated prion protein in submitted tissue samples for ‘at-risk’ individuals, including the surveillance of CJD in patients with haemophilia (C Miller, London) and the surveillance of vCJD in children and young adults with immunodeficiency (M Helbert, Manchester). The Neuropathology Laboratory undertook a large-scale prevalence study of appendix and tonsil specimens in the UK for evidence of prion infection ( Hilton et al, J Pathol 2004; Ironside et al BMJ 2006), and currently acts as a quality control centre for the immunohistochemical arm of the National Anonymous Tonsil Archive (NATA) study. Discussions are underway concerning neuropathological support for a proposed HPA study of autopsy tissues for evidence of prion infection and for a possible further study of appendicectomy specimens by HPA.
The Unit also maintains a Brain and Tissue Bank. This houses a large collection of frozen brain tissue and a wide range of other tissue samples from most forms of human prion diseases. The collection, storage and use of these materials are regulated by the Human Tissue Act and Human Tissue (Scotland) Act and have relevant consent for research use. The stored material is of major importance for studies of infectivity in specific tissues, biochemical characterisation of prion protein and prevalence studies.

These samples are also made available to researchers from across the world under the guidance of the MRC Edinburgh Brain Banks’ Steering Group, which ensures that a robust and accountable system is in place for dealing fairly with all requests for tissues for research. Details of materials provided to researchers in the past year are included in Appendix 5. The Unit has been the major provider of materials of NIBSC (National Institute for Biological Standards and Control) and WHO for use as reference standards and this need is likely to continue in the immediate future. A large quantity of vCJD tissues for the analysis of infectivity removal in prion filtration studies has been provided to R Rowher, Baltimore, USA. The Unit tissue bank is part of the Neuroprion Brain Banking project and the Medical Research Council University of Edinburgh Brain Banks are part of the European Commission-funded brain Net Europe project, which is at the leading edge in the development of brain banking and diagnostic techniques relevant to neuropathology.

The Neuropathology Laboratory takes part in a wide range of research activities ranging from those which are primarily based in pathology to collaborative projects with other groups in the Unit, particularly the Prion Protein Laboratory (identification and localisation of Type1 PrPsc in brain and other tissues, provision of tissues for CDI, PMCA analysis, and for cell culture, and collaborative analysis of iatrogenic, genetic and panencephalopathic forms of CJD), and collaborates with all leading laboratories involved in research in human prion disease in the UK, Europe, North America and Japan (T. Kitamoto, University of Sendai).
Prion Protein Laboratory
The NCJDSU Prion Protein Laboratory’s core function is the typing of abnormal prion protein in suspected cases of human prion disease. The detection of abnormal prion protein in brain autopsy and biopsy material, or in certain cases tonsil biopsy material, strongly supports a diagnosis of CJD. Prion protein typing is an integral part of the full classification of sCJD cases and is an important aid to the differentiation of vCJD from other prion diseases. It contributes to the surveillance for novel prion disease in the UK population and has a research function, contributing to a better understanding of how abnormal prion diversity might correlate with, or underlie CJD phenotypic diversity and the nature of the relationship between particular prion protein types and prion agent strain. The development of novel, sensitive and specific biochemical assays for the disease-associated form of the prion protein, that might find application as diagnostic tests and blood screening or confirmatory assays, is a major current focus of the Prion Protein Laboratory’s work.

Molecular Genetics
The NCJDSU Genetics Laboratory’s core function involves determination of PRNP-129 genotype (generally from blood samples obtained in life), which is essential for the full characterisation of cases of prion disease. Given the way in which PRNP-129 genotype affects sporadic CJD phenotype and the current association of variant CJD with MM genotype, genetic analysis can be helpful in differentiating atypical sCJD cases from vCJD. In addition, PRNP mutation screening is undertaken to exclude genetic prion disease.

CSF (Cerebro- Spinal Fluid) Laboratory
The NCJDSU CSF Laboratory plays an important role in core surveillance by providing a national CSF protein testing service, particularly in relation to 14-3-3-3. This is useful in the diagnosis of CJD and can aid case classification. Clinicians may request this test without charge at an early stage of clinical suspicion, before they formally refer the case. The laboratory thus represents an additional method of case ascertainment. Its access to referred CSF samples from a wide variety of cases (and non-cases) over 10 years has allowed significant research into the sensitivity, specificity and overall diagnostic utility of CSF protein tests in CJD. It has also been possible to correlate CSF protein data with clinical and other test data. The Unit’s CSF Laboratory has liaised with CSF laboratories
in other countries, to standardise methodologies (including organising a European-wide quality assurance for CSF 14-3-3 analysis and interpretation), to train laboratory personnel and to undertake specific research projects.

**Transmission Studies**

Animal transmission studies have a number of important roles in surveillance and research. Such studies (in selected mouse types) are the most long established method of assessing prion disease strain, and, therefore, disease classification. Animal transmission experiments also represent the only current method of determining with certainty the presence of infectivity. In addition, the use of transgenic mice expressing the human prion protein polymorphic variants allows the design and execution of a variety of experiments that address a number of important questions about prion disease. The Unit is ideally placed in its close collaboration with the Neuropathogenesis Division of the Roslin Institute (J Manson and colleagues) with its longstanding, internationally recognised, expertise in prion animal transmission and transgenics. Currently there are important research questions being addressed by transmission studies, as detailed below.
In this section, we attempt to describe the key scientific questions (and related practical issues) in relation to prion disease that the Unit is involved currently in answering, either as part of core surveillance or through research funded by specific additional research funding. Much work is done in collaboration with colleagues in the UK, Europe or internationally. The questions are grouped together relating to certain general areas. There are, inevitably, overlapping aspects to some questions and the inclusion of a question in one area and not another, while being perhaps a little arbitrary, is done to avoid unnecessary repetition.

**SURVEILLANCE**

**Question 1. Is UK surveillance of variant and sporadic CJD complete? How can this be assessed?**

As described in the Introduction (Section III), the Unit has multiple methods of case ascertainment: the principal two being referral of suspect cases by neurologists and referrals of suspected and confirmed cases by neuropathologists. An important additional method is based on the Unit’s national CSF Laboratory. Clinicians send CSF samples, particularly for 14-3-3 estimation, and this allows the Unit to identify cases that are being considered by clinicians. However, it is difficult, or even impossible, to prove that surveillance is complete. The Unit has taken various approaches to this:

- **a)** Copies of death certificates are obtained in all individuals dying of CJD in the UK (see Introduction, Section III). In all such cases in which details are not already available at the Unit, medical records are obtained and cases then classified according to the routine diagnostic criteria. In this way, data are obtained in the small (and decreasing) number of cases diagnosed as CJD, but which are not directly referred to the Unit in life.

- **b)** A National Retrospective Pathological Review of CJD and related disorders was carried out in all the major Neuropathology Laboratories in the UK over 1998-2007. The aim was to review cases of CJD which have been identified in diagnostic files back to 1970 (the earliest point of prospective clinical surveillance data) and to review
selected groups of atypical dementia cases in order to determine whether any cases of CJD (particularly variant CJD) had been misclassified or missed altogether. The results found no evidence that any cases of variant CJD has been misclassified or missed; some cases of atypical dementia were found to represent unusual cases of prion disease, one of which carried a novel mutation in the prion protein gene (Piccardo et al, J Neuropathol Exp Neurol 1998).

c) A Retrospective Death Certificate-case note review was undertaken for the period 1979-1996 in collaboration with the Office for National Statistics and the London School of Hygiene and Tropical Medicine. 1485 individual deaths in the 15-44 age range were identified. In 48%, the clinical records were traced and reviewed. No previously unknown cases of CJD were identified. (Majeed et al, BMJ 2000).

d) Regular comparisons of incidence and mortality rates for CJD between countries (taking into account variation in surveillance methodologies, including post-mortem rates). It is noteworthy that in recent years, the mortality rates for sporadic CJD in Europe have been relatively stable with no sustained change in rate in any single country.

e) Maintenance of a relatively high post mortem rate in referred cases. This essential to case confirmation, which is particular importance in clinically atypical cases, some of which may not be identified in life. As BSE infection in different human genetic backgrounds, or human infection with atypical animal TSEs, might present with novel clinico-pathological phenotypes, sustaining a high post-mortem rate in all suspected or referred cases is a major priority.

Post-mortem examinations are also a means of validating the sensitivity and specificity of clinical diagnostic criteria (which have been formulated and adopted for sporadic, variant, genetic and iatrogenic CJD). The sensitivity and specificity of the diagnostic criteria for sCJD and vCJD have been re-assessed recently by the Unit with sensitivity and specificity of 72% and 79% for the sporadic CJD criteria and 83% and 100% for the variant CJD criteria, respectively. A major objective of collaborative studies in Europe on specialist investigations is to improve the diagnostic criteria for sporadic CJD, with particular emphasis currently on validating
the utility of MRI brain scan in the diagnosis of sporadic CJD. In relation to the vCJD diagnostic criteria, of the 12 cases which did not fulfil the criteria for classification as 'probable' vCJD in life (out of 106 pathologically confirmed cases), 10 had the pulvinar sign on MRI brain scan, but three of these cases could not be classified because of preceding morbidity. Maintaining a high post-mortem rate (currently about 70% of suspect cases) is essential to achieving a high level of case ascertainment and appropriate case classification.

\(f\) Cases of vCJD may present to paediatricians and may be difficult to distinguish from other progressive neurological diseases. To address this, the Progressive Intellectual and Neurological Deterioration (PIND) Study, which is funded by the DH, was established, utilising the mechanism of the British Paediatric Surveillance Unit (BPSU). The aim being to identify all cases of progressive intellectual and neurological deterioration in children in the UK, particularly those with features suggestive of vCJD. As at February 2008, after more than 10 years surveillance, six cases of vCJD, four definite and two probable, have been identified out of 2391 patients reported with suspected PIND. Three were reported in 1999, one in 2000 and two in mid-2001.

In addition, transmission of vCJD from mother to child cannot yet be excluded as a potential route of secondary transmission and the PIND study is an important mechanism of case ascertainment should such cases occur. This aspect of the study is of particular importance as there is no direct follow-up of children of vCJD cases, which would be very difficult to justify ethically.

The PIND study also has added value in providing systematic data on progressive neurological disorders in children, including a range of very rare conditions, and this has resulted in a number of scientific publications.

\(g\) We cannot be certain that we are not missing cases of CJD in those over the age of 60 years as they may remain undiagnosed in the community or diagnosed as a general dementia without specific neurological follow up. Variant CJD cases could conceivably be unrecognised. The incidence of sporadic CJD increases with increasing age but then appears to fall in the very elderly. This could be the result of
under ascertainment in this age group, or could reflect a real decline, which would be of interest with respect to causation. CJD surveillance in the older population may also be important because of the increased frequency of surgical procedures in this age group and therefore concern about the potential for secondary transmission via contaminated surgical instruments. However, the age distribution of vCJD shows a gradual decline from a peak in individuals aged 20-35 and we believe that ascertainment is highly efficient up to the age of about 70 years. This does not suggest that significant numbers of primary cases of vCJD are being missed in the elderly population. Further issues surrounding ascertainment of sCJD are discussed in the NCJDSU’s 2006 Annual Report.

In 2004/05, a grant proposal for a pilot study was prepared with collaborators in the Institute of Public Health, Cambridge (C Brayne, Dr. D Summers), but this was not pursued because of withdrawal of support from the Primary Care Trust. To aim of the project was to determine whether a) there was unrecognised vCJD in the older population by searching for atypical dementias in the elderly, b) it was possible to set up routine systems which could detect otherwise unrecognised vCJD in the older population and c) the methodology described was practicable and could be extended to other geographical areas.

In 2006/07 we discussed the possibility of doing a similar study with collaborators in the Centre for Public Health and Primary Care Research, University of Edinburgh. However, because of the low incidence of vCJD it was not deemed a priority to pursue at this stage. Additional funding would be needed to take this further. Without a study of this type, it will not be possible to know whether we are missing cases of vCJD in the elderly. However, because of the lack of new diagnoses in the past 16 months in the younger age groups, a negative study in the elderly would be difficult to interpret. On the other hand, one role of the study was to determine whether such a methodology was feasible. This might be useful to know in advance in case of vCJD in, for example, other codon 129 genotypes, or through secondary transmission, which could potentially present in older age groups.
VARIANT CJD

Question 2. The UK primary variant CJD epidemic: What is the cause? What is the current situation? How is it changing over time?

The clinical features, neuropathology, prion protein type, molecular genotype, age, sex and geographical distribution of vCJD cases will continue to be investigated as in the Introduction (Section III). Unusual or atypical cases of CJD are of particular interest and are studied carefully, especially those from the less common codon 129 genotypes. This involves detailed assessment of the clinical phenotype, epidemiological characteristics, prion protein type and neuropathology in order to identify cases that are distinct from previous experience. If truly atypical cases are identified transmission studies in an animal model may be considered in collaboration with the Neuropathogenesis Division, Roslin Institute. An example of such a case has been described in the recent paper on CJD in adolescents (Murray et al, Journal of Neurology Neurosurgery & Psychiatry 2008). In addition, the investigation of ‘non-cases’ (those suspected of having CJD who turn out to have an alternative diagnosis) continues to be important and provides useful comparative information.

Short-term predictions of the vCJD epidemic continue to be undertaken (N Andrews, HPA) and biannual meetings are held to discuss aspects of epidemiology and mathematical modelling with colleagues at the London School of Hygiene and Tropical Medicine (P Smith, S Cousens) and Imperial College, London (A Ghani, T Garske). As a result of these meetings research strategies and publications have been discussed, planned and executed, for example, case control study of risk factors for CJD (Ward et al, Annals of Neurology 2006 and Ward et al, Annals of Neurology 2007); geographical distribution of CJD (Cousens et al, Journal of Neurology Neurosurgery & Psychiatry 1990; Cousens et al, Lancet 2001; Linsell et al, Neurology 2004); predictions through modelling of primary and secondary epidemics (Cousens et al, Nature 1997; Clarke et al, Journal of the Royal Society Interface 2007; Garske et al, Journal of the Royal Society Interface 2006) (please see Appendix 8 for a full list of collaborative publications).

Through the EUROCJD system it has been possible to provide real-time data on the incidence of vCJD internationally and to determine in all cases potential risk factors including history of residence in the UK. Of the cases of vCJD resident outside the UK at diagnosis, the majority have not lived in the UK for any significant period of time (for
example, greater than 6 months) and therefore, would appear to have acquired the BSE agent within their own country. However, this could be explained by exports of live cattle, carcasses, meat/ meat products and cattle-feed from the UK during the UK BSE epidemic. The non-UK vCJD cases appear similar in clinical features, neuropathology and molecular biology to UK cases. However, a more detailed comparison between UK and French (the country with the most vCJD cases outside the UK) vCJD cases is being undertaken. International agreement on the attribution of cases by country has been achieved and a table including up to date information on all cases of vCJD is available on the Unit website (www.cjd.ed.ac.uk). These data are of major importance for public health authorities internationally and the Unit aims to continue to provide accurate and timely data on vCJD worldwide.

The prevalence of human BSE infection in the UK population.

Key to understanding the potential burden of clinical cases of CJD (and the risk of secondary transmission) is determining the population prevalence of BSE infection. A study was undertaken examining the presence of prion protein using two monoclonal antibodies in surgically removed tonsils and appendices (Hilton et al, J Pathol 2004; Ironside et al, BMJ 2006). Three appendicectomy samples showed lymphoreticular accumulation of prion protein, giving an estimated prevalence of 237/ million (95% CI 49-692/ million). This study had its limitations and so the HPA were asked to establish a National Anonymous Tonsil Archive (NATA study) of 100 000 tonsil pairs, to be tested for abnormal prion protein. The Unit has played a role in collaboration with HPS in establishing the recruitment of tonsils from hospitals in Scotland. In addition, the Unit’s Neuropathology Laboratory currently acts as a quality control centre for the immunohistochemical arm of the National Anonymous Tonsil Archive (NATA) study. This study is on-going.

The Unit is involved in assessing and developing certain diagnostic tests that may be helpful in assessing prevalence of infection (see Questions 9 and 10 below).

The factors that govern human susceptibility to BSE infection are being investigated in a variety of ways by the Unit, both internally and through external collaboration, and are addressed in Questions 3 and 4 below.
Risk Factors for variant CJD

Case control study

Details of the methodology are provided in Appendix 6. The Multi-centre Research Ethics Committee for Scotland approved the study.

Results of case control study

Results of the first analyses of case control data examining risk factors for vCJD were published in 2006 (Ward et al, Annals of Neurology 2006). In this study we included all “definite” or “probable” vCJD cases identified in Great Britain between May 1995 and November 2003 and 922 controls recruited between 2002 and 2003. The study had 70% power to detect factors associated with a doubling of risk if their prevalence was between 10 and 90%, and 80% power to detect factors associated with a 2.2-fold increase in risk.

Reported frequent consumption of beef and beef products thought likely to contain mechanically recovered and/or head meat, including burgers and meat pies, was associated with increased risk of vCJD, as was reported frequent chicken consumption. Surgical operations were generally similarly reported for cases and controls, with the exception of a small group of minor operations, possibly attributable to under-reporting in controls. Cases and controls had similar reported occupational histories and exposure to animals.

Statistical analysis of the data revealed no convincing evidence of increased risk through medical, surgical or occupational exposure, or exposure to animals, and our findings are consistent with dietary exposure to contaminated beef products being the main route of infection of vCJD. However, this first analysis of data relied on reported histories from relatives of cases and controls, because cases are too unwell to be interviewed directly and to reduce bias controls were dealt with similarly. More accurate information can be obtained for medical and surgical risk factors from medical records directly and this is the next stage of the study (see below).

On-going work of case control study

The case control study has been funded by three consecutive research grants: 1) Department of Health 1998 – 2002, 2) DH 2002 - 2006 and 3) Scottish Government
2006 – 2008. The funding for the case-control study will cease as of 31st March 2008. With the decrease in the number of incident (new) cases of vCJD, further funding was not awarded. However, the Unit will continue to collect risk factor information of all suspect cases referred to the Unit within its core funding. If in the future it is thought necessary, funding could be sought to recruit further controls.

At present the Unit is focusing on obtaining data from general practitioner records (rather than relying on reported data from relatives) of cases and controls in order to examine the possible risk of primary and secondary transmission through medical/surgical procedures. We aim to have finished gathering these data by early summer 2008 and will continue data entry and begin the process of data ‘tidying’ and analysis. To complete double entry of these medical risk factor data (approximately 600 controls) we will need to employ an extra member of staff, which the Unit aims to fund within existing resources.

A comparison of the basic epidemiology and risk factors for cases of vCJD resident in the UK and in France is being undertaken and will be prepared for publication (A Alperovitch, J-P Brandel).

Geographical distribution
The Unit has an on-going interest in the geographical distribution of variant CJD and how this may shed light on causation. The relatively high incidence of cases of vCJD in the north of the UK compared with the south will continue to be monitored in the event of future cases of vCJD (S Cousens, P Smith). Analysis of this in 2001 showed an inconsistent correlation with consumption of foods thought to contain BSE infection and regional rates of vCJD (Cousens et al, Lancet 2001). The distribution of vCJD does not appear to be explained by social class, urban/rural mix, population density or ethnic group.

Analysis of geographical area of residence of vCJD cases is undertaken annually to detect any statistically significant clusters. A number of geographically associated cases of vCJD were investigated and a paper is in preparation (N Gill, HPA). Further investigations will be undertaken if appropriate.
Question 3: What are the clinico-pathological, genetic and molecular features of vCJD and how are they related?

The Unit has been able to characterise the clinico-pathological features of vCJD through its core surveillance activities. Specific research has been undertaken into the early clinical features of vCJD and its psychiatric manifestations (Spencer et al BMJ 2002). In collaboration with Prof N Kapur (Addenbrooke’s Hospital, Cambridge), research continues on neuropsychological aspects of variant (and sporadic) CJD. A paper updating the neuropsychological profile in vCJD has recently been submitted for publication and will be forwarded to DH if accepted for publication.

The neuropathological characteristics of vCJD have been defined by the Unit and obviously form a critical part of the differentiation of BSE-related human disease from other prion diseases. The Neuropathology and Prion Protein Laboratories have undertaken much research in this area. The distribution of disease-associated prion protein in extra-neural tissues is of particular importance, especially in vCJD. The localisation and characterisation of abnormal prion protein in the pituitary gland in sporadic and variant CJD has been published recently (Peden et al, J Gen Virol 2007) and adds to our previous tissue-based studies using PrP\textsuperscript{Sc} detection in CJD tissues. Our data is compiled and summarised in tabular form below. These studies confirm earlier findings that consistent extraneural involvement, particularly of lymphoreticular system is a characteristic feature of vCJD. However, high to moderate levels of PrP\textsuperscript{Sc} are found outside the brain in both sporadic and vCJD, but these are confined to neuronal tissues in the retina, optic nerve, trigeminal and dorsal root ganglia. Low or inconsistent levels of PrP\textsuperscript{Sc} are also found in sCJD and vCJD skeletal muscle and pituitary tissue. However, non-neuronal ocular tissues, dental tissues, some major organs and urine all appear negative at current sensitivity levels in both sCJD and vCJD. The differing pattern of tissue involvement in sCJD and vCJD might be a consequence of disease aetiology (peripheral infection in vCJD versus stochastic events in the brain in sCJD) or it might be a consequence of an intrinsic lymphotropism of the vCJD agent. However, centripetal and centrifugal dissemination of the agent is difficult to address in autopsy tissues from end-stage disease.
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Following an earlier study that failed to detect disease-associated prion protein in dental tissues in vCJD (Head et al, Brit Dental J 2003), a retrospective re-analysis of these tissues was undertaken in 2007 using a more sensitive biochemical assay, which yielded identical results. These data were provided in 2007 to Dr Peter Bennett as part of the scientific input for the revised DH Dental Risk Assessment. The Unit has provided samples of frozen dental tissues for further research, including transmission studies with Dr Neil Raven and colleagues in HPA, Porton Down.

Table 1: Tissue distribution of PrP<sup>Sc</sup> in autopsy variant and sporadic CJD tissues by a combination of immunohistochemistry, paraffin embedded tissue blotting and Western blotting

<table>
<thead>
<tr>
<th>Uniformly high</th>
<th>Sporadic CJD</th>
<th>Variant CJD</th>
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<tbody>
<tr>
<td>Intermediate</td>
<td>Optic nerve&lt;sup&gt;2&lt;/sup&gt;, Trigeminal ganglion&lt;sup&gt;1&lt;/sup&gt;, Dorsal root ganglion&lt;sup&gt;3,4&lt;/sup&gt;</td>
<td>Lymph node&lt;sup&gt;2,9&lt;/sup&gt;, Tonsil&lt;sup&gt;1,5,6&lt;/sup&gt;, Spleen&lt;sup&gt;1,4&lt;/sup&gt;, Appendix&lt;sup&gt;3&lt;/sup&gt;, Peyer’s patches&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lower or inconsistent</td>
<td>Skeletal muscle&lt;sup&gt;1&lt;/sup&gt;, Pituitary gland&lt;sup&gt;6&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>Below detection limit</td>
<td>Cornea&lt;sup&gt;2&lt;/sup&gt;, Lens&lt;sup&gt;2&lt;/sup&gt;, Vitreous body&lt;sup&gt;2&lt;/sup&gt;, Sclera&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Dental pulp&lt;sup&gt;1&lt;/sup&gt;, Gingiva&lt;sup&gt;1&lt;/sup&gt;, Alveolar nerve&lt;sup&gt;1&lt;/sup&gt;, Salivary gland&lt;sup&gt;7&lt;/sup&gt;</td>
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<td>Lung&lt;sup&gt;3&lt;/sup&gt;, Liver&lt;sup&gt;3&lt;/sup&gt;, Kidney&lt;sup&gt;3&lt;/sup&gt;, Heart&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Lung&lt;sup&gt;3&lt;/sup&gt;, Liver&lt;sup&gt;3&lt;/sup&gt;, Kidney&lt;sup&gt;3&lt;/sup&gt;, Heart&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Urine (clinical specimens)</td>
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<sup>1</sup>Head et al, Annals of Neurology 2004  
<sup>2</sup>Head et al, Investigative Ophthalmology & Visual Science 2003  
<sup>3</sup>Head et al, American Journal of Pathology 2004  
<sup>4</sup>Ritchie et al, Neuropathology & Applied Neurobiology 2004  
<sup>5</sup>Peden et al American Journal of Pathology 2004  
<sup>7</sup>Head et al, British Dental Journal 2003  
<sup>8</sup>Head et al, Neurology 2005

Prion protein typing is another critical step in the characterisation of vCJD cases and the relationship between the source of infection (BSE), PRNP genotype and prion protein type is an area of continuing research. To date all the consistent clinical and pathological features of vCJD are accompanied by the type 2B PrP<sup>res</sup> type indicating that the BSE glycoform signature remains a useful diagnostic test for BSE-related disease. The Neuropathology Laboratory has continued to improve the sensitivity of laboratory-based methods for the detection of the disease-associated form of the prion protein by improving the sensitivity of immunocytochemistry and by developing ancillary
techniques such as the PET blot technique. The Prion Protein Laboratory aims to contribute to a fuller biochemical definition of the PrP$^{\text{Sc}}$ types that occur in human prion diseases using a combination of conventional Western blotting, CDI, sucrose gradient density centrifugation and monoclonal antibody recognition. In addition, mouse transmission studies of the strain characteristics of vCJD from different geographic regions are underway.

All symptomatic cases of vCJD tested to date have been in PRNP-129 methionine homozygote (MM) individuals and the Molecular Genetics Laboratory continues to analyse the PRNP genotype of all referred cases, whenever possible. The Unit is also involved in research into possible genetic influences on prion disease susceptibility and phenotype other than the PRNP-129 polymorphism. The Laboratory reviews data on non-human TSE genomics, looking for future candidate markers in humans that could be assessed. In addition, a EUROJJD project on the identification of non-PRNP genetic influences on human prion diseases is underway (C van Duijn, Erasmus University). Preliminary results are encouraging and the project should be completed in 2008. The Unit has an ongoing collaboration with the Royal Free Hospital (M Pepys) on genetic variability in the complement system and associations with human prion disease.

Studies of genetic prion disease have also been undertaken by the Prion Protein Laboratory. Pronounced biochemical diversity has been identified in PRNP mutation cases, ranging from cases with little detectable protease-resistant PrP (in fatal familial insomnia), to mixtures of types 1 and 2 (in familial CJD), to the presence of small fragments of PrP (in a subset of Gerstmann-Straussler-Scheinker cases) and with an extreme range of glycoform ratios represented. These results suggest that mutations in PRNP result in significantly different pathological alterations of prion protein metabolism. This study is currently being prepared for publication.

The use of functional genomics to identify disease-specific changes in gene expression in the vCJD brain is being investigated. The project has shown proof of the principle that RNA of sufficient quality can be extracted from autopsy human brain specimens, and that the relative abundance of transcripts can be compared between CJD cases and other dementias and normal (sudden death) brain sample controls. This work is currently being written for in the form of a doctoral thesis.
In collaboration with EU researchers, the Unit published a study of tau protein genetics in CJD (Sanchez-Juan et al, BMC Med Genet 2007). Further work on this topic is continuing. In the Unit has also undertaken recently a collaboration with EU researchers to examine the potential role of Cathepsin D in both variant and sporadic CJD. The vCJD results are in press and the sCJD results are being prepared for publication.

**Question 4. Will BSE-related disease appear in PRNP-129 MV and VV individuals? How may this be determined?**

Neuropathological studies on an individual who died without clinical evidence of vCJD five years after a blood transfusion from a donor who subsequently died from vCJD showed evidence of vCJD infection in lymphoid tissues alone (Peden et al, Lancet 2004). This recipient was heterozygous (MV) at codon 129 in the prion protein gene. Genetic analysis of two out of three positive appendix samples in the tissue-based prevalence study in 2001-2004 showed that both were valine homozygous (VV) at codon 129 in the prion protein gene (Ironside et al, Brit Med J 2006). These findings indicate that the MV and VV genotypes appear to be susceptible to infection with BSE, but provide no evidence on what (if any) clinical and neuropathological features might result.

Therefore, it is essential that clinical, epidemiological and pathological data of human prion diseases continue to be monitored carefully, looking to see if unusual, novel, disease types are emerging. However, it is not known if clinical BSE-related disease in humans of MV or VV genotype would have the same clinico-pathological phenotype as MM-vCJD. Even if the phenotype were different, it is assumed that it would be referred under present surveillance arrangements. The key therefore is both to continue to encourage referral of any suspect cases and to make clinicians aware of the possibility that human BSE-infection might have clinical expressions different from the vCJD picture with which they are now familiar. If ‘atypical’ cases were to be identified in the UK, the Unit is ideally placed (via its extensive international connections) to rapidly ascertain whether similar cases are to be found in other countries (both higher and lower BSE risk countries).
The potential effect of prion protein gene polymorphisms on the risk of CJD and their effects on CJD phenotype are being addressed in two ways:

First, the Unit is able to correlate the genetic analyses with the clinico-pathological data. Second, as detailed further in Question 8, transgenic mice experiments are being undertaken in collaboration with the Roslin Institute. In addition, in collaboration with Erasmus University (Netherlands), the Unit has undertaken an analysis of polymorphisms in the \textit{PRNP} promoter region (as opposed to the Open Reading Frame) in CJD and controls. The results are currently being prepared for publication.

Given the potential importance of \textit{PRNP} polymorphisms in CJD, the normal population distribution of these is an important question. The Unit undertook an analysis of blood donors in two regions of the UK and then led a collaborative project looking at blood donors and other normal groups in Finland, Eire, Belgium, Greece and Iceland. This has provided a large number of normal population data on \textit{PRNP} polymorphisms, enabling comparisons between countries. The data also suggest that random sampling of blood donors gives results representative of general population sampling. Further data (concerning \textit{PRNP} variations other than the 129 M/V polymorphism) are being analysed and prepared for publication.

There are two methods of trying to confirm whether a CJD case has resulted from BSE: Prion protein typing and animal transmission experiments. Prion protein typing is not without difficulties (both theoretical and practical), but is the simplest, fastest and cheapest method. It is carried out routinely, by the Unit’s Prion Protein Laboratory, on all suspected cases from which suitable frozen tissue is available for diagnostic purposes. All autopsy proven cases of clinical vCJD thus far subjected for Western blot testing have a type 2B PrP type in the brain. This demonstrates the utility of the diglycosylated dominant BSE glycoform signature as part of the routine diagnostic process. Until combined epidemiological, clinical, and pathological evidence indicates the existence of BSE-related disease in patients with MV or VV genotypes, the biochemical phenotype of such cases remains speculative. However, three lines of evidence suggest that the BSE glycoform signature may be conserved in MV and VV individuals. First the BSE glycoform signature is present in the spleen of the asymptomatic vCJD blood transfusion recipient MV patient described by Peden et al (Lancet 2004). Second the glycoform signature is also present in vCJD infected MM, and in MV and VV humanised transgenic
mice (Bishop et al, Lancet Neurology 2005). Unfortunately BSE does not transmit efficiently to these mice, however BSE PrPSc can be amplified by the cell free PMCA assay using MM and MV (but not VV) humanised transgenic mice and the BSE glycoform signature is seen in the amplified material (Jones and Head, unpublished observation). Taken together these three lines of evidence are consistent with the BSE glycoform signature marking human BSE in non-MM individuals. Whether it is specific to such a disease, should it occur, is a different question. There have been two reports of BSE-like glycoform signatures in valine homozygous individuals with CJD (Head et al, Annals of Neurology 2001, Mead et al, Archives of Neurology 2007). Both sets of authors discussed the possibility that the cases in question resulted from BSE infection but neither group found confirmatory evidence in other aspects of the disease phenotype sufficient to link these cases to BSE exposure but recommended vigilance for future cases with a similar phenotype.

Biological strain typing by transmission to wild type and transgenic mice panels presently provide the firmest confirmation and such experiments are on-going in collaboration with Professor J Manson,(Neuropathogenesis Division, Roslin Institute).

**Question 5: Secondary, iatrogenic, vCJD in the UK: What is the risk? How is it to be managed?**

Although the numbers of UK vCJD cases are currently in decline, there are continuing concerns about the possibility of future cases, in particular secondary, cases as a result of medical or surgical transmission. The population prevalence of sub- or pre- clinical human BSE infection is a significant factor. (Sub-clinical cases are infected, but never develop clinical disease either because they are true “carriers” or because the incubation period is longer than the life expectancy of the individual; while pre-clinical cases are infected and then go onto develop clinical disease. Though both sub- and pre- clinical cases are infected, they may or may not be infectious. There is no current method of distinguishing these two states in vCJD, except by follow-up, and the terms are essentially interchangeable in this context: both relate to symptomatic infected and, therefore, potentially infectious-individuals). This is particularly important for public health as preventative measures can be put into place to limit the possibility of secondary transmission.
There are four areas of current concern: surgery (mainly neurological, ophthalmological, and gastrointestinal), dentistry, transfusion of blood components and plasma derived products. The Unit’s Brain and Tissue Bank resources have been used to study the distribution of PrP<sup>Sc</sup> in the tissues relevant to surgery and dentistry (Head et al; Br Dent J 2003; Head et al, Invest Ophthalmo Vis Sci 2003; Head et al, Am J Pathol 2004; Head et al, Br J Ophthalmol 2005) and the Bank has provided tissues for experimental infectivity studies. The tissue distribution of PrP<sup>Sc</sup> in sCJD and vCJD tissues and organs has been commented on in Question 3 above in relation to disease phenotype but it is also pertinent to evaluating risks of iatrogenic transmission. The overall greater distribution and generally higher levels of PrP<sup>Sc</sup> in vCJD peripheral tissues compared to sCJD suggests that vCJD does present greater risks of secondary transmission, particularly where lymphoreticular tissues are concerned. The risks involved in any procedure involving the retina and optic nerve would also be judged to be high by these criteria.

However, it is evident that iatrogenic transmission has occurred from tissues in which PrP<sup>Sc</sup> is readily detectable (such as brain, and pituitary) and from tissues in which PrP<sup>Sc</sup> is currently undetectable (cornea and blood) indicating that the presence of detectable PrP<sup>Sc</sup> is only one of many necessary considerations when evaluating the risk of iatrogenic transmission by a given route or procedure. Blood samples from a large number of cases of sCJD, vCJD and iatrogenic CJD have been archived at the Unit and are an important resource for possible further research in relation to tissue infectivity.

In relation to blood, the Transfusion Medicine Epidemiology Review (TMER) study is of fundamental importance (see Introduction, Section III). The effectiveness of this collaboration has allowed the identification of four cases of transfusion transmitted vCJD infection. In 2003 a case of vCJD associated with blood transfusion and the identification in 2004 of PrP<sup>Sc</sup> in the spleen and lymph node of a recipient of blood donated by someone incubating vCJD. In 2006 a further two cases of vCJD associated with blood transfusion were identified. A further instance of a potential link between a donor and a recipient with vCJD has been investigated and discussed recently at the CJD Incidents Panel. A paper has been prepared on this subject and will be forwarded to DH when completed. An analysis of patients identified by the TMER study who received a blood transfusion from an individual later dying of vCJD and who have themselves died has been completed. The question is whether any of these individuals has evidence of
vCJD clinically before death. A paper on this cohort of individuals describing cause of
death and pre-terminal clinical features is in preparation and will be forwarded to DH
when completed.

Mathematical modelling by Ghani and colleagues in collaboration with the NCJDSU has
indicated that a self-sustaining epidemic of vCJD through blood transfusion is unlikely
(Clarke et al, J R Soc Interface 2007).

The TMER study suggests that transmission of vCJD through blood transfusion is
relatively efficient and this may be because the agent strain of agent in vCJD has adapted
after inter-species transmission or because of the intravenous route of infection. This is a
critical issue for public health, because agent adaptation might lead to an increased risk of
secondary transmission through other mechanisms. A study in collaboration with the
Neuropathogenesis Division, Roslin Institute, in which the agent characteristics of blood
transfusion related cases and primary cases are compared in laboratory transmission
studies is underway (funded by the MRC). A paper on the initial results has been
submitted for publication and indicates that it is the intravenous route rather than agent
adaptation that most likely explains the efficiency of blood transfusion transmission in
vCJD.

To date, there have been no identified instances of vCJD transmission through plasma
products, but such products were prepared from donations given by people who went
onto develop vCJD (11 donors, 25 units of plasma). A paper on plasma products derived
from donors who later developed variant CJD is in preparation.

The potential for vCJD to be transmitted form person to person through contaminated
surgical instruments is a critical issue for public health. In order to examine for evidence
of such transmission the Unit obtains detailed information of past surgical procedures in
all cases of vCJD from interview with relatives, examination of hospital records and from
GP records which are obtained after death. A database has been created in which the
date, location and type of surgery are listed and close temporal and geographical links
between surgical procedures are identified. The hypothesis is that transmission via
surgical instruments will lead to patients being identified in whom surgery has been
carried out in the same hospital within a short timeframe. To date seven such pairs have
been identified but the gap between procedures and the disparate types of surgery do not suggest that ‘surgical’ transmission has occurred.

A collaborative study (A Ghani) modelling the secondary transmission of vCJD through surgery showed that a self-sustaining secondary epidemic was possible (Garske et al, J R Soc Interface 2006). Further models of transmission of vCJD through surgery, blood transfusion, dentistry and combinations of routes are being considered (A Ghani, T Garske).

Analysis of medical and surgical data from the Unit’s case control study examining risk factors for CJD (for details of case control methodology see Appendix 6) has concentrated so far on histories reported during face-to-face interviews of relatives of cases (a necessity because cases are usually too unwell to be interviewed directly) and controls (to be similar to cases so as not to introduce bias into the study). However, medical and surgical risk factor data are being collected currently from GP records for up to 600 control subjects, which will be compared with that of cases to determine whether there is evidence of secondary iatrogenic transmission for vCJD (see Question 2 above for further details). Sporadic CJD has been transmitted through neurosurgery, dura mater grafting and corneal transplantation. Because of the increased tissue distribution of vCJD compared with sCJD (abnormal prion protein has been found in lymphoreticular tissues, albeit at lower levels, in addition to central nervous system and ophthalmic tissues), there are concerns that there is increased risk of transmission of vCJD via invasive medical procedures. Recent preliminary data from animal studies (HPA, Porton Down) have indicated that the risk of transmission of vCJD through dentistry may be more than previously thought, though the study of dental tissues from cases of vCJD by the Unit have failed to demonstrate disease- associated prion protein (see Question 3).
A pilot study to assess the feasibility of accessing dental records of those with CJD and general population controls was undertaken in 2006/07. This demonstrated that dental records for controls could be obtained more often (52%) than for cases (19%), which was not surprising as information from controls was collected more recently. The main problem was identifying the dental practitioner from the information given by a close family member. Enhanced data collection using centralised dental payment schedules, which avoided this issue, was an alternative option.

A larger study involving all vCJD cases and general population controls has been funded over 12 months (commencing early 2008). Dental records of variant CJD cases and controls will be obtained, where possible, and compared with each other to determine whether two or more cases have had dental treatment at the same dental practice and within a time frame that might indicate possible transmission through contaminated dental instruments from one case to another. Dental histories of cases will also be compared with those from general population controls to examine evidence of possible risk from dental treatment. Dental Practice Boards will help identify dental practices of cases and controls and will attempt to provide dental payment schedules where necessary.

Collaboration has been established with colleagues at the MRC Biostatistics Unit, Cambridge (S Bird). A study of the potential for vCJD transmission through neurosurgery is being undertaken and a paper is being prepared for publication.

Certain individuals have been identified as being ‘at risk of CJD’ by the CJD Incidents Panel through various routes, including exposure through blood transfusion and high or medium risk surgery. Multi-centre Research Ethics Committee approval has been obtained for the follow up of this ‘at-risk’ cohort for public health and research purposes. This is extremely important in order to help elucidate various factors, which may have important public health consequences, such the incubation period, pre-clinical phase, sub-clinical phase and transmissibility of vCJD through these different routes. The project will be developed in 2008; it is being led by HPA in collaboration with the Unit, the National Blood Services, the National Prion Clinic and the National Institute for Biological Standards and Control (K Soldan, N Gill, N Connor, P Hewitt, M Painter, S Wroe, P Minor). The Unit’s involvement in the project is detailed below in Question 14.
in the Public Health section. In addition, with colleagues at HPA, the Unit has been involved (developing methodology and, when available, interpreting and disseminating results,) in developing qualitative research to determine the impact of notification of at risk of CJD status. This will involved carrying our a semi-structured interview of a sample of those who have been notified of their at risk status in order to determine what they have understood by the label ‘at risk’ and how it has affected their lives. This will help inform public health policy regarding future notifications. An investigation of the implementation by hospitals throughout Scotland of the ACDP TSE Working Group guidance relating to pre-operative risk assessment of those at risk of CJD has been undertaken and a report is being prepared (HPS, SNBTS).

**Question 6: vCJD in other countries: What is happening to vCJD outside the UK?**

**How does this aid understanding of UK vCJD?**

The initial identification of vCJD in the UK was importantly aided by the rapid availability of comparative data from other EU countries. In addition, vCJD is occurring in other countries and, through the Unit’s international collaboration, we have very good information on these cases. Many of these cases are reviewed clinically, epidemiologically, pathologically and biochemically in the Unit as part of our role as an international reference centre for prion diseases. This gives us an “early warning” of these cases and aids the public health investigation of any possible link to a UK-related exposure.

Collaborative analysis of the UK and French vCJD data may be helpful in consideration of the causation of vCJD. The presumed causal exposure to BSE in vCJD varies by geographical location of the cases. The UK cases were exposed to BSE in the UK, 22/23 of the French cases to BSE in France, 2/3 of the US cases to UK BSE while resident in the UK, the Japanese case either to BSE in Japan or through a short period of residence in the UK and the Italian case possibly to UK exports. The presumption is that all cases were infected by the same strain of BSE and a study has been initiated to confirm this hypothesis by transmitting brain material from cases of vCJD from a number of countries to panels of wild-type and human transgenic mice (J Manson). This is a collaborative study between the Unit, colleagues in surveillance systems in other countries and the Neuropathogenesis Division, Roslin Institute, and is funded through existing resources.
SPORADIC CJD

Question 7. What is the cause of sporadic CJD (sCJD)? What are the risks of secondary transmission of sCJD?

Case control studies

Risk factor information has been collected for cases of sporadic CJD since before the Unit was established in 1990. Over the years various control groups have been recruited; the method chosen depending on the resources available and anticipated validity. These have been detailed in various previous NCJDSU Annual Reports.

Since 1990 there have been five papers published examining risk factors for sCJD that the Unit has either led or collaborated in:-

1) Wientjens et al, Neurology 1996. A meta analysis of three case control studies (178 CJD cases and 333 controls). The results showed an elevated risk of CJD for those with a family history of dementia, a history of poliomyelitis, those employed as health professionals and those exposed to cows and sheep. There was no association with consumption of animal organs, including brain.

2) van Duijn et al, Lancet 1998- this compared 405 CJD cases and 405 hospital controls recruited as part of the 1993-95 EU collaborative studies of CJD in Europe. The findings suggested that genetic factors other than CJD mutations may play an important part in CJD. Iatrogenic transmission seemed rare in the population studied. There was little evidence of association between the risk of CJD and animal exposure or consumption of processed bovine meat or milk products for the period studied.

3) Zerr I et al, J Clin Epid 2000- medical risk factors were examined using the 405 CJD cases and 405 hospital controls recruited as part of the 1993-95 EU collaborative studies of CJD in Europe. The study failed to identify any common medical risk factor for CJD.

4) Ward et al, Neurology 2002- Surgical risk factors from 326 sCJD cases recruited as part of the 1993-95 EU collaborative studies of CJD in Europe were compared with 326 community controls recruited by telephone in 2000. A history of surgery was associated with risk of sCJD and the results supported the hypothesis that sCJD may result from hitherto unrecognised surgical contamination events.
5) Ward et al, Annals of Neurology 2007- Medical risk factors for 431 sCJD cases resident in the UK and referred to NCJDSU between 1998-2006 were compared with 454 general population control subjects recruited 2002-2003 (see NatCen controls in Appendix 6). This study found some evidence for a link between increased risk of sCJD and surgery, however there was no convincing evidence of temporal-geographical links between cases undergoing neurosurgery or gynaecological surgery. It concluded that it was unlikely that a high proportion of UK sCJD cases were the result of surgical transmission, but the possibility of such transmission cannot be excluded.

As for vCJD (see Questions 2 and 5 above), further work examining data from general practitioner records of cases and controls needs to be carried out in order to accurately determine risk for sCJD associated with medical or surgical procedures. This is on-going, though the priority has been given to vCJD at present.

**Blood transfusion**

An analysis of the potential for blood transfusion to be a risk factor for the development of sporadic CJD has been undertaken collaboratively by the major EU countries (coordinator M Pocchiari). A prospective study is under consideration.

**Genetic factors**

Possible genetic factors related to sCJD are being investigated by the Unit, both internally and through external collaboration (see Question 8 below).

**Geographical distribution**

The investigation of the geographical distribution of sporadic CJD by genetic and molecular subtype is on-going in the Unit.
**Question 8: What are the clinico-pathological, genetic & molecular features of sporadic CJD and how are they related?**

While this is an interesting question in its own right, it has an extremely important bearing on the Unit’s core surveillance function, particularly in relation to vCJD. This is because the most important and potentially difficult differential diagnosis of vCJD is sCJD; many initial reports of suspect cases of vCJD in the UK and elsewhere, have been found to be atypical cases of sCJD. A full characterisation of the clinico-pathological, epidemiological and molecular phenotypes of sCJD is therefore clearly essential, which the Unit has continued to carry out within the UK.

Atypical clinical presentations, disease courses and pathological findings are found in only a small percentage of sCJD cases and so are difficult to characterise, even in the UK population of 50-60 million. This is of particular importance in relation to any attempt to recognise new clinical disease phenotypes either related to BSE or, potentially, to other animal diseases. Therefore, our international collaborations have contributed greatly to the analysis of clinical, epidemiological and pathological data. For example, within the NEUROCJD collaboration, a detailed study was undertaken of particular presentations of sporadic CJD, such as pure cerebellar ataxia and Heidenhain’s syndrome (currently being prepared for publication). Studies of atypical forms and cases with young age of onset are in progress (Murray et al, J Neurol Neurosurg Psychiatry 2008). The large number of cases of sCJD accumulated within the system allowed a detailed study of the factors that separately influence disease duration (Pocchiari et al, Brain 2004).

It is well established that the clinico-pathological features of sCJD vary with PRNP-129 genotype and PrP protein type. Both the Unit’s Molecular Genetic and Protein Laboratories contribute to the full clinico-pathological-molecular characterisation of sCJD cases and research is being undertaken into these correlations. Research into possible genetic factors that affect susceptibility and disease phenotype in sCJD has been described above in Question 3.

The differentiation of atypical sCJD from vCJD is potentially aided by prion protein molecular data. However, the relationship between PrP protein type and CJD strain is not as straightforward as it initially seemed and, in particular, the Unit has been active in research into the phenomenon of the co-occurrence of prion types in individual cases of
CJD. Work carried out by the Prion Protein Laboratory used a type 1 specific antibody (12B2) to show that type 1 PrP\textsuperscript{res} is a minor component in brains of sporadic CJD cases previously classified as type 2 and also that a minority type 1 component is present in BSE brain, in all tested cases of vCJD and in vCJD transmitted to wild-type mice (Yull et al, Am J Pathol 2006). Whilst this work provoked interest and a study with similar conclusions was published by the Aguzzi laboratory (University Hospital, Zurich), the findings are somewhat controversial. To address this, the Unit has conducted a detailed study of the WHO CJD standard reference materials (available from the UK National Institute for Biological Standards and Control) to compare the PrP\textsuperscript{res} mixtures in cases of sporadic CJD that are acknowledged to be genuine mixtures, to those of vCJD where antibodies such as 12B2 are needed to detect the type 1 component. A paper is in preparation for publication. The distribution of disease-related prion protein in extra-neural tissues (such as skeletal muscle and pituitary) in cases of sCJD is an area of developing interest (Peden et al, Am J Pathol 2006; J Gen Virol 2007), which the Prion Protein Laboratory plans to continue to pursue in the future.

The protein laboratory studies have also included work on other forms of CJD. The study of iatrogenic CJD shows the presence of types 1 and/or type 2 PrP\textsuperscript{res}, similar to those found in sporadic CJD, but with a very different protein type and codon 129 genotype distribution. These data, and that on panencephalopathic CJD, have been correlated with clinico-pathological data and are currently being prepared for publication and provide valuable comparisons with the sporadic and variant forms of CJD.

The relationship between PrP\textsuperscript{res} type and agent strain is being investigated by ongoing analysis of transmission to wild-type mice in collaboration with the Neuropathogenesis Division, Roslin Institute. Studies of the transmission characteristics of subtypes of sporadic CJD in a human transgenic model have also been completed (J Manson) and provide important information on the extent of strain variation in sCJD and the influence of codon 129 genotype and prion protein type. This later project is harmonised with the EU funded HUMTRANS project which aims at identifying strain variation in all forms of human prion disease, including ‘atypical’ cases (J Manson).

Complementing animal transmission studies the Prion Protein Laboratory aims to model the transmission of human prions, and prion protein conversion, by comparing the
results of mouse transmission studies with the infection of cell cultures (including human stem cells) and the cell-free protein misfolding cyclic amplification (PMCA) method. The results of initial studies using PMCA indicate that amplification depends on both host and agent factors and that PrP\textsuperscript{res} types are amplified with fidelity from sCJD brain (Jones & Head, unpublished observation).

**DIAGNOSTIC TESTS**

**Question 9: What is the diagnostic utility of tests available presently in human prion disease? How good are the current clinical diagnostic criteria?**

**A: Clinical Diagnostic Tests**

Research has been in two main areas: tests that support the diagnosis (the EEG, MRI, CSF proteins and others) and tests that are based on disease-specific features (essentially tests for PrP\textsuperscript{Sc}). As discussed above, international collaboration allows for assessment of criteria and tests in large numbers of cases. A paper on the overall utility of specialist investigations (EEG/14-3-3/MRI) in the diagnosis of sporadic CJD has been published by the EUROJCJD group (Collins et al, Brain 2006). Members of the Unit participate in two major EU funded projects aimed at developing diagnostic markers for human prion disease: Anteprion and Prionscreen.

Analysis of EEG changes in sporadic CJD has been undertaken at the Unit over a period of years. Members of staff have classified, blind and independently, EEG tracings from large numbers of cases and non-cases in order to assess the sensitivity and specificity of EEG in the diagnosis of sporadic CJD. Preliminary findings from this work were presented at the annual EUROJCJD meeting in June 2007 and the definitive analysis should be available in mid 2008.

The Unit has undertaken detailed analysis of MRI scans over the years and has been key in developing the utility of MRI as a diagnostic aid for vCJD as well as sCJD. In addition to the issues of sensitivity and specificity of MRI signs for different forms of prion disease, there are issues of the range of abnormalities seen and the relative utility of different MRI sequences. The EUROJCJD group have completed an analysis of the sensitivity of MRI brain scans in the diagnosis of sporadic CJD, based on DWI images, including an analysis of CJD subtypes classified by codon 129 genotype and PrP type.
MRI can be an additional, helpful, test in the less typical subtypes of sCJD and may be particularly helpful in distinguishing atypical sCJD from vCJD. A paper has been submitted for publication.

A collaborative study on the specificity of MRI brain scans in the diagnosis of sporadic CJD is nearing completion. MRI scans from CJD cases and neuropathologically confirmed non-cases (initially referred as suspected CJD) have been independently scored blind to the diagnosis by two neuroradiologists (D Summers, K Kallenberg). A final analysis is expected in early 2008. A web-based study of the utility of MRI brain scans in the diagnosis of human prion disease is planned by the Unit (D Summers). A large number of MRI scans have been digitised and anonymised in anticipation of this study.

Over the last few years, the Unit’s CSF Laboratory has undertaken detailed assessments of the utility of 14-3-3, s100b and tau in prion disease investigation, both internally and in collaboration with other countries. The Unit has been part of an EU funded research project to create a database containing data on CSF levels of several proteins including 14-3-3, tau protein, S-100b and neurone-specific enolase. The database includes information such as clinical, genetic and biochemical data on patients with sporadic, variant, iatrogenic and familial CJD as well as other forms of dementia or other neurological diseases that were initially suspected as being CJD (control patients). Six European countries contribute to this database, which currently holds information on 2,976 samples. This is the largest reported collection of CSF data and enables meaningful statistical analysis to be undertaken. Data from this database have already been published and include the influence of factors such as age at onset of disease, PRNP -129 status and disease duration on the sensitivity of CSF 14-3-3 (Sanchez-Juan et al, Neurology 2006; Green et al, Eur J Neurol 2007; Sanchez-Juan et al, J Neurol 2007). A further, recent, detailed analysis of the utility of CSF markers in the diagnosis of CJD in the UK has been completed and a paper is in preparation providing information on the sensitivity and specificity of 14-3-3, S100B and tau and the comparative utility of these markers.

Despite its widespread use as a diagnostic test for sporadic CJD, very little is known about the distribution of 14-3-3 within the brain. The 14-3-3 family of proteins constitute
approximately 1% of all the soluble proteins within the central nervous system. There are seven isoforms of which five; gamma, epsilon, eta, zeta and beta are predominantly found in the central nervous system. The most commonly detected isoform in the CSF is gamma and our research examines the distribution of the gamma, eta and zeta isoforms of 14-3-3 in the brain of patients with sporadic and variant CJD, with a view to understanding why the gamma 14-3-3 isoform is preferentially released.

Abnormalities of tau protein processing are found in many forms of dementia and these abnormalities play a significant role in the neurodegenerative process. Alzheimer’s disease (AD) is associated with specific abnormalities in tau metabolism, which results in hyper-phosphorylated tau being deposited around plaques of A Beta-amyloid protein. The discovery that hyperphosphorylated tau protein deposits are also found in vCJD surrounding plaques of prion protein, suggest that tau protein may be involved in the neurodegenerative process of this condition. The Unit’s research is examining the biochemistry of the tau protein deposits found in vCJD to identify any disease-specific changes that may be exploited to develop early diagnostic tests or that could be used to monitor disease progression. One of the earlier findings by the Unit was that CSF phosphorylated tau (pT-181) protein concentrations are elevated in vCJD patients, but not sCJD. The Unit is developing currently immunoassays to other forms of phosphorylated tau in order to improve the diagnostic utility of CSF tau protein measurements (Goodall et al, JNNP 2006).

B: Clinical Criteria
Diagnostic criteria for vCJD have been amended by the EUROCJD group and an adapted version of these criteria have been adopted formally by the EU and recently by ECDC as the case definition for reporting of vCJD (see Appendix 7 for details). Diagnostic criteria for sporadic CJD have been amended several times in the light of increasing knowledge and, currently, study of the utility of including the cerebral MRI is being undertaken (detailed above). Diagnostic criteria for genetic and iatrogenic CJD have also been produced by the EUROCJD group.

C: Neuropathological & Molecular Aspects of Diagnosis
The diagnosis and sub-classification of human prion diseases on the basis of neuropathological examination is of primary relevance to surveillance not only in terms
of diagnosis of general subtypes of human prion disease, but of analysis of variations in neuropathology occurring within apparent existing groups. This is of particular importance when the potential emergence of vCJD in other genetic subgroups is concerned. There is evidence of phenotypic variability in all forms of human disease and this must always be borne in mind when individual cases are being considered, including those with apparently unusual neuropathological features. It is essential that all unusual cases are fully characterised using a combination of clinical, genetic, biochemical and pathological data. The can be supplemented by experimental transmission in laboratory animals, as was undertaken for the first cases of vCJD (Bruce et al, Nature 1997). The Unit’s experience in neuropathological analysis is unique in the UK and it is internationally recognised as a reference laboratory for the neuropathology of human prion diseases, with cases sent from all continents for further investigation and consultation.

The Unit’s work on unusual cases of sporadic CJD occurring in very young adults in the UK has been published (Murray et al, J Neurol Neurosurg Psychiatry 2008), and the Unit has been involved in confirming cases of vCJD outside the UK, particularly in the Netherlands and Portugal. A detailed retrospective analysis of cases of iatrogenic CJD occurring in growth hormone recipients has been undertaken. The neuropathology of a wide range of genetic prion diseases has been reviewed and, in collaboration with colleagues from Netherlands, a detailed analysis of cases of panencephalopathic CJD, an unusual form of sporadic CJD with severe abnormalities in the brain is being undertaken.

Abnormal prion protein typing of suspected cases of human prion disease is the core activity of the Prion Protein Laboratory. This provides valuable pathological diagnostic information and also contributes to the surveillance of novel prion disease in the UK, especially in cases where the clinical features and other investigation results (especially cerebral MRI) are not characteristic. It also provides an important research function, contributing to a better understanding of how abnormal prion diversity might correlate with, or underlie CJD phenotypic diversity, and the nature of the relationship between particular prion protein types and prion agent strain. This has included a retrospective review of the PrP types in cases with mutations in PRNP (familial CJD, Gerstmann-
Antibodies to prion protein play a vital role in diagnosis and research. The DH funded research contract, “Novel application-specific monoclonal antibodies to PrP” successfully produced the first panel of antibodies raised against native human PrP (purified from platelets) and another panel raised against alpha- and beta- form recombinant mouse PrP. This ongoing work remains a consortium arrangement between the Unit’s Prion Protein Laboratory and colleagues at the Scottish National Blood Transfusion Service (I MacGregor), the Neuropathogenesis Division, Roslin Institute (C Farquhar) and the University of Strathclyde (J Connolly). The major outcome of the study has been the development of a PrP$^\text{Sc}$-specific monoclonal antibody termed P1.1, which was made by immunising PrP knockout mice with the aggregated, toxic, PrP106-126 peptide, and screening for antibodies that react with aggregated but not monomeric PrP106-126 peptide. The antibody has a number of unexpected properties: in addition to being PrP$^\text{Sc}$-specific, it immunoprecipitates in native conditions both abnormal protease-sensitive and protease-resistant isoforms. It also shows a strong preference for type 1 PrP, binding poorly to type 2 PrP in protease-treated samples. The antibody and the method used to produce it have been the subject of a patent and have attracted both academic and commercial interest.

**Question 10: What other diagnostic tests may be useful? How are they being developed and assessed?**

The Unit is involved in research, both internally and collaboratively, into developing further diagnostic tests for prion diseases. ‘Diagnostic’ tests have several potential roles, which include helping the diagnosis of clinically suspect cases, detecting pre- or subclinical infected individuals and so contributing to population prevalence estimations, and aiding the screening of human tissues.

One major aim of the Unit’s Prion Protein Laboratory’s research work is to develop and evaluate methods, such as those using monoclonal antibodies, CDI and PMCA, for the sensitive and specific detection of PrP$^\text{Sc}$ that could serve as a much needed blood and organ/tissue donor screening assay sought by the transfusion and transplantation
services. Much of this work is being done in collaboration with colleagues at the Scottish National Blood Transfusion Service (I MacGregor, C Prowse, M Turner) and has been externally funded by competitive research grants from the DH and CSO.

The Neuropathology and Prion Protein Laboratories have also collaborated with various Neuroprion partners, including O Andreoletti in the development of novel assays for abnormal prion protein (Uro-Coste et al, PLoS Pathogens 2008), and colleagues in A Aguzzi’s laboratory, University of Zurich, for the development of novel diagnostic assays and the detection of disease-associated prion protein in the kidney. Collaboration with M Pepys, University College, London has shown that abnormal prion protein is not detectable in systemic amyloid deposits in humans, in contrast to a claim to the contrary in a model system, (Tennent et al, J Pathol 2007).

**ERAF**

A study quantifying the levels of ERAF (synonyms EDRF, AHSP) in blood from patients with CJD or haemoglobinopathies has been carried out in collaboration with the National Blood Services (DJ Anstee), the Roslin Institute (M Clinton), the Neuropathogenesis Division, Roslin Institute (J Manson) and the Unit. A paper has been accepted for publication.

**Urine test**

The Unit undertook a study of urine, using a test reported by colleagues in Israel. The initial report claimed the test detected disease-related PrP in urine and was thereby potentially useful in diagnosis. The Unit’s study failed to confirm the detection of abnormal PrP and also found poor sensitivity and specificity for CJD (Head et al, Neurology, 2005)

**Conformation Dependent Immunoassay (CDI)**

A research grant has been obtained from the Chief Scientists Office (CSO), Scottish Government, to develop CDI. This has been taken forward and a robust and sensitive CDI assay in the Prion Protein Laboratory that can detect variant CJD brain at a 10^{-5} dilution in normal human plasma without recourse to proteinase K digestion has been established. Currently this assay is being applied to peripheral organ samples from CJD patients obtained at autopsy. A denaturation profiling variation of the CDI assay is also
being used to attempt to give a better definition of abnormal prion protein isoforms in different forms of CJD.

**Protein Misfolding Cyclic Amplification (PMCA)**

A version of the PMCA technique has been established in the Prion Protein Laboratory that can amplify variant CJD PrP\(^{Sc}\) up to 1,000 times. The novelty of the assay is the use of “humanised” transgenic mouse brains (R Barron, Neuropathogenesis Division, Roslin Institute) and human platelets as a source of PrP\(^{C}\) in the amplification process. Given that both PMCA and CDI are microtitre plate assays, the two technologies have been coupled, thereby developing a potentially high through put, high sensitivity PrP\(^{Sc}\) assay (Jones et al, Journal of Pathology 2007). Supplementary funding for the development of PMCA/CDI has been secured.

**Cell culture**

An alternative approach to the cell-free amplification of human prions is the use of cell cultures. The necessary facilities to perform such studies have been established at the Unit, in part through funding from the EU Network of Excellence “NeuroPrion”. To date, these studies have explored the potential of cycling and differentiated human neurblastoma cells and follicular dendritic-like cells to support human prion replication. In collaboration with P De Sousa (University of Edinburgh Centre for Regenerative Medicine and Roslin Institute) CSO funding has been obtained to explore the potential of human stem cell lines in supporting prion replication (commenced January 2008).

**Atomic Dialectric Resonance Spectroscopy**

The Prion Protein Laboratory has collaborated with our Scottish National Blood Transfusion colleagues and the commercial company Radar World in a study that shows that atomic dialectric resonance spectroscopy can distinguish between clinical blood specimens from patients with CJD and patients considered for a diagnosis of CJD, but who were given an alternative final diagnosis (Fagge et al, Journal of Translational Medicine 2007).
**TREATMENT**

*Question 11: What are the prospects for treatment in human prion diseases? How can possible treatments be assessed?*

Trials of treatment in CJD are underway or being considered in a number of EU countries and in the USA and Japan. Through the Neuroprion Network of Excellence the Therapron group, which is chaired by the Unit, has been formed to consider harmonising methodologies in treatment trials of CJD, including entry criteria and assessment tools.

Clinicians from the Unit have been involved in the treatment of some individuals with intra-ventricular PPS (Pentosan Polysulphate). Data from PPS treated patients have been analysed in an MRC review (I Bone). The Unit has been involved in discussions with colleagues in Neurosurgery, Edinburgh, and at the Roslin Institute, Edinburgh, concerning possible PPS research in animal models. The Unit is represented on committees concerned with treatment issues, including the New Therapy Scrutiny Group, the Outcomes Measure Sub-Group and the MRC Prion-1 Trial.

**PUBLIC HEALTH**

The majority, if not all, of the surveillance and research undertaken by the Unit will ultimately have public health consequences. Therefore, each of the questions and answers listed above have implications to public health, whether it be related to risk factors for vCJD or the development of a diagnostic test using protein biochemical techniques. Rather than list out the public health implications of all the questions above, some specific questions related to key areas of the Unit’s work (surveillance and research), which currently have public health impact or are likely to in the future include:-

*(more detail of the Unit’s involvement in these areas is given in the questions/ answers referred to beneath each of the Public Health questions)*
**Question 12: If vCJD is identified in non methionine (MM) homozygotes, what are the public health implications?**

*Please also refer to the answers to questions 1, 2, 4, 5 and 6.*

Through routine surveillance and research, the Unit continues to monitor the clinical, epidemiological and pathological data of human prion diseases. One purpose of this is to determine if unusual, novel, disease types are emerging.

In addition to the public health implications of each individual case of vCJD reported by the Unit, if cases of vCJD are identified in non-methionine homozygotes (at codon 129) individuals, these will have important public health implications. If clinical cases of vCJD are found in codon 129 heterozygotes and valine homozygotes this will obviously result in increased numbers of those with the disease. However, if similar to other acquired human TSEs, such as Kuru and in human derived growth hormone recipients, this is unlikely to result in numbers of cases greater than those found in the more susceptible methionine homozygous individuals and may also result in some long (perhaps up to 40 years) incubation periods. In addition to the burden of those with the disease itself, there is the increased risk of onward, secondary transmission through, for example, blood transfusion, dentistry and surgery.

**Question 13: The public health risk of secondary transmission of CJD. How is the Unit contributing to determining this risk?**

*Please also refer to the answers to questions 1, 5 and 7.*

The Unit continues to collaborate with the UK Blood Services through the TMER to determine onward transmission of CJD. In addition, analysis through the case control study and examining tempo-geographic trends will help to determine whether surgery is responsible for transmission of vCJD. Collation and monitoring of databases of invasive medical procedures and children of vCJD cases is undertaken.

Preliminary findings from animal Studies conducted by HPA, Porton Down, have indicated that the risk of transmission of vCJD through dentistry may be greater than previously recognised. Research within the Unit during 2008 examining the dental records of vCJD cases (and ‘controls’ without CJD) will help to inform the magnitude of this risk.
In relation to modelling mathematically the potential size of the primary and secondary vCJD epidemics, the Unit actively collaborates with the HPA (N Andrews), the London School of Hygiene and Tropical Medicine (S Cousens) and Imperial College (A Ghani).

**Question 14: Those designated at risk for public health purposes. What is the Unit’s role in their follow up?**

*Please also refer to the answer to question 5.*

The Unit is a collaborator in a study lead by HPA (K Soldan) to follow up those designated ‘at risk’ of CJD (for example, through receipt of implicated blood components, donating certain blood components and contact with potentially contaminated surgical instruments) in an attempt to elucidate further the risk to the individual and to the public health.

The role of the Unit has been to contribute to the development of the study, and will be to help recruit those at risk being followed up currently by the Unit’s neurologists, to carry out neuropathology at post mortem (with relevant consent) and to link the NCJDSU database with the ‘at risk’ database (HPA) to determine possible clinical cases.

From this study, various factors, which will have important public health consequences relating to the incubation period, pre-clinical phase, sub-clinical phase and transmissibility of vCJD may be determined.

**Question 15: Is there a public health risk from the newly recognised ‘atypical’ animal prion diseases? How can this be assessed?**

*Please also refer to the answers to questions 1, 2, 3, 4, 6 and 8.*

It is not known if these ‘atypical’ TSEs in animals (atypical scrapie, H- type BSE, L- type BSE or BASE) can infect humans and, if so, whether they would have the same clinico-pathological phenotype as either variant or sporadic CJD. As discussed in Question 4, even if the phenotype were different, studies in animal models, including a primate model, indicate that infection with these agents results in a rapidly progressive prion disorder of the central nervous system and it is likely that infection in humans, should this occur would present with a progressive ‘unexplained’ neurological disorder and prion...
protein deposition in the brain. Although such cases are likely to be referred to the Unit under present surveillance arrangements, SEAC committee recommended that neurologists in the UK should be specifically asked to refer atypical cases to the Unit. The key therefore is both to continue to encourage referral of any, even only doubtfully, suspect case and for the Unit to explore atypical cases in detail—clinical features, neuropathology, prion protein type, molecular genotype, CSF biochemistry, and epidemiology, including risk factor information, will continue to be investigated as described above.

In addition, it is important to make clinicians aware of the possibility of infection that might have clinical expressions different from the CJD picture with which they are now familiar. If ‘atypical’ cases were to be identified in the UK, the Unit is ideally placed (via its extensive international connections) to rapidly ascertain whether similar cases are to be found in other countries. Atypical cases are presented at the regular EUROCD meetings, cases with unusual neuropathological features may be sent to experts in other countries for review and the Unit is often requested for opinions, on clinical, biochemical and neuropathological features, on atypical cases in other countries. Should a potential new CJD phenotype be recognised, comparative data on the occurrence, characteristics and frequency of such cases can be obtained promptly by the close collaboration between European surveillance systems (this precise mechanism was essential to the prompt recognition of variant CJD as a new phenotype in 1996).

**Question 16: Development of a diagnostic test for CJD. What is the Unit's role in the development of such a test?**

*Please also refer to the answers to questions 9 and 10.*

Development of a diagnostic test for CJD: the development of a simple, quick and cheap test for CJD that could be carried out, on for example, blood, is a major aim of the Units’ Prion Protein and Neuropathology Laboratories. If such a test were developed, it would have major public health implications, for example, in the testing of blood, organ and tissue donors, in testing those designated at risk of CJD and in diagnosing those with early clinical features. However, as with any new test developed, the ultimate use of the test will depend on many factors, including the specificity and sensitivity of the test, its cost, its acceptability etc.
Question 17: What public health measures in relation to prion diseases have been taken in different countries?

Please also refer to the answers to questions 1, 2, 4, and 6.

An analysis of public health responses in the EU to issues raised by CJD/vCJD has been completed for countries participating in the EUROCJD system (H Blystad). The initial results indicated that there is much heterogeneity between EU countries in their response to perceived public health risks and many countries would support the production of standardised guidelines. A report describing the results is in preparation.

VI APPENDICES please refer to separate document